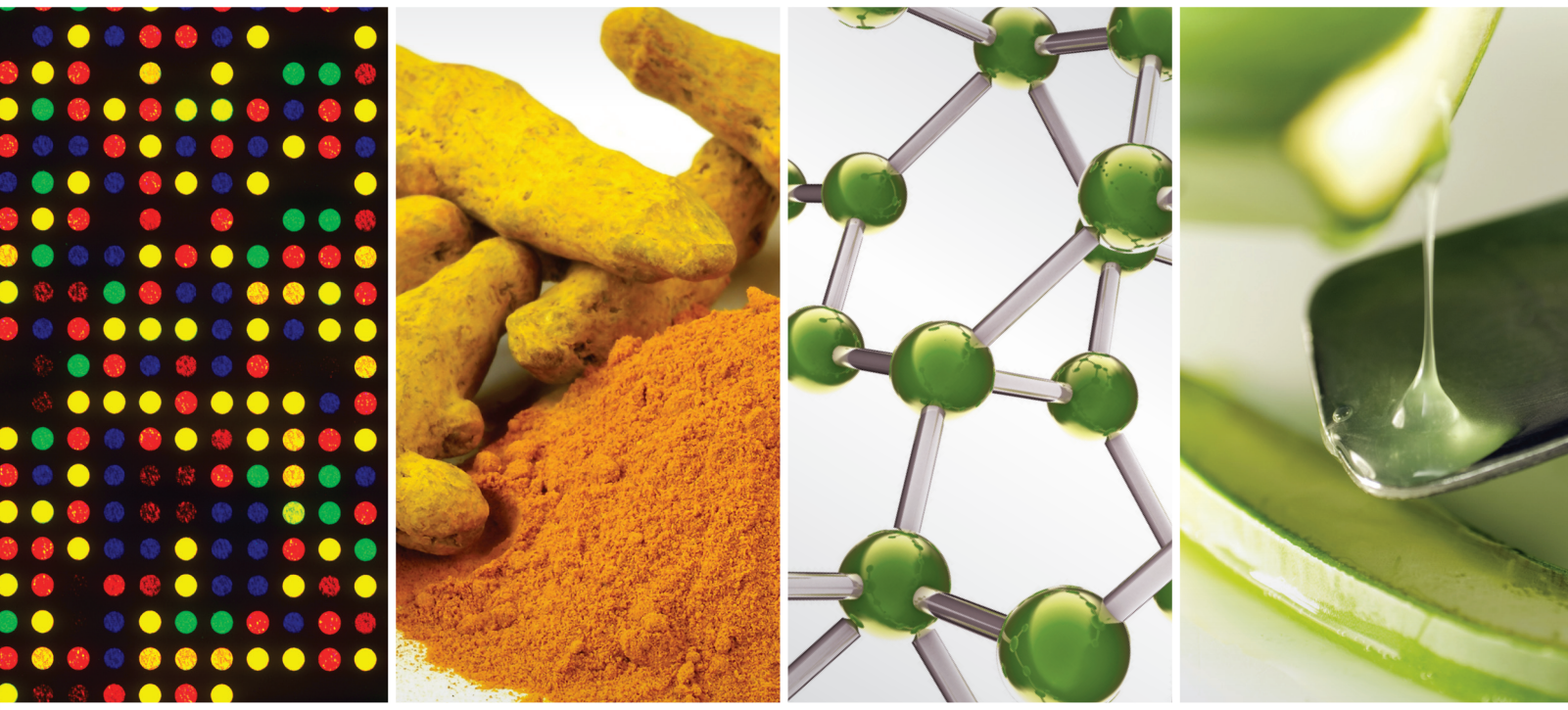
eCAM Evidence-Based Complementary and Alternative Medicine

Evidence-Based ZHENG: A Traditional Chinese Medicine Syndrome

Guest Editors: Shi-Bing Su, Aiping Lu, Shao Li, and Wei Jia



Evidence-Based ZHENG: A Traditional Chinese Medicine Syndrome

Evidence-Based Complementary
and Alternative Medicine

Evidence-Based ZHENG: A Traditional Chinese Medicine Syndrome

Guest Editors: Shi-Bing Su, Aiping Lu, Shao Li, and Wei Jia

Editorial Board

Contents

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 246538, 2 pages
doi:10.1155/2012/246538

Editorial

Evidence-Based ZHENG: A Traditional Chinese Medicine Syndrome

Shi-Bing Su,[1] Aiping Lu,[2] Shao Li,[3] and Wei Jia[4]

[1] *Research Center for Complex System of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*
[2] *Institute of Basic Research In Clinical Medicine, China Academy of Chinese Medical Sciences, Beijing 100700, China*
[3] *MOE Key Laboratory of Bioinformatics and Bioinformatics Division, TNLIST/Department of Automation, Tsinghua University, Beijing 100084, China*
[4] *Department of Nutrition, The University of North Carolina at Greensboro, Kannapolis, NC 28081, USA*

Correspondence should be addressed to Shi-Bing Su, shibingsu07@163.com

Received 5 July 2012; Accepted 5 July 2012

The traditional Chinese medicine (TCM) ZHENG, also known as TCM syndrome or TCM pattern, is an integral and essential part of TCM theory. A TCM ZHENG is in essence a characteristic profile of all clinical manifestations that can be identified by a TCM practitioner. Clinical treatments of a patient rely on the successful differentiation of a specific ZHENG. Recent advances in systems biology have allowed the application of new phenotyping technologies in the study of the ZHENG differentiation with plausible biological interpretations. Understanding of the characteristic changes in biochemistry associated with a specific TCM ZHENG will facilitate the development of ZHENG identification and a novel disease diagnostic and stratification approach that will potentially lead to personalized healthcare strategies for a range of diseases that lack therapeutic solutions. Here, we have grouped together 28 excellent papers in this field and put forward for publication in this special issue on TCM ZHENG.

Firstly, there are 3 review or research papers in this special issue addressed the concept, origin, and development of ZHENG, the recent advances in ZHENG identification and its clinical applications, and latest technologies and methods such as omics methods and data mining for ZHENG identification and outcome measurement. Two papers reviewed the clinical characterization and molecular basis of TCM ZHENG in cancer and TCM management in hepatic encephalopathy, respectively. Moreover, a review paper reviewed systematically the classification of TCM ZHENGs associated with insomnia.

In TCM, the clinical diagnosis of ZHENG relies on the gathering of clinical information through inspection, auscultation and olfaction, inquiry, and palpation. For the acquisition of ZHENG-related clinical information, 2 research articles presented the established ZHENG questionnaire in the posthepatic cirrhosis and advanced cancer patients with constipation, respectively. Moreover, the patient stratification and personalized treatment by means of the ZHENG identification and approaches in patients with allergic rhinitis and subhealthy people with fatigue were presented. Additionally, the correlation between Blood-stasis syndrome score and cardio-ankle vascular index in stroke patients was also discussed.

ZHENG is not merely an assembly of many disease symptoms but an organization of interrelated clinical manifestations following the TCM theories. The interrelated symptoms and signs of diseases in the ZHENG measurement should be analyzed using appropriate statistical tools to better understand the ZHENG classification. Six research articles of this special issue presented the data mining of ZHENG differentiation using the combination of wavelet packet transform and sample entropy, the clinical phenotypic network in angina pectoris of coronary heart disease, a multilabel learning using the relevant feature for each label algorithm in chronic gastritis, and a structural equation modeling approach in suboptimal health status.

To objectively differentiate ZHENGs, 7 research articles in this issue presented the ZHENG classification using genes, proteins, metabolites, and/or their profiles. These

are system strategies in investigating ZHENG classification and treatment evaluation by means of gene polymorphism, transcriptomics, proteomics, metabonomics, bioinformatics, and network pharmacology. The methods include IL-10 genotypes in ZHENG, the metabonomic evaluation of ZHENG classification and treatment by Chinese herbal formula, and a combined ZHENG theory and high-throughout gene chip data to predict new effects of the formula in hepatitis B-caused cirrhosis. Additionally, the molecular mechanisms of "Same ZHENG for Different Diseases" and "Different ZHENGs for Same Disease" in chronic hepatitis B and liver cirrhosis and the ZHENG classification in chronic hepatitis B patients by SELDI-based protein chip analysis were also discussed in these papers.

To experimentally evaluate ZHENG, various pharmacological models of ZHENG are to be established. In this special issue, 4 papers discussed the establishment and/or the application of ZHENG animal models, encompassing the preparation of blood-deficient syndrome model in chicken, the castration-induced kidney deficiency syndrome model in arthritic rats, the kidney-yang deficiency syndrome model in Alzheimer's disease rats, and their applications. Moreover, it was also presented that Chinese herbal medicines were used to treat the established mouse xenograft pancreatic cancer models with dampness-heat, spleen-deficiency and Blood-stasis syndromes.

In summary, the concept of TCM ZHENG, as a diagnostic approach in TCM, would provide invaluable guidance about the therapeutic choices and personalized disease management, not only in traditional medical practices but in modern healthcare systems as well. We look forward to an increasing number and sizes of clinical trials utilizing TCM ZHENG that will be conducted in the future to further promote the development of evidence-based personalized medicine.

Shi-Bing Su
Aiping Lu
Shao Li
Wei Jia

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 735078, 9 pages
doi:10.1155/2012/735078

Review Article

Classification of Insomnia Using the Traditional Chinese Medicine System: A Systematic Review

Maggie Man-Ki Poon,[1] Ka-Fai Chung,[1] Wing-Fai Yeung,[1] Verdi Hon-Kin Yau,[1] and Shi-Ping Zhang[2]

[1] *Department of Psychiatry, The University of Hong Kong, Pokfulam Road, Hong Kong*
[2] *School of Chinese Medicine, Hong Kong Baptist University, Hong Kong*

Correspondence should be addressed to Ka-Fai Chung, kfchung@hkucc.hku.hk

Received 10 January 2012; Accepted 22 March 2012

Academic Editor: Shi-Bing Su

A systematic review was conducted to examine traditional Chinese medicine (TCM) patterns commonly diagnosed in subjects with insomnia and clinical features associated with the TCM patterns, and an insomnia symptom checklist for TCM diagnostic purpose was developed based on the review. Two independent researchers searched the China Academic Journals Full-Text Database and 10 English databases. A total of 103 studies and 9499 subjects were analyzed. There was a wide variation in terminology relating to symptomatology and TCM pattern. We identified 69 patterns, with the top 3 patterns (i.e., *deficiency of both the heart and spleen, hyperactivity of fire due to yin deficiency, and liver-qi stagnation transforming into fire*) and the top 10 patterns covering 51.8% and 77.4% of the 9499 subjects, respectively. There were 19 sleep-related, 92 non-sleep-related, 14 tongue, and 7 pulse features included as diagnostic criteria of the top 10 TCM patterns for insomnia. Excessive dreaming, dizziness, red tongue, and fine pulse were the most common sleep-related, non-sleep-related, tongue, and pulse features. Overlapping symptomatology between the TCM patterns was present. A standardized symptom checklist consisted of 92 items, including 13 sleep-related, 61 non-sleep-related, 11 tongue, and 7 pulse items, holds promise as a diagnostic tool and merits further validation.

1. Introduction

Insomnia is the most common sleep complaint, with approximately 9–15% of the general population worldwide suffering from insomnia symptoms accompanied by daytime consequences [1]. Insomnia is associated with psychological distress, impaired daily functioning, and an increased risk of medical and psychiatric morbidity and mortality [2]. Although effective pharmacologic and psychological treatments for insomnia are available, their uses are limited due to concerns regarding adverse effects and feasibility in everyday clinical settings [3, 4]. Faced with the limitations of the currently available treatments, complementary and alternative medicine (CAM) has been sought to treat insomnia. A national survey in the United States showed that 4.5% of adults reported using some form of CAM for insomnia in the past year [5]. Traditional Chinese medicine (TCM), a form of CAM, is one of the oldest medical systems in the world. A population-based study in Australia showed that around 20% of adults used at least one form of TCM treatments in the past year [6]. A study in Taiwan showed that 28% of valid beneficiaries of the national health insurance filed claims for TCM treatment during the year 2002 [7].

The recognition of insomnia as a major health problem can be traced back to more than 2000 years ago in ancient Chinese medical texts [8, 9]. Based on the patient's symptoms and signs, TCM practitioners describe the patterns of bodily disharmony in terms of eight major parameters: *yin* and *yang*, *external* and *internal*, *hot* and *cold*, and *excess* and *deficiency*. Additional systems, such as *qi*, *blood* and *body-fluid* differentiation, and *zang fu* (organ) differentiation are also used [10]. The TCM patterns describe differences in etiology and pathogenesis of diseases and emphasize variation in individuals' body constitution. Although most of the TCM concepts have yet been proven by scientific method, the TCM diagnostic system continues to be practiced

nowadays. Treatment principles and specific herbal formula or acupoints are derived according to the TCM pattern. Nevertheless, the key shortcomings of the TCM diagnostic process are the lack of standardization in terminology and disagreement on pattern differentiation among Chinese medicine practitioners [11–14].

To the best of our knowledge, there has been no systematic assessment of the reliability and validity of the TCM pattern differentiation for insomnia. Although the publication of standard TCM textbooks in China can be seen as an attempt to minimize disagreement among practitioners, the recognition and acceptance of the textbooks among TCM practitioners are uncertain. Given the frequent occurrence of insomnia among patients presenting to TCM practitioners, it is important to use standardized terminology and criteria for TCM diagnosis. As a first step of the standardization, we conducted a systematic paper of TCM patterns commonly diagnosed in subjects with insomnia and gathered information on the clinical features of the TCM patterns. Based on our review, we constructed an insomnia symptom checklist which could be used as a diagnostic tool for future research and clinical purposes.

2. Material and Methods

2.1. Search Strategy. We searched the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, PsycINFO, PUBMED, Dissertation Abstracts International, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Allied and Complementary Medicine Database (AMED), National Center for Complementary and Alternative Medicine, National Institute of Health Clinical Trials Database, China Academic Journals Full-Text Database from inception to November 2008 using the grouped terms "Chinese medicine or TCM or acupunc* or acupress* or electroacupunc* or meridian* or acupoint* or tuina*" and "sleep* or insomnia* or wakeful* or sleepless* or somnambul*" and the China Academic Journals Full-Text Database using equivalent Chinese terms. The reference lists of the retrieved papers were further searched for relevant articles.

2.2. Selection Criteria. We included studies that described TCM patterns of subjects with a chief complaint of insomnia. In order to obtain a full coverage of the topic, we did not set any specification for sampling procedure, treatment method, outcome measure, and study quality. Aiming to derive a general picture of TCM pattern utilization, studies were excluded if they (1) had less than 30 subjects; (2) examined males or females only; (3) focused on individuals aged below 18 or above 70 years; (4) focused on a specific medical and psychiatric condition, a particular life transition period, or a specific TCM pattern; (5) had no statistical information regarding TCM pattern; (6) were duplicated publications. The authors (MKP and HKY) searched the databases and selected the relevant publications independently. Full papers of the relevant publications were obtained and reviewed in detail against the inclusion and exclusion criteria. Any disagreement about the eligibility of study was resolved by thorough discussion.

2.3. Data Extraction Process. For each study, the following variables were extracted: study design, sample size, mode of recruitment, sampling and diagnostic procedure, inclusion and exclusion criteria, and participants' characteristics including age, gender, and duration of insomnia. Information regarding the TCM pattern including symptoms and signs of each TCM pattern was obtained. All Chinese to English translations were deduced primarily from the *World Health Organization (WHO) International Standard Terminologies on Traditional Medicine in the Western Pacific Region* [15] and additionally from *the Traditional Chinese Internal Medicine* [16], a widely used English-language TCM textbook in China.

2.4. Construction of an Insomnia Symptom Checklist for TCM Diagnostic Purpose. The symptom checklist included clinical features of the 10 most common TCM patterns associated with insomnia. The top 10 TCM patterns were chosen because they covered roughly 80% of subjects with insomnia (Table 1). If more TCM patterns were covered, the symptom checklist would be too lengthy. Symptoms included in the checklist needed to have mentioned as clinical features of the TCM patterns in at least 10% of the reviewed studies; thus, both common and less common features would be listed. In addition, we reviewed several standard TCM textbooks for colleges and universities, including the editions of *Traditional Chinese Internal Medicine* published in 1985 [17], 1997 [18], 2003 [19], and 2007 [16] for symptoms that were not described in the reviewed studies.

3. Results

3.1. General Description of the Reviewed Studies. The search yielded 4795 potentially relevant citations, of which 3036 citations were excluded for reasons of irrelevance or duplication. A total of 1759 articles that were related to insomnia and TCM were retrieved for further review. Three hundred thirty-six articles were discussion papers, 95 were restricted to subjects aged below 18 or above 70 years, 33 focused on either males or females, 73 were limited to specific medical and psychiatric conditions, 19 focused on a particular life transition period, 145 were studying a specific TCM pattern, 264 had less than 30 subjects, 364 did not have information on TCM pattern, 310 had no statistical information regarding the frequency of individual TCM pattern, 16 were written neither in Chinese nor English, and one could not be retrieved in full text. These 1656 studies were excluded and the remaining 103 studies were included in this paper. Full details of the excluded studies are available from the authors.

The sample size of the 103 studies ranged from 30 to 856. TCM diagnosis was available in 9499 subjects. Based on the sex distribution, mean age, and number of subjects reported in each study, about 56.5% of the total sample were female and the subjects' mean age were 44.0 years. All included studies were conducted in China, and 5 (4.9%) were

Table 1: The 10 most common TCM patterns for insomnia.

TCM pattern	Chinese name	Subjects with insomnia ($N = 9499$) Number of subjects (%)
Deficiency of both the heart and spleen	心脾兩虛	2378 (25.0)
Hyperactivity of fire due to yin deficiency	陰虛火旺	1622 (17.1)
Liver-qi stagnation transforming into fire	肝鬱化火	921 (9.7)
Heart-kidney noninteraction	心腎不交	767 (8.1)
Qi deficiency of the heart and gallbladder	心膽 氣虛	544 (5.7)
Internal disturbance of phlegm-heat	痰熱內擾	466 (4.9)
Liver fire flaming upward	肝火上擾	285 (3.0)
Heart deficiency with timidity	心虛膽 怯	202 (2.1)
Stomach disharmony	胃脾不和	120 (1.3)
Stomach qi disharmony	胃氣不和	44 (0.5)

published in English-language journals. The criteria used for diagnosis of insomnia varied between studies. Twelve of the 103 studies (11.7%) were based on the *Criteria of Diagnosis and Therapeutic Effect of Diseases and Syndromes in Traditional Chinese Medicine* [20], 11 (10.7%) studies used the *Chinese Classification of Mental Disorder* [21], nine (8.7%) used the *Clinical Research Guidelines of New Chinese Herbal Medicine* [22], and one (1.0%) used the *WHO* diagnostic criteria [23]. Thirty-six (35.0%) studies based on TCM textbook or other criteria, and 34 (33.0%) did not report the diagnostic criteria used.

3.2. TCM Pattern Differentiation for Insomnia. Seventy-four different TCM patterns were reported in the 103 included studies. Similar patterns were grouped together. Thus, *heart-gall bladder deficiency and timidity* (心膽 虛怯) was grouped under *heart deficiency with timidity* (心虛膽 怯); *heart and spleen deficiency* (心脾虧損) was considered as *heficiency of both the heart and spleen* (心脾兩虛); and *stomach lost harmony* (胃脾失和) was grouped under *stomach disharmony* (胃脾不和); *stomach qi lost harmony* (胃氣失和) was grouped under *stomach qi disharmony* (胃氣不和); *phlegm-fire hindering the heart* (痰火撓心) was considered as *phlegm-fire harassing the heart* (痰火擾心). After grouping similar patterns, a total of 69 TCM patterns had been used for classification of insomnia. The most commonly presented pattern was *deficiency of both the heart and spleen* ($N = 2378$, 25.0% of the 9499 subjects), followed by *hyperactivity of fire due to yin deficiency*, *liver-qi stagnation transforming into fire*, *heart-kidney noninteraction*, *qi deficiency of the heart and gallbladder*, *internal disturbance of phlegm-heat*, *liver fire flaming upward*, *heart deficiency with timidity*, *stomach disharmony*, and *stomach qi disharmony*. The top 10 TCM patterns accounted for 77.4% of the 9499 subjects (Table 1).

3.3. Terms Relating to Sleep-Related, Non-Sleep-Related, Tongue, and Pulse Features. Thirty-seven of the included studies provided clinical features of individual TCM patterns. We examined sleep-related, non-sleep-related, tongue, and pulse features of the 10 most commonly presented TCM patterns. A total of 52 Chinese terminologies relating to sleep-related symptoms were mentioned, but many had similar meaning. For example, eight different Chinese terminologies were used to describe difficulty falling asleep and four different Chinese terms describing insomnia. After grouping similar terms, there were 19 different sleep-related symptoms. In the order of frequency, the terms included excessive dreaming, insomnia, difficulty staying asleep, difficulty falling asleep, insomnia with vexation, restless sleep, frequent awakening with a start, half asleep, sleeping late at night, nonrefreshing sleep, early-morning awakening, shallow sleep, daytime sleepiness, easy awakening from sleep with difficulty getting back to sleep, inability to sleep for the whole night, difficulty falling asleep alone, difficulty falling asleep at night, nightmare, and difficulty falling asleep with vexation.

There were 169 Chinese terminologies relating to non-sleep-related symptoms of the 10 most commonly presented TCM patterns for insomnia. After grouping similar Chinese terms, we found 92 non-sleep-related symptoms that were described in the top 10 TCM patterns for insomnia. The more frequently mentioned non-sleep-related symptoms, in the order of frequency, included dizziness, palpitation, vexation, poor memory, dry mouth, tinnitus, bitter taste, lassitude, feverish sensations in the palms, soles, and chest, fatigue, backache, timidity, reduction in luster complexion, irritability, poor appetite, constipation, oppression in the chest, reddish eyes, stuffiness in the chest and stomach, headache, tasteless, yellow urine, and sore knees. Depressed mood and weight loss were only mentioned in one article.

There were 19 Chinese terms relating to tongue features in subjects with insomnia; after grouping similar terms, it was reduced to 14. The tongue features, in the order of frequency, were red tongue, pale tongue, thin coating, yellow

coating, slimy coating, scanty coating, and white coating. There were seven pulse features in the TCM classification system related to insomnia complaints. Fine pulse was the most commonly mentioned in patients with insomnia, followed by rapid pulse, string-like pulse, weak pulse, and slippery pulse.

3.4. Comparing the 10 Most Commonly Presented TCM Patterns for Insomnia. Based on our paper, we found that most sleep-related symptoms appeared in more than one TCM pattern (Table 2). For example, excessive dreaming and difficulty falling asleep were found in seven of the 10 most commonly presented TCM patterns, while difficulty staying asleep was present in five of the top 10 patterns.

We found that dizziness, vexation, palpitation, tinnitus, and bitter taste were non-sleep-related symptoms that occurred in at least four of the top 10 TCM patterns (Table 2). Dizziness was included as a non-sleep-related symptom in *deficiency of both the heart and spleen*, *hyperactivity of fire due to yin deficiency*, *heart-kidney noninteraction*, and *stomach disharmony*. Vexation was present in all excess patterns except *stomach qi disharmony* and could be found in three deficiency patterns *hyperactivity of fire due to yin deficiency*, *qi deficiency of the heart and gallbladder*, and *heart-kidney noninteraction*. Palpitation was described in all of the deficiency patterns and *liver fire flaming upward*. Tinnitus was present in three excess patterns *liver-qi stagnation transforming into fire*, *liver fire flaming upward*, and *stomach disharmony* and two deficiency patterns *hyperactivity of fire due to yin deficiency* and *heart-kidney noninteraction*. Bitter taste was found in three excess patterns and one deficiency pattern.

The tongue feature which commonly occurred in excess patterns was red tongue (Table 2). For *liver-qi stagnation transforming into fire,* there was an addition of yellow coating, and for *internal disturbance of phlegm-heat*, there was an addition of yellow and slimy coating. However, red tongue could also occur in two deficiency patterns, *hyperactivity of fire due to yin deficiency* and *heart-kidney noninteraction*. Pale tongue was present in all deficiency patterns except *hyperactivity of fire due to yin deficiency;* for *deficiency of both the heart and spleen*, there was an additional thin coating.

The pulse feature which commonly occurred in excess TCM patterns was rapid pulse; for deficiency patterns, it was fine pulse. However, rapid pulse was also found in the two deficiency patterns, *hyperactivity of fire due to yin deficiency* and *heart-kidney noninteraction*, whereas fine pulse was also found in two excess patterns, *liver-qi stagnation transforming into fire* and *liver fire flaming upward*. There was also slight difference in pulse feature among the deficiency patterns (Table 2).

3.5. Insomnia Symptom Checklist for TCM Diagnostic Purpose. The symptom checklist took into consideration of the common and less common symptoms of the top 10 TCM patterns diagnosed in patients with insomnia (refer to the Methods section). It consisted of 92 items, including 13 sleep-related symptoms, 61 non-sleep-related symptoms, 11 tongue features, and seven pulse features (Table 3). Most of the symptoms included in the checklist were derived from the reviewed studies, with the exception of head distension, abdominal distension, dry tongue, and strong pulse, which were only listed in TCM textbooks [16–19].

4. Discussion

This is the first systematic review examining both English and Chinese literatures on the classification of insomnia using the TCM diagnostic system. We conducted an extensive review of 103 articles involving 9499 subjects to derive the common TCM patterns in the diagnosis of insomnia and the clinical features of the TCM patterns. The top 3 TCM patterns *deficiency of both the heart and spleen*, *hyperactivity of fire due to yin deficiency*, and *liver-qi stagnation transforming into fire* covered slightly more than half of the TCM patterns diagnosed in subjects with insomnia. Five of the 10 most common TCM patterns found in our review, namely *deficiency of both the heart and spleen*, *hyperactivity of fire due to yin deficiency*, *liver-qi stagnation transforming into fire*, *internal disturbance of phlegm-heat*, and *qi deficiency of the heart and gallbladder,* were listed in standard TCM textbooks in China [16–18].

We found that the terminology relating to sleep-related symptoms in the TCM classification was much more detailed than those used in the Western diagnostic systems. The insomnia symptoms mentioned in the *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition* [23] and the *WHO International Classification of Diseases 10th Edition* [23] include difficulty falling asleep, difficulty maintaining sleep, nonrefreshing sleep, and nonrestorative sleep. Although excessive dreaming, awakening with a start and restless sleep were common complaints in individuals with insomnia, they were not utilized in the Western diagnostic systems. Half asleep, going to sleep late at night, insomnia with vexation, and difficulty falling asleep with vexation were seldom mentioned in the Western literature. At present, no scientific investigation on these individual sleep symptoms has been performed; hence future studies are needed to determine their clinical significance.

Somatic symptoms are overrepresented in the TCM diagnostic system, whereas psychological symptoms are rarely mentioned. The finding is in line with the fact that Chinese patients use more somatic words to talk about emotions than Western people [24]. We found 92 different non-sleep-related signs and symptoms that were associated with the top 10 TCM patterns for insomnia. These signs and symptoms appear to reflect the imbalance or malfunctioning of various body systems, which can be causes or consequences of insomnia or both. For example, reddish eyes and reddened complexion found in *liver-qi stagnation transforming into fire* may indicate sympatho-excitation [25], whereas reduction in luster complexion seen in *deficiency of both the heart and spleen* may suggest the opposite. Supposedly, the TCM classification system utilizes somatic symptoms and tongue and pulse features to discern differences in etiology and pathogenesis of insomnia and also emphasizes variation

Table 2: Clinical features of the 10 most common TCM patterns for insomnia.

TCM patterns	Sleep-related symptoms	Non-sleep-related symptoms and signs	Tongue features	Pulse features
Excess patterns				
Liver-qi stagnation transforming into fire	**Insomnia**, difficulty falling asleep, excessive dreaming	**Vexation, irritability, bitter taste, constipation, reddish eyes, yellow urine**, headache, dizziness, hypochondriac pain, impatience, reddened complexion, thirst, poor appetite, oppression in the chest, tinnitus, hypochondriac distension, favour of drinking, reddish urine, pain in the chest and hypochondrium, frequent sighing	**Red tongue with yellow coating**	**Rapid and string-like pulse**, fine pulse
Internal disturbance of phlegm-heat	Insomnia, restless sleep	**Dizziness, vexation, bitter taste, profuse sputum, oppression in the chest, gastric stuffiness**, heavy headedness, acid regurgitation, poor appetite, belching, headache, nausea	**Red tongue with yellow and slimy coating**	**Slippery and rapid pulse**
Liver fire flaming upward	**Insomnia**, difficulty falling asleep	**Vexation, bitter taste, dry mouth, reddish eyes, tinnitus, irritability**, constipation, dizziness, dizziness with headache, dry throat, nocturnal emission, feverish sensations in the palms and soles, hypochondriac pain, impatience, reddened complexion, night sweating, palpitation and restless, aphthous stomatitis, backache, poor memory, yellow urine	**Thin coating**, **yellow coating**, red in the tip of tongue, red tongue, scanty coating, no coating	**Rapid**, string-like pulse, fine pulse
Stomach disharmony	Difficulty falling asleep, excessive dreaming, difficulty staying asleep, insomnia with vexation, restless sleep, unrefreshing sleep, insomnia, shallow sleep	**Vexation**, **belching**, **dizziness**, dry mouth, dry throat, feverish sensations in the palms, soles, and chest, night sweating, gastric stuffiness, stuffiness and pain in stomach and abdomen, sore knees, backache, hot flashes, constipation, flusteredness, poor appetite, oppression in the chest, stuffiness in stomach and abdomen, sloppy stool, tinnitus	**Slimy coating**, red tongue, scanty coating, thick coating, white coating, yellow coating	**Slippery pulse**, fine pulse, rapid pulse, string-like pulse, weak pulse
Stomach qi disharmony	*	**Abdominal distention**, **belching**	*	*

Table 2: Continued.

TCM patterns	Sleep-related symptoms	Non-sleep-related symptoms and signs	Tongue features	Pulse features
Deficiency patterns				
Deficiency of both the heart and spleen	**Excessive dreaming, difficulty staying asleep**, difficulty falling asleep, insomnia, half asleep	**Palpitation, lassitude, reduction in luster complexion, poor memory, dizziness, fatigue**, tasteless, weary limbs, poor appetite, sloppy stool	**Pale tongue with thin coating**, white thin coating	**Fine and weak pulse**
Hyperactivity of fire due to yin deficiency	**Insomnia**, difficulty staying asleep, insomnia with vexation, excessive dreaming, difficulty falling asleep	**Tinnitus, palpitation, poor memory, dizziness, feverish sensations in the palms, soles and chest, dry mouth, backache, vexation**, nocturnal emission, acid regurgitation, sore knees, sweating, dry throat, seminal emission, poor appetite, bitter taste, hot flashes, reddened cheeks	**Red tongue**, scanty coating, slimy coating, white coating, yellow coating	**Fine and rapid pulse**, slippery pulse
Qi deficiency of the heart and gallbladder	**Insomnia, excessive dreaming, frequent awakening with a start**, difficulty falling asleep, difficulty falling asleep alone, difficulty staying asleep	**Palpitation, fatigue, susceptibility to fright, dyspnea**, pale and large amount of urine, vexation in sitting and lying down, thoughtful	**Pale tongue**, thin coating	**Fine and string-like pulse**
Heart-kidney noninteraction	Insomnia, excessive dreaming, difficulty falling asleep, difficulty falling asleep with vexation, insomnia with vexation, difficulty staying asleep, restless sleep	**Backache, dizziness, tinnitus**, palpitation, vexation, feverish sensations in the palms, soles, and chest, seminal emission, night sweating, sore knees, dry mouth, susceptibility to fright, aphthous stomatitis, cold extremities, fright palpitation, irritability, reddened complexion, reddish eyes, poor memory, dry throat, hot flashes, impatience, nocturnal emission, spermatorrhea	**Red tongue**, scanty coating, thin coating, pale tongue, yellow coating, red in the tip of the tongue	**Fine and rapid pulse**, string-like pulse, sunken pulse, weak pulse
Heart deficiency with timidity	**Excessive dreaming**, **sleeping late at night**, frequent awakening with a start	**Palpitation**, **susceptibility** to fright, dyspnea, oppression in the chest, gastric stuffiness	**Pale tongue**, **thin coating**, white coating	**Fine and string-like pulse**, weak pulse

Symptoms mentioned in more than 50% of the studies that described the TCM pattern are bolded.

*No study provided information regarding sleep-related symptoms and tongue and pulse features of *stomach qi disharmony*.

Table 3: Insomnia symptom checklist for TCM diagnostic purpose.

Sleep-related symptoms ($n = 13$): Difficulty falling asleep, difficulty falling asleep alone, difficulty falling asleep with vexation, difficulty staying asleep, excessive dreaming, frequent awakening with a start, half asleep, insomnia, insomnia with vexation, restless sleep, shallow sleep, sleeping late at night, unrefreshing sleep.
Non-sleep-related symptoms ($n = 61$): Abdominal distention, acid regurgitation, aphthous stomatitis, backache, belching, bitter taste, cold extremities, constipation, dizziness, dizziness with headache, dry mouth, dry throat, dyspnea, fatigue, favour of drinking, feverish sensations in the palms, soles, and chest, flusteredness, frequent sighing, fright palpitation, gastric stuffiness, headache, **head distension**, heavy headedness, hot flashes, hypochondriac distension, hypochondriac pain, impatience, irritability, lassitude, **menstrual disturbance**, nausea, night sweating, nocturnal emission, **oliguria**, oppression in the chest, pain in the chest and hypochondrium, pale and large amount of urine, palpitation, poor appetite, poor memory, profuse sputum, reddened cheeks, reddened complexion, reddish eyes, reddish urine, reduction in luster complexion or lusterless complexion, seminal emission, sloppy stool, sore knees, spermatorrhea, stuffiness in stomach and abdomen, stuffiness and pain in stomach and abdomen, susceptibility to fright, sweating, tasteless, thirst, thoughtful, tinnitus, vexation, vexation in sitting and lying down, weary limbs, yellow urine.
Tongue features ($n = 11$): **Dry tongue**, no coating, pale tongue, red in the tip of tongue, red tongue, scanty coating, slimy coating, thick coating, thin coating, white coating, yellow coating.
Pulse features ($n = 7$): Fine pulse, rapid pulse, slippery pulse, string-like pulse, sunken pulse, **strong pulse**, weak pulse.

Symptoms only listed in standard TCM textbooks but not in the reviewed studies are bolded.

in body constitution, whereas the Western system focuses mainly on the etiology of insomnia and puts less emphasis on the pathogenesis and body constitution.

It is worthwhile to note that the TCM patterns commonly found in individuals with insomnia are not unique to insomnia. For this reason, it is rather common to find in TCM that different diseases are treated with the same formula or the same set of acupoints, when the underlying pattern is similar. For example, Gui Pi Tang is used for *deficiency of both the heart and spleen* in insomnia and in dizziness [15]. It is believed that this treatment approach is important for eradicating the underlying cause of diseases [16].

We understand that concrete evidence concerning the value of the TCM diagnostic system in the treatment of insomnia is still unavailable. The usefulness of the large number of non-sleep-related symptoms and the interrater reliability in TCM pattern differentiation are uncertain. A previous study has commented that poor diagnostic reliability can generally be traced to two different sources of uncontrolled variability [26]. The first is information variance, which occurs in the information-gathering process when different levels and types of data are collected about an individual by different interviewers. The other source of variability, criterion variance refers to the use of different sets of rules for classification purpose by different practitioners. Our study showed that the present TCM diagnostic system was subjected to the two sources of variability. There was a lack of standardization in terminology in the current TCM literature. A total of 51 Chinese terms relating to sleep-related symptoms were found, and many similar terms were used to describe non-sleep-related, tongue, and pulse features. The diagnostic criteria used for TCM diagnosis was different among TCM practitioners. It is possible that different researchers may choose the diagnostic criteria at their discretion based on their training and experiences. We consider that; unless a similar set of data is collected, standardized terminology is used, and same rule is applied it is difficult for practitioners to agree on TCM diagnosis.

The symptom checklist derived from our systematic review may reduce the information variance in the TCM diagnosis for insomnia and can be developed into a standardized tool to assess the presence and severity of the symptoms and signs in patients with insomnia. Consistency in symptom recognition between practitioners can be examined. The data can be analyzed using statistical methods such as hierarchical latent class modeling to examine the validity of TCM pattern differentiation [27]. We believe this is an important step in the scientific research of TCM treatment for insomnia.

There are strengths as well as methodologic limitations of the study. Our data were generated from a systematic review of TCM diagnosis in more than 100 articles involving almost 10000 subjects with insomnia, which provided less biased results than those derived from TCM experts. We employed broad inclusion criteria with no specification for the type of study and study quality. This approach could improve generalisability of our findings; however, the quality of data and reliability of the TCM diagnosis were uncertain. The major limitation was that the symptoms and signs of the TCM patterns were based on the description in the studies. It was uncertain whether the clinical features were established by face-to-face interview or based on the practitioners' educational background and clinical experience. In addition, there were insufficient data in the original papers to determine the pathognomonic features and the exclusion criteria that had been used for classification, especially regarding the relationships between TCM patterns and the non-sleep-related symptoms and tongue and pulse features. For example, fine pulse was expected in both *deficiency of both the heart and spleen* and *hyperactivity of fire due to yin deficiency*, but the presence of red tongue would suggest the later TCM pattern. But such exclusion criteria was not stated in the included papers.

5. Conclusion

Despite the limitations, the present study, for the first time, systematically and comprehensively summarized important data on the TCM diagnosis of patients with insomnia. We believed that while the TCM classification system had the potential to refine treatment by identifying subtle differences in etiology, pathogenesis, and body constitution, a lack of standardization in terminology and consensus on diagnostic criteria are major barriers. The insomnia symptom checklist derived from our study could be seen as a way of controlling information variance and should be used for future reliability and validity studies.

Conflict of Interests

No competing financial interests exist.

References

[1] M. M. Ohayon, "Epidemiology of insomnia: what we know and what we still need to learn," *Sleep Medicine Reviews*, vol. 6, no. 2, pp. 97–111, 2002.

[2] D. J. Taylor, K. L. Lichstein, and H. H. Durrence, "Insomnia as a health risk factor," *Behavioral Sleep Medicine*, vol. 1, no. 4, pp. 227–247, 2003.

[3] National Institutes of Health, "National institutes of health state of the science conference statement on manifestations and management of chronic insomnia in adults," *Sleep*, vol. 28, no. 9, pp. 1049–1057, 2005.

[4] C. M. Morin, R. R. Bootzin, D. J. Buysse, J. D. Edinger, C. A. Espie, and K. L. Lichstein, "Psychological and behavioral treatment of insomnia: update of the recent evidence (1998–2004)," *Sleep*, vol. 29, no. 11, pp. 1398–1414, 2006.

[5] N. J. Pearson, L. L. Johnson, and R. L. Nahin, "Insomnia, trouble sleeping, and complementary and alternative medicine: analysis of the 2002 National Health Interview Survey data," *Archives of Internal Medicine*, vol. 166, no. 16, pp. 1775–1782, 2006.

[6] C. C. L. Xue, A. L. Zhang, V. Lin, C. Da Costa, and D. F. Story, "Complementary and alternative medicine use in Australia: a national population-based survey," *Journal of Alternative and Complementary Medicine*, vol. 13, no. 6, pp. 643–650, 2007.

[7] F. P. Chen, M. S. Jong, Y. C. Chen et al., "Prescriptions of Chinese herbal medicines for insomnia in Taiwan during 2002,"

Evidence-Based Complementary and Alternative Medicine, vol. 2011, Article ID 236341, 9 pages, 2011.

[8] D. H. Tian, *Ling Shu Jing [Spiritual Axis]*, People's Health Publishing House, Beijing, China, 2005.

[9] B. Wang, *Huang Di Nei Jing Su Wen [The Yellow Emperor's Classic of Internal Medicine—Simple Questions]*, People's Health Publishing House, Beijing, China, 1979.

[10] W. Yang, F. Meng, and Y. Jiang, *Diagnostics of Traditional Chinese Medicine*, Academy Press, Beijing, China, 1998.

[11] G. G. Zhang, B. Bausell, L. Lao, W. L. Lee, B. Handwerger, and B. Berman, "The variability of TCM pattern diagnosis and herbal prescription on rheumatoid arthritis patients," *Alternative Therapies in Health and Medicine*, vol. 10, no. 1, pp. 58–63, 2004.

[12] C. J. Hogeboom, K. J. Sherman, and D. C. Cherkin, "Variation in diagnosis and treatment of chronic low back pain by traditional Chinese medicine acupuncturists," *Complementary Therapies in Medicine*, vol. 9, no. 3, pp. 154–166, 2001.

[13] B. Zell, J. Hirata, A. Marcus, B. Ettinger, A. Pressman, and K. M. Ettinger, "Diagnosis of symptomatic postmenopausal women by traditional Chinese medicine practitioners," *Menopause*, vol. 7, no. 2, pp. 129–134, 2000.

[14] J. J. Y. Sung, W. K. Leung, J. Y. L. Ching et al., "Agreements among traditional Chinese medicine practitioners in the diagnosis and treatment of irritable bowel syndrome," *Alimentary Pharmacology and Therapeutics*, vol. 20, no. 10, pp. 1205–1210, 2004.

[15] World Health Organization, *WHO International Standard Terminologies on Traditional Medicine in the Western Pacific Region*, World Health Organization, Western Pacific Region, 2007.

[16] B. Peng and J. Xie, *Traditional Chinese Internal Medicine*, People's Medical Publishing House, Beijing, China, 2nd edition, 2007.

[17] B. Y. Zhang, *Traditional Chinese Internal Medicine*, Shanghai Scientific and Technical Publishers, Shanghai, China, 1985.

[18] Y. Y. Wang, *Traditional Chinese Internal Medicine*, Shanghai Scientific and Technical Publishers, Shanghai, China, 1997.

[19] Z. Y. Zhou, *Traditional Chinese Internal Medicine*, China Press of Traditional Chinese Medicine, Beijing, China, 2003.

[20] State Administration of Traditional Chinese Medicine of the People's Republic of China, *Criteria of Diagnosis and Therapeutic Effect of Diseases and Syndromes in Traditional Chinese Medicine*, Nanjing University, Nanjing, China, 1994.

[21] Chinese Society of Psychiatry, *The Chinese Classification and Diagnostic Criteria of Mental Disorder (CCMD)*, Chinese Society of Psychiatry, Jinan, China, 2001.

[22] Ministry of Health of the People's Republic of China, *Clinical Research Guidelines of New Chinese Herbal Medicine*, Ministry of Health of China, Beijing, China, 1997.

[23] World Health Organization, *ICD-10 International Statistical Classification of Diseases and Related Health Problems*, World Health Organization, Geneva, Switzerland, 10th edition, 2007.

[24] J. L. Tsai, D. I. Simeonova, and J. T. Watanabe, "Somatic and social: Chinese Americans talk about emotion," *Personality and Social Psychology Bulletin*, vol. 30, no. 9, pp. 1226–1238, 2004.

[25] S. Mellander, P. O. Andersson, L. E. Afzelius, and P. Hellstrand, "Neural beta-adrenergic dilatation of the facial vein in man. Possible mechanism in emotional blushing," *Acta Physiologica Scandinavica*, vol. 114, no. 3, pp. 393–399, 1982.

[26] J. Endicott and R. L. Spitzer, "A diagnostic interview. The schedule for affective disorders and schizophrenia," *Archives of General Psychiatry*, vol. 35, no. 7, pp. 837–844, 1978.

[27] N. L. Zhang, S. Yuan, T. Chen, and Y. Wang, "Statistical validation of traditional Chinese medicine theories," *Journal of Alternative and Complementary Medicine*, vol. 14, no. 5, pp. 583–587, 2008.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 521780, 7 pages
doi:10.1155/2012/521780

Research Article

The Exploration of Disease Pattern, Zheng, for Differentiation of Allergic Rhinitis in Traditional Chinese Medicine Practice

Sienhung Yang,[1,2] Hsingyu Chen,[1] Yihsuan Lin,[1,3] and Yuchun Chen[4]

[1] *Division of Chinese Internal Medicine, Center for Traditional Chinese Medicine, Chang Gung Memorial Hospital, No. 123, Dinghu Road, Guei-shan, Taoyuan 33378, Taiwan*
[2] *School of Traditional Chinese Medicine, College of Medicine, Chang Gung University, No. 259, Wen-Hwa 1st Road, Guei-shan, Taoyuan 333, Taiwan*
[3] *Graduate Institute of Clinical Medical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan*
[4] *Department of Medical Research and Education, National Yang-Ming University Hospital, I-Lan, Taiwan*

Correspondence should be addressed to Sienhung Yang, dryang@ms1.hinet.net

Received 8 January 2012; Accepted 27 April 2012

Academic Editor: Shi-Bing Su

Pattern, or "zheng," differentiation is the essential guide to treatment with traditional Chinese medicine (TCM). However, the considerable variability between TCM patterns complicates evaluations of TCM treatment effectiveness. The aim of this study was to explore and characterize the relationship between patterns and the core patterns of allergic rhinitis. We summarized 23 clinical trials of allergic rhinitis with mention of pattern differentiation; association rule mining was used to analyze TCM patterns of allergic rhinitis. A total of 205 allergic rhinitis patients seen at Chang Gung Memorial Hospital from March to June 2005 were included for comparison. Among the 23 clinical trials evaluated, lung qi deficiency and spleen qi deficiencies were the core patterns of allergic rhinitis, accounting for 29.50% and 28.98% of all patterns, respectively. A higher prevalence of lung or spleen qi deficiency (93.7%) was found in Taiwan. Additionally, patients with lung or spleen qi deficiency were younger (27.99 ± 12.94 versus 58.54 ± 12.96 years) and the severity of nasal stuffiness was higher than among patients with kidney qi deficiency (1.35 ± 0.89 versus 0.62 ± 0.65; $P < 0.05$). Lung and spleen qi deficiencies are the core patterns of allergic rhinitis and determining the severity of nasal stuffiness is helpful in differentiating the TCM patterns.

1. Introduction

Traditional Chinese medicine (TCM) has been used for centuries in China and more recently has been widely studied and applied throughout the world [1, 2]. "Pattern differentiation and treatment" has an important role in TCM treatment. With this approach, a diagnosis is established through four examinations: visual inspection, smelling and listening, inquiry, and palpation, followed by TCM interventions such as use of herbal medicine, acupuncture, moxibustion, and massage [3, 4].

Pattern differentiation, or "zheng," is a unique TCM concept that summarizes the nature, location, and pattern of diseases corresponding to the World Health Organization's definition [4]. According to each individual pattern, the specific TCM treatment can be prescribed precisely to maximize its effectiveness [5–7]. However, successful use of pattern differentiation depends primarily on TCM doctors' subjective judgment, which is based upon classical TCM principles, education, and clinical experience. Thus, the practice of pattern differentiation can vary considerably among individual physicians [8]. In addition, there is little agreement between textbook guidelines for TCM pattern differentiation and its actual use in practice [9]. Finding ways to incorporate TCM knowledge into clinical practice and eliminating variability is an important issue in evidence-based investigations [9].

Due to the considerable variability in individual practices, it can be difficult to summarize TCM clinical data by conventional statistical techniques, and thus a number

of data mining methods, such as association rule mining (ARM) and cluster analysis, are used to acquire TCM knowledge from large-scale clinical data [3, 10, 11]. ARM is a modern data mining tool developed to explore the relationships between a wide range of factors, and it is widely applied to TCM prescription analysis [10, 12]. Moreover, ARM can effectively pinpoint the core TCM formula from a large prescription database by analyzing the relationship between TCM formulas [11]. In addition to TCM prescription, ARM is also used to analyze disease comorbidities and TCM patterns, and the advantages in reducing the complexity of TCM patterns have been well demonstrated [13, 14].

Allergic rhinitis, a common immunologic disorder, affects 10% to 20% of the world's population [15]. It involves type 2 CD4 T lymphocyte activation with cytokine secretion, producing an increased number of eosinophils and mast cells. Certain drugs used in Western medicine (WM), such as H_1-antihistamines, leukotriene receptor antagonists, intranasal corticosteroids, and even short-term oral corticosteroids, have been used to block disease progression and relieve symptoms [15]. In Taiwan, allergic rhinitis is one of the most common reasons for TCM visits, due to concern about side effects from long-term use of Western medications and the prospect of fewer side effects with TCM treatment [2, 16].

Several TCM treatments have been beneficial for allergic rhinitis, and the results of many studies have outlined the possible mechanisms for suppressing allergic reactions [17–21]. Nonetheless, the effectiveness of different TCM treatments is still unclear because no large-scale survey on TCM pattern differentiation of allergic rhinitis has yet been done.

The aim of this study was to explore the core TCM patterns of allergic rhinitis by using ARM and to compare these results with a hospital-based database to identify crucial factors to differentiate the patterns of allergic rhinitis. Depending upon the results of this study, future studies could focus on the most important TCM patterns, and different treatments could then be designated for specific TCM patterns.

2. Materials and Methods

2.1. Construction of the Clinical Trial Database. First, we conducted an extensive search of several databases, including PubMed, MEDLINE, Web of Science, Scopus, and the China Academic Journals Full-Text Database (CJFD). Keywords searched included "allergic rhinitis," "bi qiu," "chronic rhinitis," "pattern differentiation," "syndrome differentiation," "zheng," and "clinical trials." "Bi qiu" is the TCM disease corresponding to allergic rhinitis in WM. The full text of the search results was accumulated and critiqued by all authors of this study, and disagreements were resolved by consensus. After critical appraisal, the essential elements, including case number, gender, age, diagnostic criteria, and distribution of TCM patterns, were extracted from the eligible clinical trials manually. All these elements were entered into the computerized database.

2.2. Association Rule Mining (ARM). ARM, a data mining technique developed in the 1990s, has been widely used in medical research to explore the relationships among TCM prescriptions, disease comorbidities, and TCM patterns [13, 14]. The detailed algorithm has been thoroughly described and presented in previous studies, and IBM DB2 Intelligent Miner 9.1 software (IBM Corporation, Armonk, NY) was used to perform ARM of the clinical trials database [22]. Two decisive factors, support and confidence, were used to demonstrate relationships between patterns. Support was defined as the prevalence of a certain relationship among the whole database, and conditional probability of coexistence of pattern A and B given only pattern A was related to confidence. Depending on the threshold formed both by the support and confidence factors, the significant relationship between pattern A and B was established. It was an iterative process to decide the proper value of support and confidence factors and, in this study, support and confidence factors were set to 1% and 20%, respectively. These values were agreed upon by all authors in this study. Additionally, a diagram was drawn of associations between all patterns to clarify the relationships between TCM patterns and the core patterns of allergic rhinitis.

2.3. Hospital-Based Clinical Data Acquisition. To compare ARM results from the clinical trials database and practical clinical data, we used an established database of allergic rhinitis patients in the TCM outpatient service at CGMH. The definitive diagnosis of allergic rhinitis and TCM patterns was confirmed by Dr. Yang. Detailed data, including TCM patterns, age, gender, parents' health history, patients' personal health history, residence, serum IgE levels, results of MAST (Multiple Allergen Simultaneous Test panel) tests, and symptom severity, were recorded in this database. All data were collected with informed consent, and the records from March to June 2005 were extracted for further analysis. The process of data collection and analysis was approved by the Institutional Review Board (IRB) of CGMH.

2.4. Statistical Analysis of Characteristics of TCM Patterns. To examine the differences in characteristics among TCM patterns Student's t-test and one-way analysis of variance (ANOVA) were used for numerical data, and chi-square statistics were applied to categorical data. Only results of statistics with a P value less than 0.05 were deemed to be significant.

3. Results

3.1. Description of Clinical Trials of TCM Patterns. A total of 114 studies were found by the search strategy, and after detailed appraisal, 23 studies were eligible for inclusion in the study. All 23 studies were done in China and had been published in Chinese. Studies with English titles are listed as examples in the Appendix. From the 23 eligible studies, 2589 patients were identified, and a patient-pooled database was constructed. Fifteen patterns composed of one or more organs and the nature of disease were identified. Lung qi

Table 1: All TCM patterns of 23 clinical studies for allergic rhinitis.

Patterns	Number of patients	Percentage
Lung qi deficiency	620	23.95%
Spleen qi deficiency	589	22.75%
Lung yang deficiency pattern with wind-cold assailing the lung	382	14.75%
Phlegm-heat obstructing the lung	232	8.96%
Dampness-phlegm obstructing the lung	210	8.11%
Kidney yang deficiency	185	7.15%
Kidney qi deficiency	104	4.02%
Dual deficiency of the lung-spleen qi	57	2.20%
Dual deficiency of the spleen-kidney qi	54	2.09%
Qi stagnation and blood stasis	46	1.78%
Dual deficiency of the lung-kidney qi	42	1.62%
Lung-kidney yin deficiency	27	1.04%
Lung-spleen yang deficiency	19	0.73%
Dual deficiency of qi and yin of lung	17	0.66%
Blood stasis	5	0.19%
Total	2589	

Table 2: The 10 most common relationships of TCM patterns among 23 clinical studies for allergic rhinitis.

Relationship of pattern	Support	Confidence
Lung with qi deficiency	29.50%	47.35%
Spleen with qi deficiency	28.98%	97.63%
Lung with cold	15.40%	24.72%
Heat with lung	8.61%	100.00%
Phlegm-dampness with lung	7.79%	100.00%
Lung with qi deficiency	7.64%	48.70%
Kidney with cold	7.05%	44.92%
Lung and spleen with qi deficiency	4.71%	86.99%
Kidney and spleen with qi deficiency	2.00%	100.00%
Kidney and lung with qi deficiency	1.78%	64.00%

deficiency was the most common pattern (23.95%), followed by spleen qi deficiency (22.75%), and lung yang deficiency with wind-cold assailing the lung (14.75%). More than half of patients were classified into the qi deficiency pattern in these trials. In contrast, blood stasis, dual deficiencies of qi and yin of lung, and lung-spleen yang deficiency were the least-recognized patterns, and all had a prevalence of less than 1% (Table 1).

3.2. ARM of TCM Patterns. After applying ARM, we identified the 10 most common relationships between the locations and nature of disease patterns (Table 2). The lung, followed by the spleen, was the most common site of disease, whereas qi deficiency was the most common nature of disease. More than half (58.48%) of all pattern combinations were composed of lung or spleen qi deficiency. Nearly all locations or cases of allergic rhinitis were connected to the lung, spleen, and qi deficiency, and strong interactions were also found.

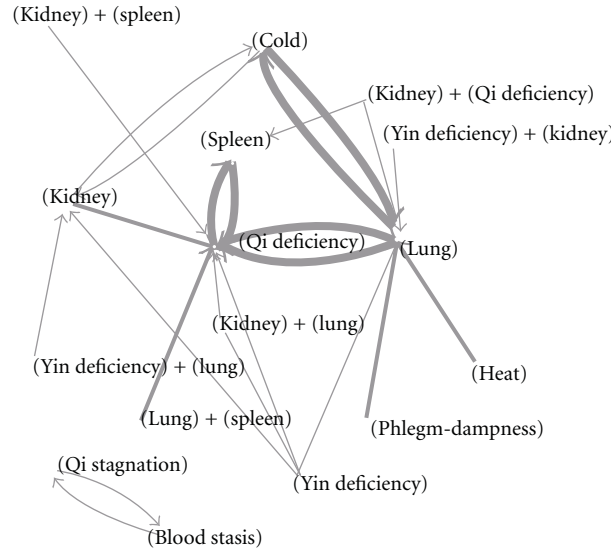


Figure 1: The associations between different TCM patterns of allergic rhinitis. Note: the width of connection lines represents the prevalence of the associations.

The central role of the lung and spleen can be seen in a diagram of relationships between patterns (Figure 1).

Additionally, high confidence, as high conditional probability, was found among three conditions: "heat with lung," "phlegm-dampness with lung," and "kidney and spleen with qi deficiency." It is assumed that, for patients with allergic rhinitis, once heat or phlegm-dampness was found, the nature of these two diseases would always be combined with lung, forming a pattern. More interestingly, qi stagnation and blood stasis were strongly associated, and neither had any relationship with major organs, such as lung, spleen, or even kidney. Despite the fact that this group's prevalence was only 0.19%, it may represent special mechanisms or manifestations of allergic rhinitis.

3.3. Pattern Analysis in Hospital-Based Surveillance. Using the well-established allergic rhinitis patient database at CGMH, TCM pattern analysis showed these patients could be divided into 3 groups: those with lung qi deficiency, dual deficiency of lung-spleen qi, and kidney qi deficiency (Table 3). Similar to the results of clinical study reviews done in China, 93.7% of patients had patterns composed of lung, spleen, and qi deficiency, and the percentage was higher than in the clinical trials. Among all the patients' characteristics, patients diagnosed with kidney deficiencies were significantly older than the other two groups—57.37 years versus 27.99 years—whereas no differences were found in serum IgE levels, results of MAST allergy tests, or other factors (Table 3).

3.4. Relations between TCM Patterns and Symptoms. TCM pattern differentiation was mainly based on clinical symptoms and therefore analysis of patients' symptom severity provided decisional information for pattern differentiation.

Table 3: Characteristics of TCM patterns of 205 allergic rhinitis patients at Chang Gung Memorial Hospital.

	Lung qi deficiency	Dual deficiency of the lung-spleen qi	Kidney qi deficiency	*P* value
Number of cases	137	55	13	
Age, mean ± SD†	29.07 ± 13.17	25.29 ± 12.03	57.53 ± 12.96*	<0.0001
Patients gender				0.690
Male	58	27	6	
Female	79	28	7	
Parents' history of allergic diseases				0.234
None	70	24	9	
One	58	24	2	
Both	9	7	2	
Personal history				
Asthma	16	6	2	0.903
Atopic dermatitis	21	12	1	0.370
Urbanization level				0.423
Urban	93	32	9	
Rural area	44	23	4	
IgE level (IU/mL) mean ± SD†	335.05 ± 456.07	420.90 ± 778.82	255.744 ± 433.59	0.494
MAST allergy test (positive/negative)	60/19	25/12	2/4	0.070
Symptom severity, mean ± SD†	4.33 ± 2.08	4.64 ± 2.05	3.15 ± 2.23	0.072

*P value < 0.0001 compared to another two groups.
†SD: standard deviation.

Table 4: Relations between severity of symptoms and TCM patterns.

	Lung qi deficiency or spleen qi deficiency‡ $N = 192$	Kidney qi deficiency $N = 13$	*P* value
Total score, mean ± SD†	4.42 ± 2.07	3.15 ± 2.23	0.068
Sneezing, mean ± SD†	1.39 ± 0.98	1.08 ± 1.19	0.278
Running nose, mean ± SD†	1.68 ± 1.02	1.46 ± 1.05	0.453
Stuffiness, mean ± SD†	1.35 ± 0.89	0.62 ± 0.65	0.004*

Key: *P value < 0.05; †SD: standard deviation; ‡combination of "lung qi deficiency" and "dual deficiency of the lung-spleen qi" groups.

Higher symptom severity scores, equivalent to more severe symptoms, were noted in the lung qi deficiency group and dual deficiency of the lung-spleen qi group, compared to the kidney qi deficiency group, although this was not statistically significant (Table 3). Nevertheless, the differences in symptom severity became more obvious when lung and spleen qi deficiency were combined due to symptom similarity, and compared with the kidney qi deficiency group (Table 4). Moreover, "stuffiness," one of the most bothersome effects of allergic rhinitis, was found to be more severe in the lung or spleen qi deficiency group than in the kidney qi deficiency group (Table 4).

4. Discussion

To the best of our knowledge, this is the first study to investigate the TCM patterns of clinical trials and to provide comparisons of clinical hospital-based data and severity of symptoms. The use of TCM has become much more widespread in recent years and many more interventions guided by TCM theory are being integrated into modern medicine [1, 2, 9]. TCM treatments, including herbal medicine, acupuncture, moxibustion, and massage, are administered according to TCM patterns, or "zheng" [23]. TCM patterns are composed of the cause, nature, and location of diseases, and differentiation of patterns is largely dependent upon clinical symptoms [3, 24]. Because of the complexity and plurality of clinical symptoms, and the nature and location of diseases, such as the Chinese medicine theory of five viscera and six bowels, the variability of pattern differentiation is extremely high. Thus, agreement on patterns of the same disease is usually low [8, 9].

From the viewpoint of evidence-based medicine, in future studies, it will be particularly important to summarize TCM patterns and to explore core patterns of disease. ARM is an appropriate statistical method for summarizing disease patterns and exploring core patterns and the nature and locations of diseases because it examines not only the prevalence of a pattern but also the strength of relations between and within patterns [14]. In this study, combinations of lung, spleen, and qi deficiencies were found to be the most crucial part of TCM patterns of allergic rhinitis. The results are consistent among clinical trials and hospital-based clinical data, and disclose valuable, evidence-based information for further investigation.

Qi deficiency has been proved to be crucial to allergic rhinitis in previous studies, and two famous qi-tonifying Chinese herbal products, Bu-zhong-yi-qi-tang and Xiang-sha-liu-jun-zi-tang, have had marked therapeutic effects on allergic rhinitis, even without pattern differentiation [18–20]. The mechanisms of immunomodulation of qi-tonifying

agents include decreasing serum IgE, interleukin-4 (IL-4), interleukin-5 (IL-5), and gamma interferon (IFN-γ), increasing interleukin-10 (IL-10), and suppressing cyclooxygenase 2 mRNA expressions [18–20]. As a result, the imbalance of type 1 and type 2 helper T lymphocyte cells is reversed and allergic rhinitis symptoms are alleviated [18, 20]. IL-4 and IL5 with helper T-lymphocytes switch from type 1 to type 2, and subsequently high IgE secretion has been proved to be the cardinal pathogenesis of an allergic reaction [25–27]. The effective reversal of activation of an allergic reaction by qi-tonifying agents shows the possible relationship between qi deficiency and serum cytokine level, and, perhaps, the pathogenesis of qi deficiency of allergic rhinitis.

Lung and spleen are the two important locations of diseases and are highly related to qi deficiency, forming TCM patterns. The function of lung, from the viewpoint of TCM, includes control of respiration, qi domination, and fluid regulation, and these functions are highly related to the nose and skin [4]. The most common symptoms of allergic rhinitis, such as sneezing, runny nose, and stuffiness, and possible subsequent critical illness in the form of asthma have been shown to be associated with the nose and entire respiratory tract and share the similar pathogenesis [15]. Moreover, immunomodulation of allergic diseases by lung-tonifying agents such as *Astragalus membranaceus* and *Cordyceps militaris* has been widely reported [28, 29]. Owing to the remarkably similar disease behavior and pathogenesis, the lung, rather than other organs, represents the most important organ in pattern differentiation of allergic rhinitis.

The spleen, from the viewpoint of TCM, dominates transformation of food to energy, similar to WM's view of the gastrointestinal tract's function [4]. The gastrointestinal system has been thought to be associated with allergic diseases and the underlying mechanism may be related to activation of eosinophils and type 2 helper T lymphocytes, with increasing IgE levels [30, 31]. Thus, by modifying intestinal bacterial flora and subsequent systemic immunomodulation, symptoms of allergic rhinitis may be relieved [32]. Additionally, a spleen-tonifying TCM formula has been found to be effective for alleviating allergic rhinitis symptoms [33]. These facts reveal the close relationship between spleen deficiency and allergic reactions, and through modulating gastrointestinal function by TCM herbal products, allergic disorders may be alleviated.

Yin and yang deficiencies are less commonly identified than qi deficiency in clinical trials, and they were also absent in the surveillance at our hospital. Yin deficiency was a specialized TCM pattern characterized by decreased body fluids, and it was diagnosed when patients complained about dryness of the mouth, throat, and nasal passages, or constipation. Additionally, a reddish tongue with scanty coating and a fine, rapid pulse were commonly seen among such patients. Moreover, symptoms of yang deficiency among allergic rhinitis patients included manifestations of qi deficiency with prominent fear of cold, cold extremities, clear nasal discharge, pale face, and an enlarged tongue with a white, slick coating. Both lung yin and yang deficiencies were noted in the late stage of the clinical course of allergic rhinitis, and they usually developed when qi deficiency, the early stage of allergic rhinitis, was not properly treated. Therefore, it is reasonable that combinations of qi and yin deficiency or yang deficiency were less frequently found among allergic rhinitis patients.

Additionally, combination of qi stagnation and blood stasis was a special pattern in this study. Although the prevalence was low, about 1.78%, a strong association with allergic rhinitis was found (Tables 1 and 2). Also, this group of patients seemed to be isolated from other patients (Figure 1). In other words, once qi stagnation was diagnosed, blood stasis was always also diagnosed, and vice versa. Qi stagnation and blood stasis among allergic rhinitis patients had a chronic course, and patients had a purplish or purple-spotted tongue and a stringy, choppy pulse. Due to the unusual characteristics, a different pathogenesis was suspected among these patients and therefore further studies were warranted.

The severity of nasal stuffiness, one of the common symptoms of allergic rhinitis, is definitely different between lung or spleen qi deficiency and kidney qi deficiency groups. In this study, the patients in the kidney qi deficiency group were older than those in the lung or spleen qi deficiency group. This finding is similar to that of previous studies. Currently, nasal stuffiness is thought to be caused by eosinophil and mast cell infiltration with subsequent airway remodeling. It is believed to be related to certain neuropeptides, and its severity decreases with aging [34]. From TCM's viewpoint, metabolism and transport of body fluids largely depend on lung and spleen [4] and therefore nasal stuffiness, caused by nasal cavity mucosa edema and swelling due to allergic reaction, is easily found in patients with lung and spleen qi deficiency with disturbed body fluid transport. Additionally, the prominent immunologic disorder found among lung and spleen qi deficiency patients may also be the cause of severe nasal stuffiness. Based on this significantly different symptom among the two groups, nasal stuffiness can be used as an inclusion or exclusion criteria for patient selection, and different treatment plans are able to be individually provided for the specific groups.

Though the clinical data are closely comparable to the summarized results of clinical trials for allergic rhinitis, there are still some limitations to this study. First, the quality of clinical trials is heterogeneous. Some population characteristics, such as gender, age, or detailed manifestations of allergic rhinitis, are not provided in every trial, and therefore selection bias may exist. To effectively eliminate this bias, only the most representative trials of allergic rhinitis were included in this study after strict evaluation. Although the number of cases was considerably reduced, the result of ARM is highly reliable, since trials enrolled in this study firmly focus on TCM patterns of allergic rhinitis. Second, the definition of TCM patterns is not exactly the same among these studies, and the basis of pattern differentiation includes Chinese expert consensus on allergic rhinitis in 1997 and 2004, and a textbook of TCM otolaryngology. This disadvantage was largely overcome by examining the descriptions of patterns in every trial and validating them by TCM doctors. Furthermore, results of statistical analysis on

Table 5: Clinical trials of allergic rhinitis included in this study*.

Study	Number of patients	Age (yr; range)	Patterns description (cases)
Yang et al. [35]	216	36.2; 7–63	Lung qi deficiency: 100 Spleen qi deficiency: 71 Kidney qi deficiency: 45
Liu et al. [36]	242	42.6†	Lung yang deficiency pattern with wind-cold assailing the lung: 167 Phlegm-heat obstructing the lung: 23 Spleen qi deficiency: 32 Kidney yang deficiency: 20
Tang et al. [37]	70	28.95†	Lung yang deficiency pattern with wind-cold assailing the lung: 24 Phlegm-heat obstructing the lung: 16 Spleen qi deficiency: 20 Kidney yang deficiency: 14
Qiu et al. [38]	256	32.52; 7–70	Lung qi deficiency: 124 Phlegm-heat obstructing the lung: 32 Spleen qi deficiency: 72 Kidney yang deficiency: 28
Lu et al. [39]	106	31, 4–82	Lung qi deficiency: 60 Dual deficiency of the lung-spleen qi: 21 Dual deficiency of the lung-kidney qi: 25

*Studies without titles or an abstract in English are not listed in this table.
†Range of age was not provided by the authors.

large-scale pooled clinical trials are similar to the consensus among TCM experts and thus can overcome the variability of patterns differentiation.

5. Conclusion

Core TCM patterns were explored in this study by applying ARM to clinical trials of allergic rhinitis, and the summarized result is comparable to hospital-based data. A younger patient population and greater severity of nasal stuffiness were associated with the most important patterns, lung or spleen with qi deficiency. Future investigations of TCM treatment for allergic rhinitis can be designed on the basis of these results, and may help define a specific TCM pattern.

Appendix

For more details see Tables 5 and 6.

Table 6: Symptom severity assessment of allergic rhinitis.

Symptoms	Presentation	Score
Sneezing	None	0
	1–5 times/day	1
	6–10 times/day	2
	>10 times/day	3
Runny nose	None	0
	1–5 times/day	1
	6–10 times/day	2
	>10 times/day	3
Stuffiness	None	0
	Mild, without mouth-breathing	1
	Moderate, with occasional mouth-breathing	2
	Severe, with frequent mouth-breathing	3

Acknowledgment

The authors especially thank Dr. Peifang Cai for collecting clinical data for this study.

References

[1] D. Normile, "The new face of traditional Chinese medicine," *Science*, vol. 299, no. 5604, pp. 188–190, 2003.
[2] F. P. Chen, T. J. Chen, Y. Y. Kung et al., "Use frequency of traditional Chinese medicine in Taiwan," *BMC Health Services Research*, vol. 7, article 26, 2007.
[3] H. Zhao, J. Chen, N. Hou et al., "Discovery of diagnosis pattern of coronary heart disease with Qi deficiency syndrome by the *T*-test-based Adaboost algorithm," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 408650, 7 pages, 2011.
[4] World Health Organization, *WHO International Standard Terminologies On Traditional Medicine in the Western Pacific Region*, World Health Organization, Geneva, Switzerland, 2007.
[5] A. P. Lu and K. J. Chen, "Integrative medicine in clinical practice: from pattern differentiation in traditional Chinese medicine to disease treatment," *Chinese Journal of Integrative Medicine*, vol. 15, no. 2, article 152, 2009.
[6] C. Lu, Q. Zha, A. Chang, Y. He, and A. Lu, "Pattern differentiation in traditional chinese medicine can help define specific indications for biomedical therapy in the treatment of rheumatoid arthritis," *Journal of Alternative and Complementary Medicine*, vol. 15, no. 9, pp. 1021–1025, 2009.
[7] S. P. Yun, W. S. Jung, S. U. Park et al., "Hwangryunhaedogtang (huanglianjiedutang) treatment for pathological laughter after stroke and importance of patterns identification: a preliminary study," *The American Journal of Chinese Medicine*, vol. 35, no. 5, pp. 725–733, 2007.
[8] G. G. Zhang, W. L. Lee, L. Lao, B. Bausell, B. Handwerger, and B. Berman, "The variability of TCM pattern diagnosis and herbal prescription on rheumatoid arthritis patients," *Alternative Therapies in Health and Medicine*, vol. 10, no. 1, pp. 58–63, 2004.
[9] V. Scheid, T. Ward, and V. Tuffrey, "Comparing TCM textbook descriptions of menopausal syndrome with the lived experience of London women at midlife and the implications for

Chinese medicine research," *Maturitas*, vol. 66, no. 4, pp. 408–416, 2010.

[10] Y. Feng, Z. Wu, X. Zhou, Z. Zhou, and W. Fan, "Knowledge discovery in traditional Chinese medicine: state of the art and perspectives," *Artificial Intelligence in Medicine*, vol. 38, no. 3, pp. 219–236, 2006.

[11] H.-Y. Chen, Y. H. Lin, J. C. Wu et al., "Prescription patterns of Chinese herbal products for menopausal syndrome: analysis of a nationwide prescription database," *Journal of Ethnopharmacology*, vol. 137, no. 3, pp. 1261–1266, 2011.

[12] F. P. Chen, M. S. Jong, Y. C. Chen et al., "Prescriptions of Chinese herbal medicines for insomnia in Taiwan during 2002," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 236341, 9 pages, 2011.

[13] Y. M. Tai and H. W. Chiu, "Comorbidity study of ADHD: applying association rule mining (ARM) to National Health Insurance Database of Taiwan," *International Journal of Medical Informatics*, vol. 78, no. 12, pp. e75–e83, 2009.

[14] L. Zhang, Q. M. Zhang, Y. G. Wang, D. L. Yu, and W. Zhang, "The TCM pattern of the six-zang and six-fu organs can be simplified into the pattern of five-zang and one-fu organs," *Journal of Traditional Chinese Medicine*, vol. 31, no. 2, pp. 147–151, 2011.

[15] J. L. Broek, J. Bousquet, C. E. Baena-Cagnani et al., "Allergic rhinitis and its impact on asthma (ARIA) guidelines: 2010 revision," *Journal of Allergy and Clinical Immunology*, vol. 126, no. 3, pp. 466–476, 2010.

[16] Y. Y. Kung, Y. C. Chen, S. J. Hwang, T. J. Chen, and F. P. Chen, "The prescriptions frequencies and patterns of Chinese herbal medicine for allergic rhinitis in Taiwan," *Allergy*, vol. 61, no. 11, pp. 1316–1318, 2006.

[17] D. K. Ng, P. Y. Chow, S. P. Ming et al., "A double-blind, randomized, placebo-controlled trial of acupuncture for the treatment of childhood persistent allergic rhinitis," *Pediatrics*, vol. 114, no. 5, pp. 1242–1247, 2004.

[18] S. H. Yang, C. Y. Hong, and C. L. Yu, "Decreased serum IgE level, decreased IFN-γ and IL-5 but increased IL-10 production, and suppressed cyclooxygenase 2 mRNA expression in patients with perennial allergic rhinitis after treatment with a new mixed formula of Chinese herbs," *International Immunopharmacology*, vol. 1, no. 6, pp. 1173–1182, 2001.

[19] S. H. Yang, C. Y. Hong, and C. L. Yu, "The stimulatory effects of nasal discharge from patients with perennial allergic rhinitis on normal human neutrophils are normalized after treatment with a new mixed formula of Chinese herbs," *International Immunopharmacology*, vol. 2, no. 12, pp. 1627–1639, 2002.

[20] S. H. Yang and C. L. Yu, "Antiinflammatory effects of Buzhong-yi-qi-tang in patients with perennial allergic rhinitis," *Journal of Ethnopharmacology*, vol. 115, no. 1, pp. 104–109, 2008.

[21] S. H. Yang, C. L. Yu, Y. L. Chen, S. L. Chiao, and M. L. Chen, "Traditional Chinese medicine, Xin-yi-san, reduces nasal symptoms of patients with perennial allergic rhinitis by its diverse immunomodulatory effects," *International Immunopharmacology*, vol. 10, no. 8, pp. 951–958, 2010.

[22] T. J. Chen, L. F. Chou, and S. J. Hwang, "Application of a data-mining technique to analyze coprescription patterns for antacids in Taiwan," *Clinical Therapeutics*, vol. 25, no. 9, pp. 2453–2463, 2003.

[23] X. Zhang and X. Sun, "Discussion on the five basic syndrome patterns and their corresponding treating principles and prescriptions," *Journal of Traditional Chinese Medicine*, vol. 27, no. 4, pp. 310–314, 2007.

[24] R. Q. Chen, C. M. Wong, K. J. Cao, and T. H. Lam, "An evidence-based validation of traditional Chinese medicine syndromes," *Complementary Therapies in Medicine*, vol. 18, no. 5, pp. 199–205, 2010.

[25] D. H. Broide, M. M. Paine, and G. S. Firestein, "Eosinophils express interleukin 5 and granulocyte macrophage-colony-stimulating factor mRNA at sites of allergic inflammation in asthmatics," *The Journal of Clinical Investigation*, vol. 90, no. 4, pp. 1414–1424, 1992.

[26] P. Chomarat and J. Banchereau, "Interleukin-4 and interleukin-13: their similarities and discrepancies," *International Reviews of Immunology*, vol. 17, no. 1–4, pp. 1–52, 1998.

[27] L. J. Rosenwasser, "Mechanisms of IgE inflammation," *Current Allergy and Asthma Reports*, vol. 11, no. 2, pp. 178–183, 2011.

[28] A. Nalbantsoy, T. Nesil, O. Yilmaz-Dilsiz, G. Aksu, S. Khan, and E. Bedir, "Evaluation of the immunomodulatory properties in mice and in vitro anti-inflammatory activity of cycloartane type saponins from Astragalus species," *Journal of Ethnopharmacology*, vol. 139, no. 2, pp. 574–581, 2012.

[29] G. X. Gao, Q. M. Li, and H. H. Shen, "Effect of Astragali-Cordyceps Mixtura on TGF-β/Smad signal pathway in the lung of asthma airway remodeling," *Journal of Ethnopharmacology*, vol. 125, no. 1, pp. 68–74, 2009.

[30] C. Cingi, D. Demirbas, and M. Songu, "Allergic rhinitis caused by food allergies," *European Archives of Oto-Rhino-Laryngology*, vol. 267, no. 9, pp. 1327–1335, 2010.

[31] S. Jyonouchi, T. A. Brown-Whitehorn, and J. M. Spergel, "Association of eosinophilic gastrointestinal disorders with other atopic disorders," *Immunology and Allergy Clinics of North America*, vol. 29, no. 1, pp. 85–97, 2009.

[32] K. Ivory, S. J. Chambers, C. Pin, E. Prieto, J. L. Arqués, and C. Nicoletti, "Oral delivery of *Lactobacillus casei* Shirota modifies allergen-induced immune responses in allergic rhinitis," *Clinical and Experimental Allergy*, vol. 38, no. 8, pp. 1282–1289, 2008.

[33] C. E. Clark, E. Arnold, T. J. Lasserson, and T. Wu, "Herbal interventions for chronic asthma in adults and children: a systematic review and meta-analysis," *Primary Care Respiratory Journal*, vol. 19, no. 4, pp. 307–314, 2010.

[34] H. L. Thulesius, H. O. Thulesius, and M. Jessen, "What happens to patients with nasal stuffiness and pathological rhinomanometry left without surgery?" *Rhinology*, vol. 47, no. 1, pp. 24–27, 2009.

[35] L. Yang, W. Y. Fu, X. N. Li et al., "Allergic rhinitis differentiation and analysis of relationship between immune globulin and allergens," *China Modern Medicine*, vol. 18, pp. 11–12, 2011 (Chinese).

[36] F. T. Liu, X. F. Jiang, and J. H. Dai, "Survey on the traditional Chinese medicine syndromes analysis of allergic rhinitis in Yili," *Chinese General Practice*, vol. 14, pp. 1337–1339, 2011 (Chinese).

[37] Y. Y. Tang, Y. S. Zeng, Y. Chen et al., "The patterns differentiation of allergic rhinitis and their association with the plasma levels of IL-4 and INF-γ," *Chinese Otorhinolaryngol Journal of Integrative Medicine*, vol. 15, pp. 9–11, 2007 (Chinese).

[38] B. S. Qiu, S. Z. Wang, and Y. P. Zhong, "Study on correlativity of syndrome types of allergic rhinitis with their clinical characteristics," *Journal of Traditional Chinese Medicine*, vol. 45, pp. 373–375, 2004 (Chinese).

[39] D. W. Lu, M. H. Wang, and P. B. Zhao, "The relative studies between traditional Chinese medicine types of allergic rhinitis and corresponding indicators of allergology," *Chinese Otorhinolaryngological Journal of Integrative Medicine*, vol. 4, pp. 112–114, 1996 (Chinese).

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 509705, 9 pages
doi:10.1155/2012/509705

Research Article

Xiaopiyishen Herbal Extract Granule Improves the Quality of Life among People with Fatigue-Predominant Subhealth and Liver-Qi Stagnation and Spleen-Qi Deficiency Syndrome

Xiao-lin Xue,[1] Xiu-yan Wu,[1] Jian-min Xing,[2] Li Li,[3] Yan Zhao,[1] Jia-jia Wang,[1] Ya-jing Zhang,[1] Qing-bo Wang,[4] Yu Tang,[5] Guan-ru Li,[1] Ping Han,[6] Zhen Li,[7] Wen-ping Wang,[8] and Tian-fang Wang[1]

[1] *Department of Diagnostics of Traditional Chinese Medicine, Preclinical School, Beijing University of Chinese Medicine, Beijing 100029, China*
[2] *Center of Evidence-Based Medicine, Preclinical School, Beijing University of Chinese Medicine, Beijing 100029, China*
[3] *Preventive Treatment and Health Management Center, The First Affiliated Hospital of Henan University of TCM, Zhengzhou 450000, China*
[4] *Department of Acupuncture and Moxibustion, The First Affiliated Hospital of Henan University of TCM, Zhengzhou 450000, China*
[5] *Department of Rehabilitation Medicine, The Affiliated Hospital of Liaoning University of TCM, Liaoning Province, Shenyang 110032, China*
[6] *Health Examination Centre, Beijing Xiao Tang Shan Hospital, Beijing 102211, China*
[7] *Department of Endocrinology, The First Affiliated Hospital of Henan University of TCM, Zhengzhou 450000, China*
[8] *Clinical Trial Institution, The Affiliated Hospital of Liaoning University of TCM, Liaoning Province, Shenyang 110032, China*

Correspondence should be addressed to Tian-fang Wang, tianfangwang2000@yahoo.com.cn

Received 14 January 2012; Revised 1 May 2012; Accepted 8 May 2012

Academic Editor: Aiping Lu

To observe the effects of Xiaopiyishen Herbal Extract Granule (XPYS-HEG) on the quality of life in people with fatigue-predominant subhealth (FPSH) and liver-qi stagnation and spleen-qi deficiency syndrome, the participants were allocated randomly to the treatment group (XPYS, $n = 100$) and the control group (placebo, $n = 100$) in this study. The study period was 18 weeks (6 weeks for the intervention and 12 weeks for followup). The results show that there were no differences between the two groups for the scores of eight factors on the SF-36 (Chinese version of the SF-36 universal quality-of-life scale) at baseline. Compared with the baseline score, intervention with XPYS-HEG led to a significant increase in scores for the factor of bodily pain at the end of the 6th week. Compared with the score at the end of the 6th week, the score for the factor of mental health in the XPYS group significantly increased at the end of the 18th week. Therefore, XPYS-HEG could partially improve the quality of life for people with FPSH and liver-qi stagnation and spleen-qi deficiency syndrome, which can ease bodily pain, stimulate a positive mood, and ease a negative mood.

1. Introduction

Fatigue is a common health-related complaint, a frequent complication of diseases, and the chief symptom of chronic fatigue syndrome (CFS) or fatigue-predominant subhealth (FPSH). Chronic fatigue syndrome is an illness characterized by disabling fatigue lasting at least 6 months, accompanied by several other symptoms [1, 2].

Subhealth (also referred to as suboptimal health) is a new concept that has been described by Chinese scholars [3]. Subhealth refers to a status between healthy and diseased states and is characterized by reduced vitality, function, and adaptive capacity lasting for at least 3 months. Subhealth is not considered a health condition and does not meet the clinical and subclinical diagnostic criteria of some diseases at this stage [4].

Various uncertain and abnormal physical and mental complaints presented by individuals with subhealth status have no conclusive laboratory markers. The main symptoms of subhealth include fatigue, sleeping disorders, amnesia, bodily pain, anxiety, and depression.

Subhealth has become a public health challenge in China because a surprisingly large number of Chinese people suffer with this condition. The prevalence of subhealth was found to be high among college and university staff according to a regional survey in China.The survey suggested that individuals with subhealth status were more likely to be women and middle aged. The most common risk factors were occupational stress, psychological factors, bad habits and behaviors, lack of relaxation and physical exercise, working extended hours, and air and noise pollution [5, 6].

People with subhealth report a poor quality of life. Therefore, it has become increasingly important to identify effective intervention methods. TCM is characterized by the concept of holism, which promotes the use of numerous intervention methods and plays an important role in the treatment of subhealth status.

The condition of subhealth status maybe divided into various types according to the predominant symptoms. Fatigue-predominant subhealth is characterized by obvious fatigue and represents the most frequently reported type of subhealth status [7]. In our prior study [8, 9], we reported that liver-qi stagnation and spleen-qi deficiency syndrome is the most common TCM syndrome observed in people with FPSH. This syndrome is marked by hypochondriac pain, depression or irritation, abdominal distention, loose bowel, and lassitude. The life quality of people with FPSH is impaired; therefore, the improvement of the quality of life is an important goal of the TCM interventions investigated in our study [10].

In a previous investigation, the total score of the Fatigue Scale 14 was used to evaluate the efficacy and safety of Xiaopi Yishen herbal extract granule (XPYS-HEG) in the treatment of people with FPSH due to liver-qi stagnation and spleen-qi deficiency. The fatigue status and the grade of liver-qi stagnation and spleen-qi deficiency syndrome were also recorded. It was reported that XPYS-HEG relieved fatigue and other symptoms associated with liver-qi stagnation and spleen-qi deficiency syndrome [11].

The purpose of the paper was to analyze the effect of XPYS-HEG on the quality of life in people with FPSH and liver-qi stagnation and spleen-qi deficiency syndrome.

2. Methods

2.1. Study Protocol. A placebocontrolled multicenter clinical trial with a randomized, double-blinded, and parallel design was completed by 3 participating centers across mainland China (Beijing, Henan, Liaoning). Randomization schedules were generated by a statistician using the statements of PROC PLAN of SAS (version 9.1.3) and assigning equal numbers of patients to each of the groups. Block sizes of 2 and 4 were used to balance the assignments across groups and to prevent decoding of the system. Assignments were stratified within the centers. The allocations were placed in numbered opaque envelopes to be opened by the doctors in the presence of the participants. The process of disclosed blinding included two steps. The first disclosure was performed after completion of the reviewing of the data, that is, only disclosing the code A or B of each case to the statistician. The second was performed after completing the statistical analysis and report, that is, disclosing the corresponding group of A and B. The removal of the blinding during the process of this trial was executed by the researchers when serious adverse event occurred or the participant needed emergency medical treatment. This trial was intended to be terminated if all of the allocations were disclosed or if the proportion of patients for whom the blinding was removed exceeded 20%.

2.2. Participants. The sample size of 200 was estimated using the formulation of $n = (U_\alpha + U_\beta)^2 \times 2P \times (1 - P)/(P_1 - P_0)^2$ [12] with type I error 0.05 and type II error 0.1. The participants were recruited among patients receiving a physical check up and outpatients during the period of March 2008 and February 2009 at Beijing Xiao Tang Shan Hospital (80 cases), the First Affiliated Hospital of the Henan College of Chinese Medicine (60 cases) and the Affiliated Hospital of the Liaoning University of Chinese Medicine (60 cases).

2.3. Diagnostic Criteria

2.3.1. Diagnostic Criteria for FPSH. According to the "Clinical Guidelines of Chinese Medicine on Subhealth," the research group developed the following diagnostic criteria for FPSH [13]: (1) chief complaint: persistent or recurrent fatigue lasting more than 3 months; (2) exclusion: a disease that may lead to fatigue, with no obvious abnormalities detected through a routine physical examination; (3) total scores reaching 3 points or more on the Fatigue Scale 14 (FS-14) [14, 15]. The routine physical examinations include routine analyses of the blood, urine and stool, blood pressure, liver and kidney function, blood lipid profile, fasting blood sugar, abdominal B ultrasound, an ECG, and a chest X-ray.

2.3.2. TCM Differentiation Standard. In accordance with the "criteria for the diagnosis and evaluation of the therapeutic effects of treatments of diseases and syndromes in traditional Chinese medicine," "Differential diagnosis of the syndromes of Chinese medicine" released in 1995, which were issued by the State Administration of Traditional Chinese Medicine of the People's Republic of China, and "The guiding principles for the clinical study of new drugs for use in traditional Chinese medicine" released in 2002, combined with the characteristics of subhealth, the standards of liver-qi stagnation and spleen-qi deficiency syndrome in FPSH are formulated as follows: (1) chest or hypochondriac fullness, distending pain, or wandering pain; (2) low mood, irritability, or emotional instability; (3) reduced appetite, abdominal distension/relieved by pressure, loose stools, or diarrhea; (4) alternate loose and dry stools; (5) enlarged and tooth-marked tongue. Participants can be diagnosed with liver-qi

stagnation and spleen-qi deficiency syndrome if they meet (1) or (2) and (3) or (4) or (5), excluding the apparent thermal effects, such as a red tongue with a yellow coating (refer to pulse, such as wiry pulse, moderate pulse, weak pulse, or feeble pulse).

2.4. Inclusion Criteria. The inclusion criteria for this study were as follows: (1) meet the diagnostic criteria described above for FPSH and liver-qi stagnation and spleen-qi deficiency syndrome according to the FPSH of TCM syndrome differentiation standards; (2) between 18 and 60 years of age; (3) education: junior high school and above; (4) no fatigue interventions (including antifatigue healthcare supplements) taken within the past month; (5) participants signed informed consent.

2.5. Exclusion Criteria. The exclusion criteria were as follows: (1) upper respiratory tract infection, trauma, acute medical history, and so forth. Within the past week (2) pregnant or lactating women and women planning a pregnancy within the next six months; (3) a history of mental illness or family history of psychiatric disorders.

2.6. Dropout Criteria. Participants were considered as dropouts when he/she did not complete the entire observation period. Participants who stopped taking the medication with less than one treatment cycle were not counted as dropouts.

2.7. Ethics and Consent. The study protocol conforms to the Helsinki Declaration [16] and the research regulations for Chinese clinical trials. The Ethics Committee of the Affiliated Dongzhimen Hospital of the Beijing University of Chinese Medicine reviewed and approved the study protocol. All participants were required to provide written informed consent before participation in the study.

2.8. Treatment. A total of 200 participants were randomly divided into the XPYS-HEG intervention group (XPYS, $n = 100$) and the placebo group ($n = 100$). The participants were given XPYS-HEG (10 g Radix Astragali, 2 g Radix Ginseng, 6 g Pericarpium Citri Reticulatae, 6 g Rhizoma Cyperi, 6 g Radix Angelicae, and 6 g Fructus Lycii) or placebo with same packaging as that used for XPYS-HEG. The participants took one bag half an hour after breakfast and one bag half an hour after dinner for 6 continuous weeks. The components of the placebo are dextrin and caramel fabricated based on the color of XPYS-HEG.

2.9. The Quality Control for XPYS-HEG. The XPYS-HEG is provided by the Beijing Kangren Tang Pharmaceutical Co., Ltd. The production procedure is as follows: selection of the genuine regional drug, implementation of modern pharmaceutical technology, the use of Chinese herbal fragments as raw materials according to traditional processing methods, preparing the granular formulation after single-herb extraction, concentration, and drying with decoction as a standard. The quality standards are higher than the Codex standard for enterprise internal control standards. The 95% ethanol extract of the roast milkvetch root granule is not less than 24%. Ginseng granules containing ginsenoside Rg1 ($C_{42}H_{72}O_{14}$) and ginsenoside Re ($C_{48}H_{82}O_{18}$) shall not be less than 0.80%, and the total ginsenoside Rb1 ($C_{54}H_{92}O_{23}$) shall not be less than 0.18%. Tangerine peel granule contains hesperidin not below 1%. The 95% ethanol extract of nutgrass galingale rhizome processed with vinegar is not less than 10%. The ferulic acid content of the angelica formula particle is not less than 0.030%. Wolfberry polysaccharides of the barbary wolfberry fruit granule contain no less than 1.26%.

2.10. Evaluation. To evaluate the quality of life, we used the Chinese edition of the universal quality-of-life scale for the SF-36 (The Short Form-36 Health Survey), which was developed by the American Medical Outcomes Study Group. The SF-36 includes eight factors: physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotional, and mental health. Higher scores correspond to a better quality of life.

The measures were assessed at the baseline, at the end of intervention at the 6th week, and at the posttreatment followups conducted at the end of the 12th and 18th weeks. We compared the scores for the eight factors of the SF-36 between baseline and the end of 6th week and among the end of 6th, 12th, and 18th weeks to assess the intervention efficacy and long-term intervention effect on the quality of life of XPYS-HEG in treatment of people with FPSH and liver-qi stagnation and spleen-qi deficiency syndrome in TCM.

2.11. Data Entry and Statistical Analysis. A database was built by Epidata 3.0, and the data were entered twice by two different people.

According to the principle of intention-to-treat, two data sets were used to test the difference between the drug and the placebo: full analysis sets (FAS), including all 200 participants, and per-protocol sets (PPS), including 197 participants (excluding 3 cases due to vomiting after drinking, suffering from intestinal adhesion, and catching cold and without following the treatment plan) [11] (see flow chart of the randomized controlled trial in Supplementary Materials, Supplementary Materials will be available online at doi: 10.1155/2012/509705).

The mean and standard deviation are presented for the quantitative data, and the frequency and the percentage are presented for the numerical data. A P value of 0.05 was considered significant. A Student's t-test or chi-squared test was used to test the differences among the characteristics of demography and the baseline of the SF-36 between the two groups. To detect changes in the SF-36, repeated-measures analysis of variance (ANOVA) models were used. All analyses were performed using the Statistical Package for the Social Sciences (SPSS for Windows version 17.00 SPSS Inc., Chicago, Ill).

2.12. Quality Control

2.12.1. The Training before Clinical Trials. The researchers had a full understanding of the clinical trial scheme and measures, and they proceeded according to the outlined scheme. The researchers observed adverse events or unexpected side effects and followed up in these cases.

2.12.2. Compliance. Compliance was established by explaining the trial to the participants and obtaining informed consent, interviewing the participants once every two weeks, verifying that the drug was taken, issuing the drug in the amount required for two weeks, and keeping records of the drugs issued to the participants.

3. Results

3.1. Characteristics of Demography and Baseline. There were no differences between the groups for the baseline measures of gender, ethnicity, marital status, occupation, or educational status ($P > 0.05$, see [11]) (see Table 1 in Supplementary Materials) or among the scores of the eight factors on the SF-36 ($P > 0.05$, see Table 1).

3.2. Comparison of the Intervention Effect between Groups. At the end of the 6th week, the analyses were conducted for the FAS and PPS with the objective data. A repeated-measures ANOVA examining the score changes of bodily pain according to the SF-36 from baseline to 6 weeks showed a significant treatment × time interaction (FAS: $P = 0.007$, PPS: $P = 0.005$) (see Table 2), changes in the score for role physical showed a significant treatment effect (FAS: $P = 0.032$, PPS: $P = 0.026$), and all eight factors showed significant time effects ($P < 0.01$).

3.3. Comparison of the Long-Term Clinical Effect between the Groups. Changes in the scores for the eight factors of the SF-36 at the end of the 6th, 12th, and 18th weeks were examined using repeated-measures ANOVA for the FAS and PPS. There was a significant treatment × time interaction for mental health (FAS: $P = 0.017$, PPS: $P = 0.025$) (see Table 3). Furthermore, there were significant time effects ($P < 0.05$), but no treatment × time interaction, and the other factors showed significant time effects ($P < 0.01$) with the exception of social function. There were significant treatment effects detected for five factors ($P < 0.05$), excluding physical functioning, bodily pain, and mental health.

4. Discussion

Quality of life includes various domains, such as physical functioning, mental status, social association, and bodily feeling. With the changes in the medical model, patient-reported outcomes (PRO), such as quality of life, are increasingly used as outcome assessments. Quality of life is regarded as an important PRO for people with FPSH and could also be used as an outcome measure to examine the effectiveness of therapies for people with FPSH [10].

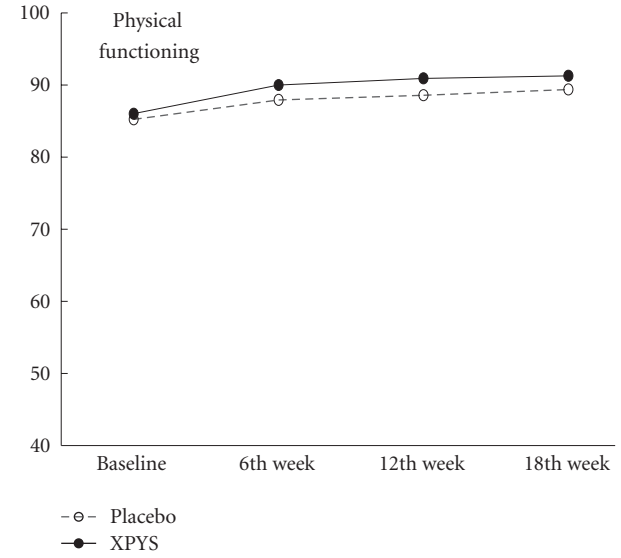


Figure 1: The trends of the changes in the scores for physical functioning (PPS).

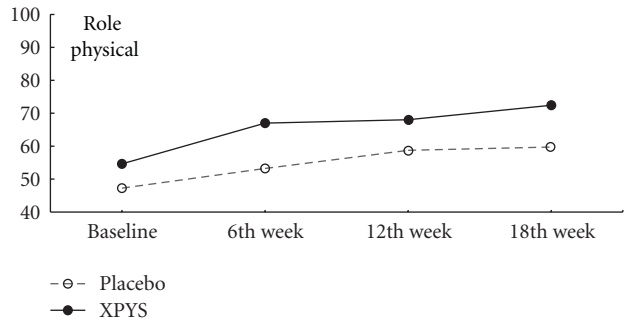


Figure 2: The trend of the changes in the scores for role physical (PPS).

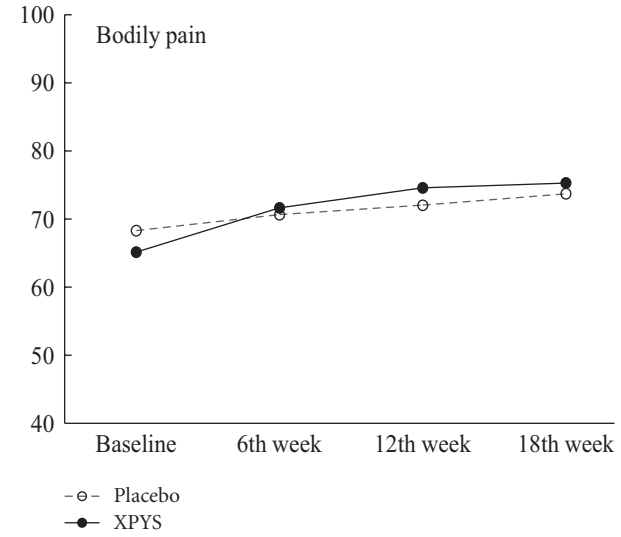


Figure 3: The trend in the change in scores for bodily pain (PPS).

Table 1: Baseline comparison of factor scores for the SF-36 between the two groups ($\overline{\chi} \pm s$).

		Placebo	XPYS	Z value	P value
Physical functioning	FAS	85.25 ± 11.53	85.70 ± 12.79	−0.509	0.610
	PPS	85.25 ± 11.53	86.03 ± 12.52	−0.644	0.520
Role physical	FAS	47.25 ± 36.39	54.25 ± 35.90	−1.376	0.169
	PPS	47.25 ± 36.39	54.64 ± 36.14	−1.434	0.152
Bodily pain	FAS	68.29 ± 14.59	65.54 ± 13.12	−1.513	0.130
	PPS	68.29 ± 14.59	65.13 ± 12.83	−1.643	0.100
General health	FAS	45.69 ± 13.66	45.68 ± 16.19	−0.158	0.874
	PPS	45.69 ± 13.66	45.24 ± 16.03	−0.345	0.730
Vitality	FAS	50.05 ± 13.46	51.60 ± 15.17	−0.661	0.538
	PPS	50.05 ± 13.46	51.29 ± 15.14	−0.457	0.648
Social functioning	FAS	70.75 ± 17.70	72.13 ± 16.17	−0.511	0.610
	PPS	70.75 ± 17.70	72.42 ± 16.23	−0.652	0.514
Role emotional	FAS	47.00 ± 35.17	52.33 ± 33.59	−1.079	0.281
	PPS	47.00 ± 35.17	52.58 ± 33.62	−1.113	0.266
Mental health	FAS	54.96 ± 14.54	56.76 ± 13.41	−0.838	0.402
	PPS	54.96 ± 14.54	56.99 ± 13.38	−0.942	0.346

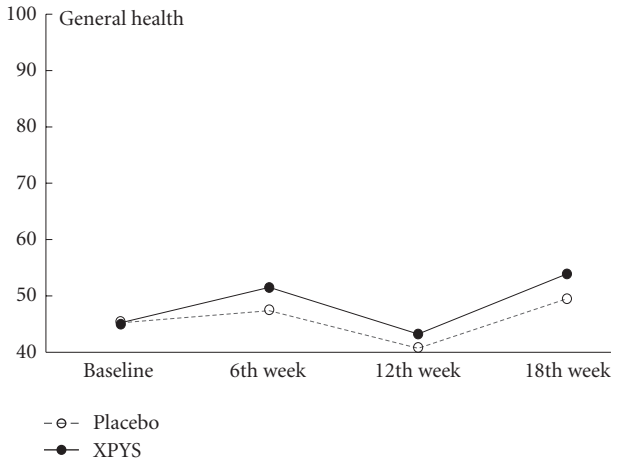


Figure 4: The trend in the change in scores for general health (PPS).

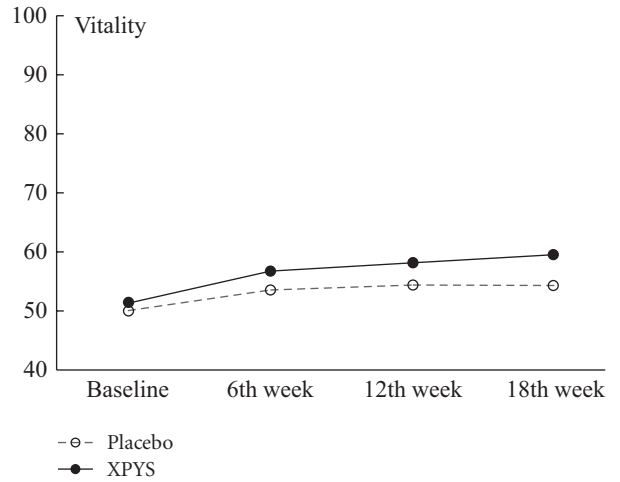


Figure 5: The trend for the change in scores for vitality (PPS).

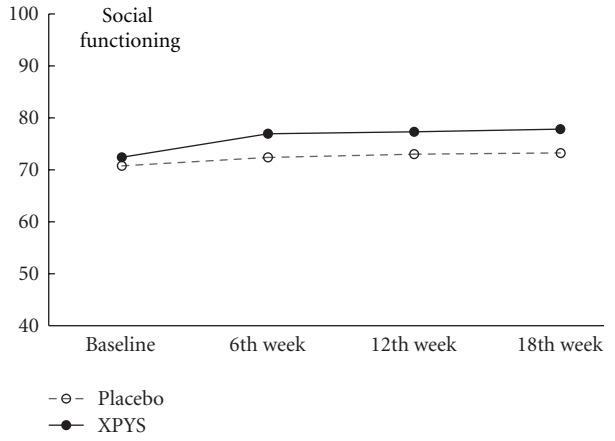


Figure 6: The trend for the change in scores for social function (PPS).

The results of this study demonstrated that the scores for eight factors on the SF-36 changed statistically from baseline to the end of the 6th week of treatment in the two groups, and the changes exhibited an increasing trend (see Table 1 and Figures 1, 2, 3, 4, 5, 6, 7, and 8). Our findings suggest that the quality of life of the participants could be improved as a result of the intervention. When comparing the scores of the eight factors at the end of the 6th week between the two groups, we found that the score for the factor of role physical in the XPYS group was significantly higher than that in the placebo group. However, there was no obvious score change for the factor of role physical in the two groups according to the treatment × time interaction. Compared with the baseline score, the score change for bodily pain in the XPYS group was more obvious than in the placebo group according to the treatment × time interaction at the end of the 6th week. However, there was

Table 2: The comparison of factor scores from the SF-36 from baseline to the end of the 6th week between the two groups ($\overline{\chi} \pm s$).

		Group	Baseline	6th week	Time effect		Treatment × time interaction		Treatment effect	
					F	P	F	P	F	P
Physical functioning	FAS	Placebo	85.25 ± 11.53	87.95 ± 10.28	23.501	0.000*	0.777	0.379	0.585	0.445
		XPYS	85.70 ± 12.79	89.60 ± 8.22						
	PPS	Placebo	85.25 ± 11.53	87.95 ± 10.28	23.313	0.000*	0.844	0.359	1.110	0.293
		XPYS	86.03 ± 12.52	90.00 ± 7.32						
Role physical	FAS	Placebo	47.25 ± 36.39	53.25 ± 36.18	24.286	0.000*	2.848	0.093	4.686	0.032*
		XPYS	54.25 ± 35.90	66.50 ± 33.75						
	PPS	Placebo	47.25 ± 36.39	53.25 ± 36.18	23.950	0.000*	2.880	0.091	5.033	0.026*
		XPYS	54.64 ± 36.14	67.01 ± 33.57						
Bodily pain	FAS	Placebo	68.29 ± 14.59	70.65 ± 14.03	36.080	0.000*	7.468	0.007*	0.195	0.660
		XPYS	65.54 ± 13.12	71.84 ± 12.16						
	PPS	Placebo	68.29 ± 14.59	70.65 ± 14.03	36.804	0.000*	8.025	0.005*	0.380	0.538
		XPYS	65.13 ± 12.83	71.63 ± 11.98						
General health	FAS	Placebo	45.69 ± 13.66	47.59 ± 13.71	7.929	0.005*	2.146	0.145	2.006	0.158
		XPYS	45.68 ± 16.19	51.70 ± 13.34						
	PPS	Placebo	45.69 ± 13.66	47.59 ± 13.71	8.249	0.005*	2.370	0.125	1.475	0.226
		XPYS	45.24 ± 16.03	51.53 ± 13.21						
Vitality	FAS	Placebo	50.05 ± 13.46	53.55 ± 14.71	27.812	0.000*	1.164	0.282	1.671	0.198
		XPYS	51.60 ± 15.17	56.90 ± 15.17						
	PPS	Placebo	50.05 ± 13.46	53.55 ± 14.71	28.076	0.000*	1.348	0.247	1.355	0.246
		XPYS	51.29 ± 15.14	56.75 ± 15.23						
Social functioning	FAS	Placebo	70.75 ± 17.70	72.38 ± 15.82	10.143	0.002*	2.235	0.137	1.867	0.173
		XPYS	72.13 ± 16.17	76.63 ± 14.40						
	PPS	Placebo	70.75 ± 17.70	72.38 ± 15.82	9.898	0.002*	2.189	0.141	2.264	0.134
		XPYS	72.42 ± 16.23	76.93 ± 14.25						
Role emotional	FAS	Placebo	47.00 ± 35.17	55.33 ± 33.58	21.020	0.000*	0.115	0.735	2.002	0.159
		XPYS	52.33 ± 33.59	62.00 ± 29.60						
	PPS	Placebo	47.00 ± 35.17	55.33 ± 33.58	20.385	0.000*	0.105	0.746	2.136	0.146
		XPYS	52.58 ± 33.62	62.20 ± 29.12						
Mental health	FAS	Placebo	54.96 ± 14.54	57.64 ± 14.64	8.009	0.005*	3.024	0.084	0.172	0.679
		XPYS	56.76 ± 13.41	57.40 ± 13.04						
	PPS	Placebo	54.96 ± 14.54	57.64 ± 14.64	7.861	0.006*	2.876	0.091	0.291	0.590
		XPYS	56.99 ± 13.38	57.65 ± 12.98						

no obvious score change in the two groups from the end of the 6th week to the end of the 12th and 18th weeks. This result suggested that XPYS-HEG could ease bodily pain and influence the effects on work and housework that result from bodily pain in people with FPSH and liver-qi stagnation and spleen-qi deficiency syndrome. However, there was no a long-term effect.

The scores for seven of the factors on the SF-36 changed significantly between the two groups during the follow-up period, with the exception of social functioning from the end of the 6th week to the end of the 12th and 18th weeks. This finding indicated that there was a long-term effect of at least 18 weeks on quality of life in the two groups. The scores for the factors of general health and mental health are also worth noting. Compared with the baseline scores and the scores at the end of the 6th week, the scores of these two factors decreased at the end of the 12th week, but they increased at the end of the 18th week. The two factors that were related to the level of recognition of health among the participants were influenced by the varying conditions. When comparing the scores of the eight factors at the end of the 18th week between the two groups, we found that the scores for the factors of role physical, general health, vitality, social functioning, and role emotional in the XPYS group were significantly higher than those in the placebo group. However, there were no obvious changes in the scores for these factors between the two groups based on the comparison of the treatment × time interaction. Compared with the scores at the end of 6th week, the change in the score for mental health in the XPYS group was more obvious than that in the placebo group according to the treatment × time interaction at the end of the 12th and 18th weeks. However, there was no obvious change in

Table 3: The comparison of factor scores from the SF-36 from the end of the 6th week to the end of the 12th and 18th weeks between the two groups ($\overline{\chi} \pm s$).

			6th week	12th week	18th week	Time effect		Treatment × time interaction		Treatment effect	
						F	P	F	P	F	P
Physical functioning	FAS	Placebo	87.95 ± 10.28	88.60 ± 9.40	89.40 ± 8.36	5.992	0.003*	0.306	0.737	1.943	0.165
		XPYS	89.60 ± 8.22	90.50 ± 8.03	90.85 ± 8.91						
	PPS	Placebo	87.95 ± 10.28	88.60 ± 9.40	89.40 ± 8.36	5.996	0.003*	0.305	0.738	3.336	0.069
		XPYS	90.00 ± 7.32	90.93 ± 7.05	91.29 ± 8.05						
Role physical	FAS	Placebo	53.25 ± 36.18	58.75 ± 34.88	59.75 ± 35.34	7.273	0.001*	1.471	0.232	6.347	0.013*
		XPYS	66.50 ± 33.75	67.50 ± 32.08	71.75 ± 30.91						
	PPS	Placebo	53.25 ± 36.18	58.75 ± 34.88	59.75 ± 35.34	7.252	0.001*	1.445	0.238	6.963	0.009*
		XPYS	67.01 ± 33.57	68.04 ± 31.82	72.42 ± 30.51						
Bodily pain	FAS	Placebo	70.65 ± 14.03	72.04 ± 13.24	73.71 ± 12.72	15.083	0.000*	1.223	0.297	1.242	0.266
		XPYS	71.84 ± 12.16	74.70 ± 11.97	75.38 ± 10.71						
	PPS	Placebo	70.65 ± 14.03	72.04 ± 13.24	73.71 ± 12.72	15.194	0.000*	1.293	0.277	1.053	0.306
		XPYS	71.63 ± 11.98	74.58 ± 11.82	75.28 ± 10.51						
General health	FAS	Placebo	47.59 ± 13.71	40.71 ± 9.73	49.53 ± 12.90	140.896	0.000*	0.948	0.389	5.783	0.017*
		XPYS	51.70 ± 13.34	43.56 ± 9.94	53.95 ± 13.07						
	PPS	Placebo	47.59 ± 13.71	40.71 ± 9.73	49.53 ± 12.90	142.937	0.000*	1.266	0.284	5.340	0.022*
		XPYS	51.53 ± 13.21	43.26 ± 9.33	53.92 ± 12.89						
Vitality	FAS	Placebo	53.55 ± 14.71	54.40 ± 14.55	54.30 ± 14.74	4.233	0.016*	1.527	0.220	4.525	0.035*
		XPYS	56.90 ± 15.17	58.25 ± 14.13	59.60 ± 13.48						
	PPS	Placebo	53.55 ± 14.71	54.40 ± 14.55	54.30 ± 14.74	4.302	0.015*	1.592	0.206	4.225	0.041*
		XPYS	56.75 ± 15.23	58.14 ± 14.17	59.54 ± 13.50						
Social functioning	FAS	Placebo	72.38 ± 15.82	73.00 ± 15.76	73.25 ± 16.19	0.807	0.447	0.025	0.975	4.432	0.037*
		XPYS	76.63 ± 14.40	77.00 ± 13.96	77.50 ± 13.76						
	PPS	Placebo	72.38 ± 15.82	73.00 ± 15.76	73.25 ± 16.19	0.807	0.448	0.025	0.975	5.126	0.025*
		XPYS	76.93 ± 14.25	77.32 ± 13.78	77.84 ± 13.56						
Role emotional	FAS	Placebo	55.33 ± 33.58	59.33 ± 34.36	60.00 ± 34.49	9.058	0.000*	1.524	0.220	4.152	0.043*
		XPYS	62.00 ± 29.60	66.33 ± 27.01	71.33 ± 28.04						
	PPS	Placebo	55.33 ± 33.58	59.33 ± 34.36	60.00 ± 34.49	9.169	0.000*	1.617	0.201	4.514	0.035*
		XPYS	62.20 ± 29.12	66.67 ± 26.35	71.82 ± 27.36						
Mental health	FAS	Placebo	57.64 ± 14.64	40.24 ± 11.11	57.96 ± 13.42	1045.885	0.000*	4.148	0.017*	0.782	0.378
		XPYS	57.40 ± 13.04	42.52 ± 11.08	60.48 ± 13.20						
	PPS	Placebo	57.64 ± 14.64	40.24 ± 11.11	57.96 ± 13.42	1152.427	0.000*	3.767	0.025*	0.983	0.323
		XPYS	57.65 ± 12.98	42.52 ± 11.11	60.83 ± 13.08						

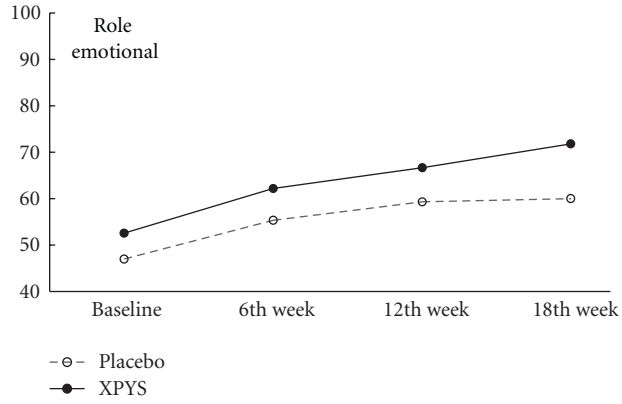


Figure 7: The trend for the change in scores for role emotional (PPS).

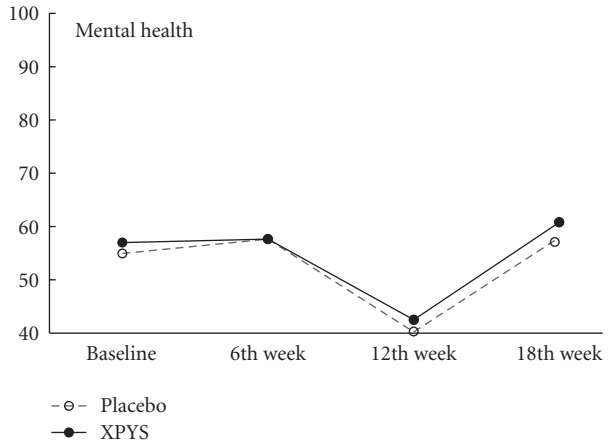


Figure 8: The trend for the change in scores for mental health (PPS).

the scores in the two groups from baseline to the end of the 6th week. This result suggested that XPYS-HEG had a slow-acting effect that could stimulate a positive mood and ease a negative mood.

XPYS-HEG is composed by Radix Astragali, Radix Ginseng, Pericarpium Citri Reticulatae, Rhizoma Cyperi, Radix Angelicae, and Fructus Lycii. Radix Astragali and Radix Ginseng are used to replenish spleen qi; Pericarpium Citri Reticulatae and Rhizoma Cyperi are applied to soothe liver qi; Radix Angelicae and Fructus Lycii nourish the blood. According to the theory of TCM, the effect of XPYS-HEG on the quality of life in participants is explained as follows. (1) The spleen governs the muscles, flesh and the four limbs. Replenishing the spleen qi and nourishing the blood can nourish the four limbs. The liver governs the sinews, and when the liver obtains blood, the sinews stretch, which may explain why XPYS-HEG could ease both bodily pain and influence the work and housework resulting from bodily pain among people with FPSH and liver-qi stagnation and spleen-qi deficiency syndrome. (2) Dispersing and discharging functions of the liver can regulate emotion. The regulation of emotion requires a long period, which may explain why XPYS-HEG was observed to stimulate a positive mood and ease a negative mood at the end of the 18th week during the posttreatment follow-up period.

Additionally, according to Figures 5 and 7, there were changes in the trends for the scores of the factors for vitality and role emotional, which increased gradually in the XPYS group, but decreased gradually or were maintained in the placebo group. Further research is required to identify the long-term effect of XPYS-HEG for improving vitality and easing functional constraints resulting from a negative mood.

5. Conclusions

XPYS-HEG could partially improve the quality of life for people with FPSH and liver-qi stagnation and spleen-qi deficiency syndrome. XPYS-HEG may ease bodily pain, stimulate a positive mood and ease a negative mood.

Acknowledgments

The paper was sponsored by the "Eleventh Five-year Plan" of China State Science & Technology Ministry (2006-BAI13B05), National Basic Research Program "973 Plan" of China State Science & Technology Ministry (2011CB-505105), Innovative Team Project of Beijing University of Chinese Medicine (2011-CXTD-08), and Research Base Development Project of Beijing University of Chinese Medicine (2011-JDJS-09).

References

[1] G. P. Holmes, J. E. Kaplan, N. M. Gantz et al., "Chronic fatigue syndrome: a working case definition," *Annals of Internal Medicine*, vol. 108, no. 3, pp. 387–389, 1988.

[2] K. Fukuda, S. E. Straus, I. Hickie, M. C. Sharpe, J. G. Dobbins, and A. Komaroff, "The chronic fatigue syndrome: a comprehensive approach to its definition and study," *Annals of Internal Medicine*, vol. 121, no. 12, pp. 953–959, 1994.

[3] T. Sun, T. F. Wang, and L. X. Wu, *On Subhealth*, China Press of Traditional Chinese Medicine, Beijing, China, 2007.

[4] China Association of Chinese Medicine, *Clinical Guidelines of Chinese Medicine on Sub-health*, Medicine in China Press of Traditional Chinese Medicine, Beijing, China, 2006.

[5] Y. M. Xie, B. Y. Liu, and H. Y. Piao, "Exploration on the common characters of sub-healthy people based on clinical epidemiology," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 26, no. 7, pp. 612–616, 2006.

[6] C. X. Fan, S. Y. Wang, L. Zhu, Y. J. Xiao, and S. B. Ma, "Study on the prevalence and risk factors of "subhealth" status in college and university staff in Guangdong province," *Zhonghua Liu Xing Bing Xue Za Zhi*, vol. 24, no. 9, pp. 774–777, 2003.

[7] T. F. Wang and X. L. Xue, "Sub-health state and chronic fatigue syndrome," *Zhonghua Liu Xing Bing Xue Za Zhi*, vol. 28, no. 1, pp. 77–79, 2008.

[8] Y. J. Zhang, T. F. Wang, X. L. Xue, J. J. Wang, G. R. Li, and P. Han, "Characteristics of traditional chinese medicine syndromes and their element distributions in sub-health status: a modern literature review," *Journal of Chinese Integrative Medicine*, vol. 6, no. 12, pp. 1290–1293, 2008.

[9] T. F. Wang, J. J. Wang, X. L. Xue et al., "Distribution characteristics of traditional Chinese medicine syndromes and their elements in people with subhealth fatigue," *Journal of Chinese Integrative Medicine*, vol. 8, no. 3, pp. 220–223, 2010.

[10] X. L. Xue, T. F. Wang, Y. J. Zhang et al., "Construction of effectivenenss evaluation system for traditional Chinese medicine interventions in subhealth," *Journal of Chinese Integrative Medicine*, vol. 7, pp. 201–204, 2009.

[11] T. F. Wang, X. L. Xue, Y. J. Zhang et al., "Effects of Xiaopi Yishen herbal extract granules in treatment of fatigue-predominant subhealth due to liver-qi stagnation and spleen-qi deficiency: a prospective, randomized, placebo-control led and double-blind clinical trial," *Journal of Chinese Integrative Medicine*, vol. 9, no. 5, pp. 515–524, 2011.

[12] J. P. Liu, "Sample size calculation for clinical trais," *Chinese Journal of Integrated Traditional and Western Medicine*, vol. 23, pp. 536–538, 2003.

[13] Y. J. Zhang, T. F. Wang, X. L. Xue, J. J. Wang, and G. R. Li, "Exploration on diagnostic standardization of fatigue-predominant sub-health in combining between traditional Chinese and Western medicine," *Beijing Journal of Traditional Chine Meicine*, vol. 28, pp. 269–270, 2009.

[14] T. Chalder, G. Berelowitz, T. Pawlikowska et al., "Development of a fatigue scale," *Journal of Psychosomatic Research*, vol. 37, no. 2, pp. 147–153, 1993.

[15] Z. J. Zhang, "Behavioral medicine scale manual," Chinese Medicine Multimedia Press, 2005.

[16] World Medical Association Declaration of Helsinki, "Ethical principles for medical research involving human subjects," *Journal of the American Medical Association*, vol. 284, pp. 3043–3045, 2000.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 783923, 8 pages
doi:10.1155/2012/783923

Review Article

Clinical Distribution and Molecular Basis of Traditional Chinese Medicine *ZHENG* in Cancer

Zhen Chen[1, 2] and Peng Wang[1, 2]

[1] *Department of Integrative Oncology, Fudan University Shanghai Cancer Center, Shanghai 200032, China*
[2] *Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China*

Correspondence should be addressed to Peng Wang, wangp413@yahoo.com.cn

Received 13 January 2012; Revised 30 May 2012; Accepted 4 June 2012

Academic Editor: Shi-Bing Su

In traditional Chinese medicine (TCM) clinical practice, *ZHENG* (also known as syndrome) helps to guide design of individualized treatment strategies. In this study, we investigated the clinical use of *ZHENG* in TCM-treated cancer patients by systematically analyzing data from all relevant reports in the Chinese-language scientific literature. We aimed to determine the clinical *ZHENG* distributions in six common cancers (lung, liver, gastric, breast, colorectal, and pancreatic) with the expectation of uncovering a theoretical basis for TCM *ZHENG* as a clinical cancer treatment. In addition, we also reviewed the molecular basis underlying *Xue-Yu* (blood stasis), *Shi-Re* (dampness-heat), *Yin-Xu* (Yin deficiency), and *Pi-Xu* (spleen deficiency) *ZHENG* that are commonly found in cancer patients. The results from our summary study provide insights into the potential utility of TCM *ZHENG* and may contribute to a better understanding of the molecular basis of TCM *ZHENG* in cancer.

1. Introduction

Traditional Chinese medicine (TCM) has been practiced and recorded in the medical literature for thousands of years. It is considered unique among the world's ancient traditional medicines based upon its integrative use of physiological and holistic theories; for example, a key aim of TCM is to regulate and maintain proper body functions by modulating and exploiting interactions between the patient and their environment. The rich history of TCM has prompted a recent surge in clinical research efforts to evaluate its efficacy as an alternative strategy to the largely pharmaceutical-based approaches used in developed countries to prevent and treat many types of disease, including cancers.

It has been reported that over 90% of modern Chinese cancer patients received some form of TCM during their treatment regimen [1]. The rates of TCM used by health care providers and interest by patients outside of China are continuing or rise annually, especially within the field of oncology [2]. Application of TCM as an adjuvant cancer therapy has been reported to enhance the efficacy of both chemo- and radiotherapy and to help reduce adverse effects of each [3, 4]. Furthermore, the Chinese herbal medicines used in TCM have recently been recognized as an important source for novel drug development, including anticancer drugs [5]. Therefore, western medicine practitioners and researchers are, now more than ever, open to exploring the potential of TCM to enhance conventional treatment of cancer patients [6].

The concept of *ZHENG* occupies an important position in the TCM system and is key to recognizing a patient's disease state and developing an effective, individualized treatment strategy. *ZHENG* is a kind of pathology of the disease development of a body in a certain stage, including the disease wherefrom, the cause, the feature, and the conflicts between healthy energy and evils. It reflects the nature of pathological change at a certain stage and reveals the intrinsic quality of disease more completely, profoundly, and accurately than symptoms. Therefore, the diagnosis of TCM *ZHENG* is to differentiate a disease by analyzing and synthesizing the information, symptoms, and patients' physical status collected through four types of diagnostic methods: inspection, auscultation and olfaction, inquiry, and palpation. According to the combination of diagnostic

methods used, different types of *ZHENG* are possible for a single disease, and all may be equally effective. This feature provides flexibility and ready diversification to the disease-targeting therapy, allowing for the treating clinician to take advantage of the patient's personality and mental and spiritual desires to achieve high rates of compliance and completion. Therefore, TCM *ZHENG* differentiation must also be applied to the new TCM efforts being used in cancer patients worldwide.

The purpose of this study was to identify the clinical usage of *ZHENG* TCM in Chinese cancer patients by systematically searching the relevant Chinese-language medical and scientific literature collections. After analyzing the clinical distribution, the molecular basis underlying TCM *ZHENG* was considered in an attempt to better understand its usefulness in future clinical practice.

2. Literature Search for Publications on TCM *ZHENG* in Chinese Cancer Patients

We searched the four major electronic databases of Chinese-language medical and scientific literature (China National Knowledge Infrastructure (CNKI), Chinese Scientific Journal Database (VIP), Wanfang Database, and Chinese BioMedical Literature Database (CBM)) for publications between 2000 and 2011 that were related to "Zhong Yi" (traditional Chinese medicine), "*ZHENG*," and "Ai" (cancer). More than 20,000 papers on TCM *ZHENG* in cancer were initially identified and included clinical observations, individual or small-scale case reports, large-scale clinical experiences, and experimental animal studies.

3. Investigation of TCM *ZHENG* in Cancer Patients by Publication Year and Cancer Type

As shown in Figure 1, there was a dramatic increase in the number of annual publications of TCM *ZHENG* in cancer patients during the past ten years. Among these articles, 32.2% (700 out of 2175) were related to lung cancer, 22.9% to liver cancer, 19.4% to gastric cancer, 12.1% to breast cancer, 5.9% to colon cancer, 1.7% to pancreatic cancer, and 10.3% to a variety of other types of cancer. This cancer type distribution is consistent with the incidence of cancers in China. It has been reported that the four most frequently diagnosed cancers in Chinese men over the past ten years involved lung, stomach, liver, and colon; Chinese women, however, were most frequently diagnosed with cancers of the breast, lung, stomach, and colon. The incidence of pancreatic cancer in Chinese men and women ranked 8th and 9th, respectively, but produced high mortality (nearly equal to incidence) in both sexes. This result suggests that TCM has been widely applied, as at least one form of treatment, for Chinese cancer patients in modern medical practice. Furthermore, the practice of TCM *ZHENG* in cancer patients has increased steadily over the past decade.

4. Clinical Distributions of TCM *ZHENG* in Chinese Patients with Common Cancers

The six most common types of cancer reported in the studies included in this summary analysis were lung, liver, gastric, breast, colorectal, and pancreatic—collectively accounting for 89.7% of all the publications. We attempted to systematically identify and analyze the clinical *ZHENG* distribution in these six types of cancer. We searched the collection of initially identified relevant studies to identify clinical trials and case series that provided information on ≥ 10 cases with *ZHENG* description. A total of 144 articles were selected for clinical distribution analysis. The annual distribution frequencies of TCM *ZHENG* for each type of cancer were calculated. The cancer types with *ZHENG* frequency over 10% are presented in Figure 2.

The number of publications describing TCM *ZHENG* in lung cancer increased dramatically from the year 2000 ($n = 8$, in total) to the end of 2011 ($n = 85$, in total). Among these publications, 32 reported results from clinical trials or case series with *ZHENG*-based TCM. Summary analysis indicated that *Qi-Yin-Liang-Xu*, *Fei-Pi-Qi-Xu*, *Yin-Xu-Nei-Re*, *Qi-Zhi-Xue-Yu,* and *Tan-Re* were the most common *ZHENGs* in lung cancer (Figure 2). The number of publications describing TCM *ZHENG* in other types of cancer (liver, gastric, breast, colorectal, and pancreas) also increased dramatically over the past decade. As shown in Figure 2, by the end of 2011, a total of 26 articles had reported data on TCM *ZHENG* in liver cancer, 19 on gastric cancer, 21 on breast cancer, 29 on colorectal cancer, and 17 on pancreatic cancer. The frequency distribution of *ZHENG* for each of these types of cancer was calculated. The results indicated that the main *ZHENGs* for liver cancer were *Xue-Yu*, *Pi-Xu*, *Gan-Shen-Yin-Xu*, *Qi-Zhi*, and *Gan-Dan-Shi-Re*, which accounted for 94.3% of the total. The main *ZHENGs* for gastric cancer were *Pi-Xu*, *Yu-Du-Zu-Zhi*, *Gan-Wei-Bu-He*, *Qi-Xue-Liang-Xu*, *Tan-Shi*, and *Wei-Re-Shang-Yin*, which accounted for 93.9% of the total. The main *ZHENGs* for breast cancer were *Qi-Yin-Liang-Xu*, *Qi-Xue-Liang-Xu*, and *Gan-Qi-Fan-Wei*, which accounted for 90.5% of the total. The main *ZHENGs* for colorectal cancer were *Shi-Re-Yun-Jie*, *Qi-Xue-Liang-Xu*, *Pi-Shen-Yang-Xu*, *Yu-Du-Zu-Zhi*, *Gan-Shen-Yin-Xu*, and *Han-Shi-Kun-Pi*, which accounted for 84.5% of the total. The main *ZHENGs* for pancreatic cancer were *Shi-Re*, *Pi-Xu*, and *Xue-Yu*, which accounted for 82.8% of the total.

5. The Molecular Basis for Common *ZHENGs* in Cancer

5.1. Xue-Yu ZHENG (Blood Stasis). *Xue-Yu ZHENG* is one of the common syndromes in TCM, characterized by cyanosis (of skin, lips, nails, and/or tongue), ecchymosis and petechia, and irregular pulse (detected by palpation as thin, unsmooth, deep, taut, knotted, slow, or intermittent). In addition, other common clinical signs include blackish complexion, dry skin, and purpura. The *Xue-Yu* status was recently shown to be related with changes of hemorheological properties,

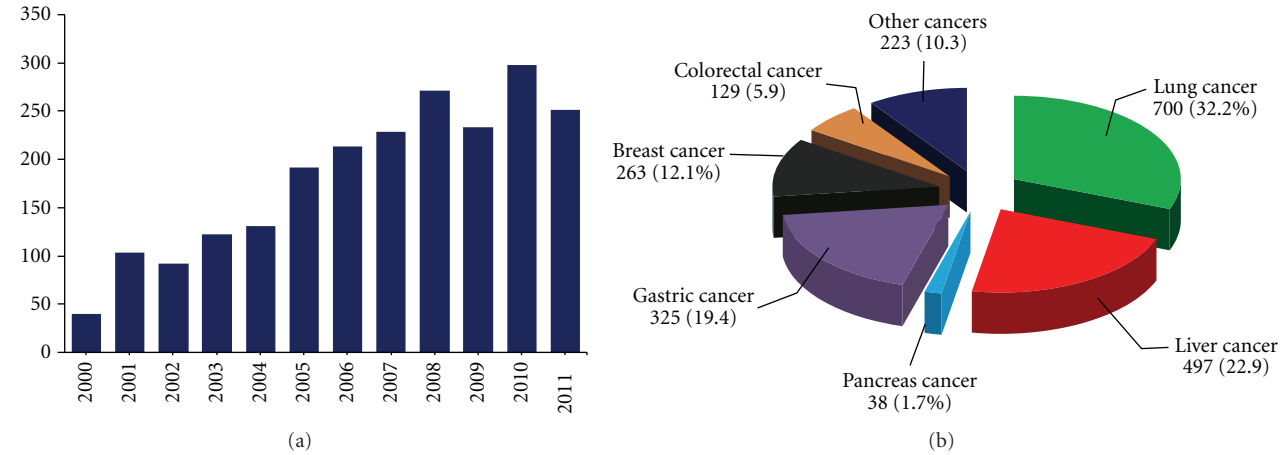


Figure 1: Annual publications on TCM *ZHENG* in cancer. (a) A total of 2175 papers were retrieved by searching the terms "Zhong Yi" (traditional Chinese medicine), "*ZHENG*," and "Ai" (cancer) through the main Chinese electronic databases, including China National Knowledge Infrastructure (CNKI), Chinese Scientific Journal Database (VIP), Wanfang Database, and Chinese BioMedical Literature Database (CBM), and then analyzed by calculating the annual publications from January 1, 2000 to November 13, 2011. (b) The distributions of cancer types among all the publications. *To November 13, 2011.

such as high-blood viscosity, increased erythrocyte aggregation, increased blood sedimentation, decreased erythrocyte deformability, and decreased hematocrit [7].

Xue-Yu is associated with many diseases, including cancer. Epidemiological investigation has revealed that *Xue-Yu* is one of the most prominent *ZHENGs* in patients with cancer, especially for those with liver, lung, and pancreatic cancer; the results from our summary analysis agreed with this reported pattern (Figure 2). TCM treatment of cancer patients with *Xue-Yu* using traditional Chinese herbs has shown satisfactory efficacy in clinical practice in China. Since 1990, several retrospective clinical studies have reported strong statistical correlation between tumor metastasis and *Xue-Yu ZHENG*; treating or controlling tumor metastasis, while *Huo-Xue-Hua-Yu (promoting blood circulation and removing blood stasis)* has been advocated as a potential therapeutic approach [8, 9]. There are several reasons accounting for this theory. One is that cancer patients usually show *Xue-Yu ZHENG*. For example, patients with liver cancer usually exhibit bluish tendon on abdomen, scaly skin, a darkened complexion on the face, a hump below the costal region, and a purple-colored tongue and complain of a localized pricking pain in the region corresponding to the liver [10]. These symptoms are indicators of *Gan-Xue-Yu* (blood stasis in the *Gan*) and should be treated with the aim of *Huo-Xue-Hua-Yu* (as described above). Another reason is that cancer patients with *Xue-Yu ZHENG* usually present with microcirculation disturbance [11]. For example, Liu et al. observed that lung cancer patients with *Xue-Yu ZHENG* had significantly higher fibrinogen content than their counterparts without *Xue-Yu ZHENG*; moreover, the increased fibrinogen was found to be correlated with increased metastasis [8]. Another observational study from 105 patients with liver cancer demonstrated that the presence of *Xue-Yu ZHENG* was associated with a worse prognosis; it was unclear whether treatment for *Huo-Xue-Hua-Yu* in these patients significantly affected patient survival [12]. The third reason is that a tumor-mediated hypercoagulable state may exist and functionally complicate the disease state. The tumor-mediated hypercoagulable state is known to promote expression of tissue factor (TF) on the surfaces of tumor cells and macrophages, cell surface phospholipids that support coagulation activation, other tumor-mediated factors that trigger platelet activation and support accumulation, and tumor-induced endothelial cell factors that activate coagulation [13]. Furthermore, recently published preclinical data has suggested that activation of coagulation can promote tumor growth and angiogenesis. Since clinical hypercoagulable status is associated with adverse cancer prognosis, treatment with anticoagulation agents may prolong survival in certain types of cancer [14].

Even though a definitive link between cancer and *Xue-Yu ZHENG* has not yet been identified, some studies have shown evidence that *Huo-Xue-Hua-Yu* treatment may promote cancer metastasis. A prospective randomized controlled trial in 60 nasopharyngeal carcinoma cases conducted by Han et al. showed that integrated *Huo-Xue-Hua-Yu* herbs treatment with radiotherapy in nasopharyngeal carcinoma patients was associated with a 2.67-fold increase in distant metastasis, as compared to patients receiving radiotherapy alone [15]. In addition, preclinical studies showed that some *Huo-Xue-Hua-Yu* medicines, such as Danshen (*Red-rooted salvia root*), Chishao (*Red paeony root*), Danggui (*Chinese angelica*), Honghua (*Indian azalea leaf*), Jixueteng (*Suberect spatholobus stem*), Awei (*Chinese asafoetida*), and Chuanxiong (*Szechuan lovage rhizome*), could promote lung metastasis in liver cancer xenografted mouse models [16]. Our group previously established a xenograft tumor mouse model with *Xue-Yu ZHENG* to evaluate the effect of *Xue-Yu ZHENG* on tumor metastasis. We found that mice with the *Xue-Yu ZHENG* developed less metastasis than their counterparts without *Xue-Yu* [17–20]. However, when the

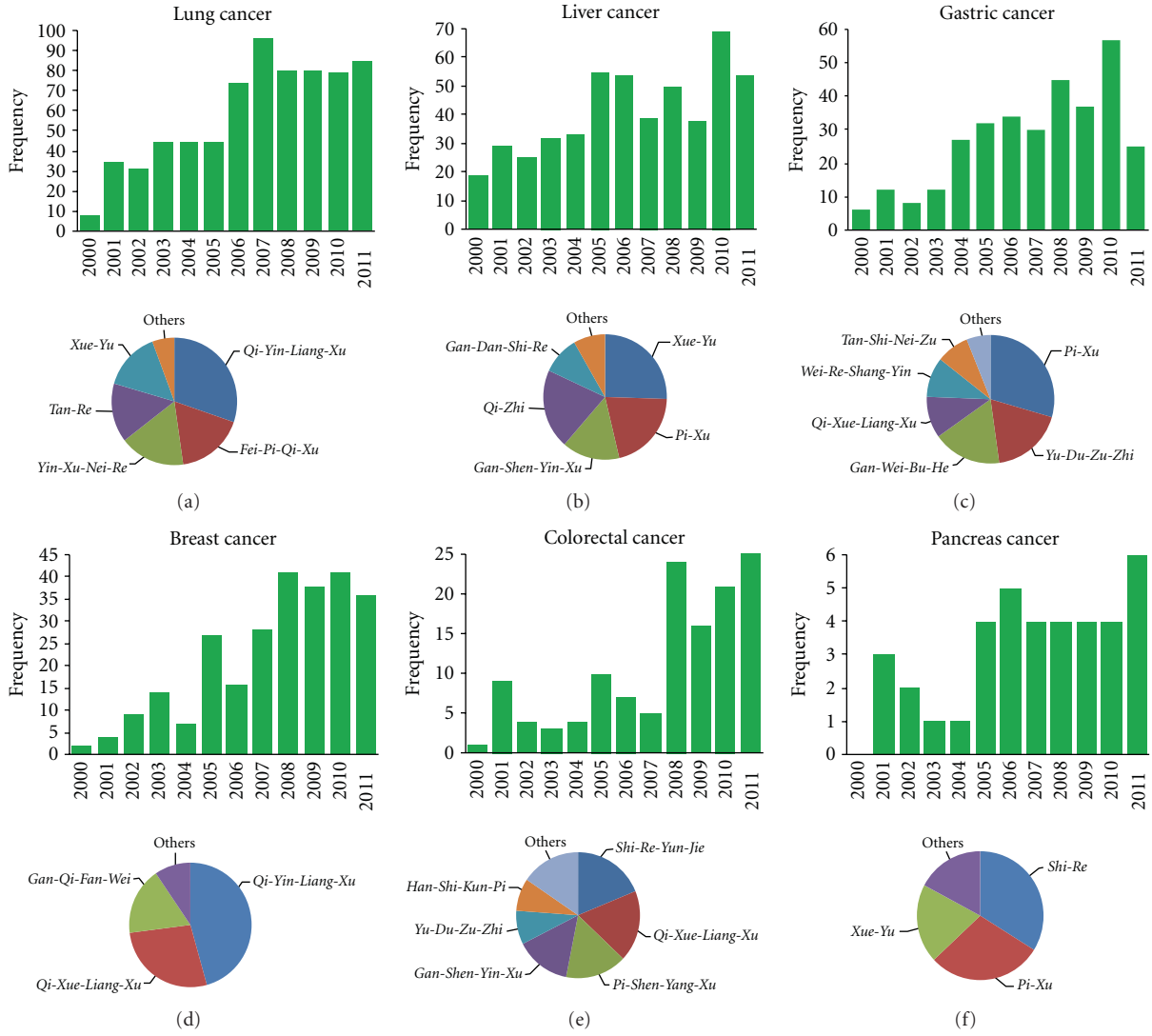


Figure 2: Clinical distributions of TCM *ZHENG* in common cancers. Annual publications for each common cancer were calculated and presented as a histogram. Publications involved with clinical trials and case series, where information on more than 10 cases with *ZHENG* description was available, were further selected. Thirty-two articles reported on lung cancer, 26 on liver cancer, 19 on gastric cancer, 21 on breast cancer, 29 on colorectal cancer, and 17 on pancreatic cancer. Finally, for each type of cancer, the distribution frequency of *ZHENG* was calculated and presented in pie chart. Note: *Qi-Yin-Liang-Xu*, deficiency of both *Qi* and *Yin; Fei-Pi-Qi-Xu*, lung-spleen *Qi* deficiency; *Yin-Xu-Nei-Re*, *Yin* asthenia and internal heat; *Tan-Re*, phlegm-heat; *Xue-Yu*, blood stasis; *Pi-Xu*, spleen deficiency; *Gan-Shen-Yin-Xu*, liver-kidney *Yin* deficiency; *Qi-Zhi*, *Qi* stagnation; *Gan-Dan-Shi-Re*, liver-gallbladder dampness-heat; *Yu-Du-Zu-Zhi*, stagnation of blood stasis and toxin; Gan-Wei-Bu-He, liver-stomach disharmony; *Qi-Xue-Liang-Xu*, deficiency of both *Qi* and blood; *Yin-Xu-Nei-Re*, Yin deficiency due to stomach heat; *Tan-Shi-Nei-Zu*, stagnation of phlegm-dampness; *Gan-Qi-Fan-Wei*, liver *Qi* invading stomach; Shi-Re-*Yun-Jie*, stagnation of dampness-heat; *Pi-Shen-Yang-Xu*, asthenic splenonephro-yang; *Yu-Du-Nei-Zu*, stagnation of blood stasis and toxin; *Han-Shi-Kun-Pi*, cold-dampness disturbing spleen.

tumor-bearing mice with *Xue-Yu ZHENG* were treated with individual *Huo-Xue-Hua-Yu* herbs, such as Danshen (*Red-rooted salvia root*) and Shensanqi (*Sanchi*), we found that Shensanqi treatment suppressed liver metastasis [19, 20] while Danshen treatment promoted liver metastasis [19]. Therefore, the correlation between *ZHENG* and cancer cells needs to be further studied in order to gain a more comprehensive understanding of its effects on the complex processes of tumor growth and metastasis.

5.2. Shi-Re ZHENG (Dampness-Heat). *Shi-Re ZHENG* is caused by dysfunction of the *Pi* ("spleen") and *Wei* ("stomach") due to retention of dampness and heat in the body. The occurrence of *Shi-Re* is usually based on water and wetness. The water and wetness can change into heat if they are stored in the body for long periods, and the combination of water and wetness and heat may cause *Shi-Re ZHENG*. *Shi-Re* is characterized by epigastric or abdominal oppression, lack of appetite, heavy body weight, thirst with little/no desire to

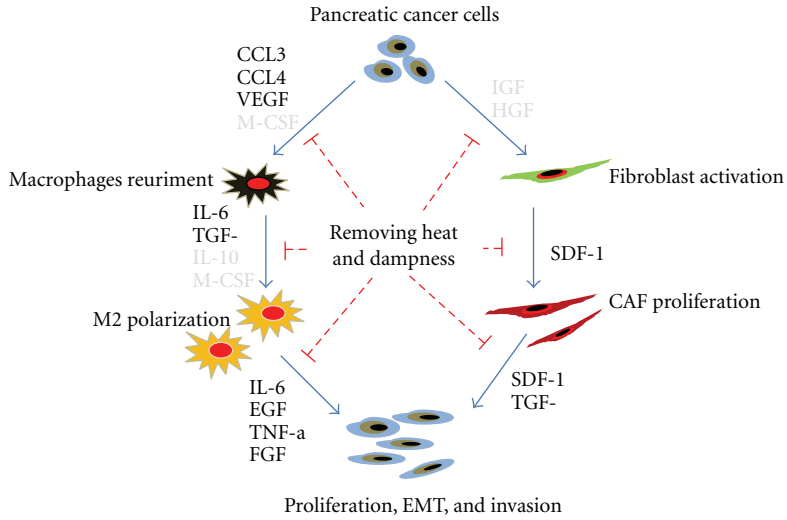


Figure 3: A schematic cartoon portraying the molecular basis of *Shi-Re ZHENG* in pancreatic cancer, based on our previous studies. It has been proposed that tumors with *Shi-Re ZHENG* exhibited altered cancer-associated myofibroblast (CAF) proliferative activities and tumor-associated macrophage (TAM) infiltration, which led to altered levels of CAF- and TAM-derived secreted cytokines (such as SDF-1, VEGF, TGF-1β, IL-6, CCL3, CCL4, CCL5, TNF-α, IL-8, and bFGF). The presence of *Shi-Re ZHENG* has impact on tumor growth. Chinese herbs for *Qing-Re-Hua-Shi* (removing heat and dampness) inhibited cancer cell proliferation, invasion, and *in vivo* metastasis through modification of the tumor microenvironment. Cytokines that are marked in bold have been confirmed by our previous studies.

drink, abdominal pain, loose stools, nausea, vomiting, fever, headache, red tongue body with a yellow sticky coat, and/or slippery rapid pulse.

Shi-Re ZHENG has been associated with many diseases, especially those involving the gastrointestinal (GI) tract. The potential molecular basis of *Shi-Re ZHENG* has attracted much research attention, although it is far from clear. Recently, *Shi-Re ZHENG* has been implicated in a broad range of inflammatory conditions, including eczema, psoriasis, cystitis, urethritis, gastroenteritis, vaginitis, cervicitis, meningitis, conjunctivitis, rheumatoid arthritis, and allergic reactions [21–25]. In addition, *Shi-Re ZHENG* was found to correlate with changes in expression of inflammation cytokines. For example, Liu and Wang showed that the serum levels of tumor necrosis factor-α (TNF-α) and interleukin-13 were significantly higher in rats with ulcerative colitis complicated with *Shi-Re ZHENG*, as compared with those without *Shi-Re* [26]. Likewise, Jiang et al. observed that in a total of 63 patients with chronic hepatitis B, 27 cases were diagnosed with *Shi-Re ZHENG*, and patients with *Shi-Re* had higher levels of TNF-α and tissue inhibitor of metalloproteinases (TIMP)-1 [27].

It has been reported that symptoms of *Shi-Re ZHENG* are commonly seen in patients with GI cancer, including cancers of the duodenum, colon, liver, pancreas, and gallbladder. Just as we have shown in Figure 2, *Shi-Re* has been reported as one of the most common *ZHENGs* in liver, colorectal, and pancreatic cancers. However, there is still not a clear understanding of the biological validity of *Shi-Re* and the possible mechanisms of *ZHENG* in cancer. Our research group has previously established a pancreatic cancer xenograft mouse model with *Shi-Re ZHENG* [28]. We found that *Shi-Re ZHENG* mice exhibited altered cancer-associated myofibroblast (CAF) proliferative activities and tumor-associated macrophage (TAM) infiltration, which led to altered levels of CAF- and TAM-derived secreted cytokines (such as, SDF-1, VEGF, TGF-1β, IL-6, CCL3, CCL4, CCL5, TNF-α, IL-8, and bFGF). The presence of *Shi-Re ZHENG* has also been shown to impact tumor growth. Chinese herbs for *Qing-Re-Hua-Shi* (removing heat and dampness) were found to inhibit cancer cell proliferation through modification of the components of the tumor microenvironment [28–30]. These findings suggested that *Shi-Re* is associated with altered tumor microenvironment (Figure 3).

5.3. Yin-Xu ZHENG (Yin Deficiency). *Yin-Xu* represents insufficiency of body fluid. It is characterized by dryness in the throat and/or mouth, perspiration during sleep, tinnitus, dizziness, fatigue, insomnia, red tongue body with no coating on, and pulse that is thin, fine, or floating and empty. *Yin-Xu* may occur in many organs, including the stomach, lung, liver, kidney, or heart. Symptoms of *Yin-Xu ZHENG* are commonly seen in cancers of the liver, lung, breast, stomach, and colon (Figure 2). However, only a few publications in the literature have studied the molecular basis of *Yin-Xu*. Shen et al. observed that in patiens with lung cancer, *Yin-Xu* was correlated with changes in the cytokine expression profile [31–34]. They also showed that lung cancer tissue with *Yin-Xu ZHENG* exhibited dysregulated expression of TNF-α, IL-lα, IFN, IL-2, IL-8, and IL-1Rα, as compared with that without *Yin-Xu ZHENG*. Thus, the molecular basis of *Yin-Xu ZHENG* is believed to involve components of the inflammatory cytokines network.

5.4. Pi-Xu (Spleen Deficiency). For this *ZHENG*, the word "spleen" does not refer to the organ, as in western medicine. It is a term used to describe an entire group of physiological functions. Based on the so-called *Pi-Wei theory* (also called *spleen-stomach theory)*, the *Pi* ("*spleen*") governs molecular transport and transformation since the *Pi* transforms food into nutrients, which are the sources of *Qi* and blood, and distributes the nutrients to the limbs and other organs. Hence, the theory of "*Pi* being acquired foundation" has emerged. This theory postulates that when there is *Pi-Xu*, the digestion process is perturbed, causing abdominal discomfort and making the person feel tired. Since the *Pi* would normally keep the body fluids flowing in their respective pathways, signs of *Pi-Xu ZHENG* are hemorrhage, swelling, and bruising.

Pi-Xu has been shown to be involved with dysfunction of the vegetative nervous system of the GI tract, immune pathways, and endocrine processes. It can also mediate the distribution and content of fecal bacteria flora and gut-associated microbiota, including ulcer- and inflammation-causing *Helicobacter pylori*, as well as trace elements involved in blood and muscle metabolism [35]. Patients with different cancer types, in addition to the GI type, may present with *Pi-Xu* at various stages of the disease. Because many if not all cancers share at least some pathophysiological features, it is possible that they may be treated by an intervention approach based on a single principle but with flexibility to allow emphasis on different aspects of the disease in different patients.

Extensive research has been carried out to determine the molecular basis of *Pi-Xu* in cancer. Since the 1960s, a group led by Yu Erxin has performed a series of investigations in liver cancer patients to investigate the potential molecular components of *Pi-Xu* [36, 37]. These efforts have identified a correlation between *Pi-Xu* and immunological dysfunction [38]. Liver cancer xenograft mice with *Pi-Xu* were shown to have significantly less total T cells and T helper (Th) cell lymphocytes, but more inhibitory T cells, than their counterparts without *Pi-Xu*. Furthermore, when these *Pi-Xu* mice were treated with Dangshen (*Pilose asiabell root*) and Huangqi (*Pilose asiabell root*) combination therapy, the level of Th cell-expressed CD4 was elevated significantly. Thus, it is believed that *Pi*-fortifying prescriptions may enhance proliferation of splenic cells and significantly increase auto-antibody secretory cell number, thereby enhancing the cytotoxic action of lymphocytes. Indeed, it has been shown that administration of *Pi*-fortifying therapy to ConA-stimulated mice promotes splenic cells to secrete cytokines, such as IL-2 [38]. Likewise, clinical observation in patents with liver cancer showed that patients with *Pi-Xu* were treated with *Pi*-fortifying therapy the activities of both natural killer cells and lymphokine-activated killer cells were restored [39].

Pi-Xu has been correlated with the abnormal energy metabolism that occurs in tumor cells. Observational study from 40 cased with liver cancer showed that liver cancer patients with *Pi-Xu* exhibited decreased serum levels of cyclic adenosine monophosphate (cAMP), while those patients with *Shi-Re* or *Xue-Yu* showed no significant changes in cAMP level [40]. Liver cancer xenograft mice with *Pi-Xu* also showed decreased serum and splenic cAMP levels, and increased cGMP and cAMP/cGMP ratio; intriguingly, the condition was not improved or resolved by treatment with the *Pi*-fortifying prescriptions [41]. These findings were also observed in patients with gastric cancer [42]. In addition to its effects on immune-related mechanisms and energic metabolism, the *Pi*-fortifying prescriptions was also shown to mediate the patterns of trace elements [43, 44]. Patients with various chronic diseases and *Pi-Xu* present with altered expression and distribution patterns of trace elements, including Cu, Zn, and Fe [45]. In gastric cancer patients with *Pi-Xu*, the levels of Cu and Zn are significantly changed, in particular [46]. Therefore, *Pi-Xu* is a now considered as a multisystem functional impairment.

6. Prospects and Challenges

In TCM, the medicines are prescribed according to *ZHENG*, and *ZHENG* remains the essence of TCM treatment. However, there are some important issues that deserve mentioning. First, as TCM *ZHENG* differentiation is usually based upon the treating physician's intuition and personal experience, results differ from physician to physician and from clinic to clinic. Thus, *ZHENG* differentiation has a low reproducibility. To date, no unified criteria have been published for *ZHENG* differentiation, and it remains one of the main obstacles to widespread application of TCM in the clinical and research settings. Second, in this summary analysis, we emphasized the important position of *ZHENG* since it helps to guide the design of an individual's treatment regimen. We believe that the results of this study may help provide a theoretical basis for clinical diagnosis and treatment. However, we also recognize that when used as the sole treatment for cancer, TCM *ZHENG* does not consistently produce satisfactory therapeutic efficacy. Recently, there has been much interest in the potential clinical utility of "analogous *ZHENG* existing in the same disease" for improving TCM in clinical practice [47], especially for cancer patients. Thus, a strategy combining *ZHENG* differentiation and disease diagnosis is considered promising for future cancer treatment.

While much research has attempted to elucidate the molecular basis of the cancer-associated *ZHENGs*, the available data are subject to several limitations that must be considered when contemplating the utility of TCM *ZHENG* as a cancer therapy. First, TCM is focused on alleviating a particular disease or condition, while the *ZHENG* is based on systemic and holistic concepts. Therefore, a system's biology approach may be the optimal way to research the clinical utility and therapeutic efficacy of TCM *ZHENG*. Second, TCM is practiced with respect to the rules of "treating the same disease with different methods" and "treating different diseases with the same methods". In our summary analysis, we found the same molecular basis underlying the same *ZHENG* in different diseases. However, we should also emphasize that molecular differences that are disease- or diagnosis-specific, while sharing a *ZHENG*, may prove particularly important in designing effective individualized treatment regimens. This

notion is consistent with the current understanding that combination of *ZHENG* differentiation and disease diagnosis yields improved treatment efficacy. Third, we point out that a comprehensive profile of *ZHENG*-specific molecules has yet to be identified, and the correlation between *ZHENG* and molecules has yet to be firmly established. Finally, it is important to remember that *ZHENG* is now considered as a multisystem and multiorgan functional impairment. Although modern technologies have been applied to *ZHENG* research, we are far from obtaining a clear understanding of the exact molecular basis of *ZHENG*. We are hopeful that future integration of modern technologies and continued research may eventually promote *ZHENG* research.

7. Conclusions

In this study, we systematically identified the collected body of research on TCM *ZHENG* in cancer patients. The sources of these data were the publically available Chinese language scientific and medical literature databases. We first summarized the clinical *ZHENG* distribution among six common cancer types, including lung, liver, gastric, breast, colorectal, and pancreatic, which may help to provide a theoretical basis for TCM as a clinical cancer treatment. We then considered the molecular basis of *Xue-Yu*, *Shi-Re*, *Yin-Xu*, and *Pi-Xu ZHENGs* that are commonly present in different types of cancer, which may contribute to a better understanding of the potential of TCM *ZHENG* for supplementing modern therapeutic strategies for cancer.

Acknowledgments

This study was supported by National Science Foundation of China (81001061); Shanghai Nature Science Fund, Shanghai, China (09ZR1406800); Doctoral Programs Foundation of Ministry of Education of China (20090071120076); Shanghai Science and Technology Committee Rising-Star Program (11QA1401300); Medical Talents Training Program of Health Bureau of Shanghai (XYQ2011008); Shanghai Municipal Health Bureau Grant (KY148).

References

[1] Z. Chen, K. Gu, Y. Zheng, W. Zheng, W. Lu, and X. O. Shu, "The use of complementary and alternative medicine among Chinese women with breast cancer," *Journal of Alternative and Complementary Medicine*, vol. 14, no. 8, pp. 1049–1055, 2008.

[2] K. H. Chang, R. Brodie, M. A. Choong, K. J. Sweeney, and M. J. Kerin, "Complementary and alternative medicine use in oncology: a questionnaire survey of patients and health care professionals," *BMC Cancer*, vol. 11, article 196, 2011.

[3] M. McCulloch, C. See, X. J. Shu et al., "Astragalus-based Chinese herbs and platinum-based chemotherapy for advanced non-small-cell lung cancer: meta-analysis of randomized trials," *Journal of Clinical Oncology*, vol. 24, no. 3, pp. 419–430, 2006.

[4] C. X. Pan, R. S. Morrison, J. Ness, A. Fugh-Berman, and R. M. Leipzig, "Complementary and alternative medicine in the management of pain, dyspnea, and nausea and vomiting near the end of life: a systematic review," *Journal of Pain and Symptom Management*, vol. 20, no. 5, pp. 374–387, 2000.

[5] T. Efferth, P. C. Li, V. S. Konkimalla, and B. Kaina, "From traditional Chinese medicine to rational cancer therapy," *Trends in Molecular Medicine*, vol. 13, no. 8, pp. 353–361, 2007.

[6] J. Liu, X. Li, L. Ma, and V. Fonnebo, "Traditional Chinese medicine in cancer care: a review of case reports published in Chinese literature," *Forsch Komplementmed*, vol. 18, no. 5, pp. 257–263, 2011.

[7] C. F. Zhang, "Exploring of blood viscosity in cancer patients," *China Joumal of ModelTI Medicine*, vol. 14, no. 7, pp. 73–75, 2004.

[8] Y. H. Liu, Y. W. Zhang, G. G. Liang, and K. Q. Yang, "Experimental and clinical study on tumor metastasis and microscopic blood stasis," *Xian Dai Zhong Yi*, vol. 4, no. 33, pp. 224–228, 1996.

[9] Y. F. Chen and S.H. Zhang, "Promoting blood circulation and Removing blood stasis in cancer treatment," *Journal of Shanxi College of Traditional Chinese Medicine*, vol. 29, no. 2, pp. 62–63, 2006.

[10] Y. F. Wu, "Correlation factors analysis on hepatic blood stagnation type of primary liver cancer," *Shanxi Journal of Traditional Chinese Medicine*, vol. 23, no. 1, pp. 63–65, 2007.

[11] W. H. Yang, L. M. Liu, H. S. Zhao, and L. C. Wu, "Micro-circulation analysis in liver cancer with blood stasis," *Chinese Journal of Integrated Traditional and Western Medicine on Liver Diseases*, vol. 9, no. 6, pp. 10–11, 1999.

[12] X. Z. Chen, H. Q. Tian, X. Q. Huang, G. W. Liang, and Z. Q. Huang, "Effect of Ganji formula on blood stasis in patients with advanced liver cancer," *Shan Xi Zhong Yi*, vol. 26, no. 9, pp. 891–893, 2005.

[13] S. A. Mousa, "Anticoagulants in thrombosis and cancer: the missing link," *Expert Review of Anticancer Therapy*, vol. 2, no. 2, pp. 227–233, 2002.

[14] S. Ahmad and A. A. Ansari, "Therapeutic roles of heparin anticoagulants in cancer and related disorders," *Medicinal Chemistry*, vol. 7, no. 5, pp. 504–517, 2011.

[15] J. Q. Han, Y. T. Chen, and Y. Y. Man, "Clinical study on effect of combined treatment of fuchunpian with radiotherapy on nasopharyngeal carcinoma," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 15, no. 12, pp. 710–712, 1995.

[16] X. T. Li, Y. Q. Wang, and N. W. Fu, "Effects of herbs for promoting blood circulation and removing blood stasis on lung metastasis in liver cancer xenografted mice," *Journal of Traditional Chinese Medicine*, vol. 21, no. 8, p. 75, 1980.

[17] Z. Chen, L. M. Liu, and Y. B. He, "Correlation between TXB2, 6-Keto-PGF1alpha and liver metastasis in rats model with blood stasis," *Zhong Xi Yi Jie He Xue Bao*, vol. 1, no. 3, pp. 199–201, 2003.

[18] Z. Chen, L. M. Liu, and Y. B. He, "Effects of blood stasis on W256 tumor growth and liver metastasis," *Chinese Archives of Traditonal Chinese Medicine*, vol. 21, no. 6, pp. 866–872, 2003.

[19] P. F. Chen, L. M. Liu, Z. Chen, Y. F. Xu, and C. S. Xie, "Study on blood stasis of liver metastasis of cancer and the intervention of shen sanqi and danshen," *Journal of Zhejiang Chinese Medical University*, vol. 31, no. 5, pp. 552–553, 2007.

[20] P. F. Chen, L. M. Liu, Z. Chen, S. Y. Lin, W. X. Song, and Y. F. Xu, "Effects of ethanol extracts of Panax notoginseng on liver metastasis of B16 melanoma grafted in mice," *Zhong Xi Yi Jie He Xue Bao*, vol. 4, no. 5, pp. 500–503, 2006.

[21] X. B. Zheng, H. B. Liu, Y. L. Feng, and S. X. Dai, "Huangqin decoction lowers the number of mast cells in ulcerative colitis in rats with dampness and heat syndrome," *Nan Fang Yi Ke Da Xue Xue Bao*, vol. 31, no. 2, pp. 252–255, 2011.

[22] J. L. Yuan, H. Zhang, L. Wang et al., "Biochemical characteristics of traditional Chinese medicine syndromes and their elements in patients with hepatitis B cirrhosis," *Zhong Xi Yi Jie He Xue Bao*, vol. 9, no. 4, pp. 374–381, 2011.

[23] X. B. Zheng, Y. L. Feng, H. B. Liu, and S. X. Dai, "Effect of huangqin decoction on CD4+T Cell and its co-stimulator factors for unlcerative colitis rat with damp-and-heat syndrome," *Chinese Journal of Experimental Traditional Medical Formulae*, vol. 17, no. 1, pp. 169–172, 2011.

[24] N. J. Cui, L. Hu, and S. X. Lao, "Relationship between Pi-Wei damp-heat syndrome with expressions of nuclear factor-kappaBmRNA and heat shock protein 70 mRNA in patients with chronic gastritis," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 30, no. 1, pp. 18–21, 2010.

[25] X. H. Li and G. Q. Xie, "The correlation study between damp-heat syndrome and pro-inflammatory cytokines in primary nephrotic syndrome," *Liaoning Journal of Traditional Chinese Medicine*, vol. 38, no. 6, pp. 1096–1098, 2011.

[26] W. Liu and Y. Z. Wang, "Effects of Reyusan on serum levels of tumor necrosis factor-a and Interleukin-13 in rats with ulcerative colitis complicated with damp-heat syndrome of stomach and intestine," *Journal of Anhui TCM college*, vol. 29, no. 6, pp. 56–58, 2010.

[27] K. P. Jiang, Y. Tao, H. T. Hu et al., "A clinical study on the serum levels of TGF-b1 and TIMP-1 in patients with chronic B hepatitis in TCM types damp-heat," *Chinese Journal of Integrated Traditional and Western Medicine on Liver Diseases*, vol. 17, no. 5, pp. 264–265, 2007.

[28] H. Y. Dai, P. Wang, L. Y. Feng et al., "The molecular mechanisms of traditional Chinese medicine *ZHENG* syndromes on pancreatic tumor growth," *Integrative Cancer Therapies*, vol. 9, no. 3, pp. 291–297, 2010.

[29] H. Q. Ouyang, L. M. Liu, Z. Chen, J. M. Luo, and E. X. Yu, "Effects of Qingyi Huaji decoction on serum levels of interleukin-6, interleukin-8 and tumor necrosis factor-α in nude mice bearing pancreatic tumors," *Zhong Xi Yi Jie He Xue Bao*, vol. 8, no. 7, pp. 655–661, 2010.

[30] J. H. Yin, W. D. Shi, X. Y. Zhu, Z. Chen, and L. M. Liu, "Qingyihuaji formula inhibits progress of liver metastases from advanced pancreatic cancer xenograft by targeting to decrease expression of Cyr61 and VEGF," *Integrative Cancer Therapies*, vol. 11, no. 1, pp. 37–47, 2012.

[31] W. X. Shen, Y. Sun, and S. R. Zhang, "Study on relation between IL-1a, Il-1b, IL-1Ra gene mRNA expression and Yin-deficiency syndrome in lung cancer," *Bulletin of Medical Research*, vol. 29, no. 8, pp. 5–7, 2000.

[32] H. Zheng, W. X. Shen, X. Y. Liu et al., "An experimental study of the gene expression profile of cytokines in Yin deficiency syndrome of different diseases," *Journal of Basic and Clinical Oncology*, vol. 19, no. 6, pp. 448–450, 2006.

[33] X. Y. Liu, W. X. Shen, Y. M. Liu et al., "Inflammatory cytokines profile in lung cancer with Yin-deficiency syndrome," *Yi Xue Yan Jiu Za Zhi*, vol. 35, no. 3, pp. 75–76, 2006.

[34] W. X. Shen, Y. Sun, and S. R. Zhang, "Correlation between IL-8, Il-10 and Yin-deficiency syndrome," *Bulletin of Medical Research*, vol. 32, no. 5, pp. 62–64, 2003.

[35] X. N. Wu, "Current concept of Spleen-Stomach theory and Spleen deficiency syndrome in TCM," *World Journal of Gastroenterology*, vol. 4, no. 1, pp. 2–6, 1998.

[36] E. X. Yu and L. N. Lv, "clinical and molecular mechanism of Spleen-nourishing and Qi-regulating therapy for liver cancer," *Journal of Traditional Chinese Medicine*, vol. 43, no. 7, pp. 28–30, 1987.

[37] P. Wang, W. X. Huang, and L. M. Liu, "Overview of clinical and experimental study on Spleen-nourishing and Qi-regulating therapy for liver cancer," *Shanghai Journal of Traditional Chinese Medicine*, vol. 39, no. 5, pp. 60–62, 2005.

[38] E. X. Yu, L. N. Lv, and Q. D. Wang, "Effects of Spleen-nourish ing and Qi-regulating herbs on immunofunction of liver tumor xenografted mice with Spleen-deficiency," *Tumor*, vol. 3, no. 4, pp. 168–170, 1983.

[39] H. Y. Huang and E. X. Yu, "Effect of Spleen-nourish ing herbs on NK and LAK cells and IL-2 activities," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 13, no. 4, pp. 253–255, 1993.

[40] M. Z. Song and E. X. Yu, "Serum cAMP levels in liver cancer with different TCM syndromes," *Shanghai Journal of Traditional Chinese Medicine*, vol. 23, no. 4, pp. 40–43, 1981.

[41] S. L. Zhang, G. S. Yin, G. X. Wang, and E. Li, "Effects of Spleen-nourishing decoction on plasma and spleenic cAMP levels in rats," *Zhong Xi Yi Jie He Za Zhi*, vol. 10, no. 11, pp. 672–674, 1990.

[42] G. Y. Yin, "A study of the relation of intestinal metaplasia, gastric carcinoma and the Spleen deficiency syndrome using histochemical staining of the gastric mucosa and determination of cAMP and cGMP," *Zhong Xi Yi Jie He Za Zhi*, vol. 6, no. 7, pp. 395–397, 1986.

[43] J. Wang, M. L. Zhang, and X. F. Qiu, "The efect of Spleen-reinforcing therapy on the transporting and distribution Of ^{59}Fe metablism of Spleen-deficiency-sufering mice in the organs," *Journal of Hubei College of TCM*, vol. 9, no. 3, pp. 20–22, 2007.

[44] J. W. Ma, G. Hao, and J. Li, "Serum level of Zn, Cu and Mg in patients with Spleen deficiency," *Zhejiang Journal of Traditional Chinese Medicine*, vol. 25, no. 10, p. 471, 1990.

[45] G. M. Dong, "Changes of trace elements in gastric juice in peptic ulcer patients with Spleen deficiency syndrome," *Shi Yong Zhong Xi Yi Jie He Za Zhi*, vol. 8, no. 2, pp. 68–69, 1995.

[46] G. Z. Yin and X. Z. Xu, "Correlation between gastric mucosa Zn, cAMP, DNA and 3H-TdRLCT in gastric carcinoma with Spleen deficiency," *Jiang Su Yi Yao*, vol. 16, no. 8, pp. 408–412, 1989.

[47] W. J. Wang, "Pay more attention to 'Analogous syndrome complex in the same disease and treating the same disease with analogous methods '," *Zhong Xi Yi Jie He Xue Bao*, vol. 6, no. 5, pp. 441–445, 2008.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 739642, 7 pages
doi:10.1155/2012/739642

Research Article

The Quintessence of Traditional Chinese Medicine: Syndrome and Its Distribution among Advanced Cancer Patients with Constipation

Chung-Wah Cheng,[1] Annie O. L. Kwok,[2] Zhao-Xiang Bian,[3] and Doris M. W. Tse[4]

[1] *Yan Chai Hospital, Hong Kong Baptist University Clinical Centre for Training and Research in Chinese Medicine (West Kowloon), Kowloon, Hong Kong*
[2] *Department of Medicine & Geriatrics, Caritas Medical Centre, Kowloon, Hong Kong*
[3] *Clinical Division, School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong*
[4] *Department of Medicine & Geriatrics, Intensive Care Unit (ICU), Caritas Medical Centre, Kowloon, Hong Kong*

Correspondence should be addressed to Zhao-Xiang Bian, bzxiang@hkbu.edu.hk and Doris M. W. Tse, mwtse@ha.org.hk

Received 11 January 2012; Accepted 6 April 2012

Academic Editor: Shi-Bing Su

Constipation is a common problem in advanced cancer patients; however, specific clinical guidelines on traditional Chinese medicine (TCM) syndrome (*Zhang*) are not yet available. In this cross-sectional study, the TCM syndromes distribution and their common symptoms and signs among 225 constipated advanced cancer patients were determined. Results showed that 127 patients (56.4%) and 7 patients (3.1%) were in deficient and excessive patterns, respectively, while 91 patients (40.4%) were in deficiency-excess complex. The distributions of the five syndromes were: *Qi* deficiency (93.3%), *Qi* stagnation (40.0%), blood (*Yin*) deficiency (28.9%), Yang deficiency (22.2%), and excess heat (5.8%). Furthermore, age, functional status, and level of blood haemoglobin were factors related to the type of TCM syndrome. A TCM prescription with the functions on replenishing the Deficiency, redirecting the flow of *Qi* stagnation and moistening the dryness caused by the blood (*Yin*) deficiency can be made for the treatment of advance cancer patients with constipation. Robust trials are urgently needed for further justifying its efficacy and safety in evidence-based approaches.

1. Introduction

Traditional Chinese medicine (TCM) has been in use for curing diseases and promoting the health of human for thousands of years in China. Its theory is derived from Chinese ancient philosophy and completed as the result of long-term clinical practices. Nowadays, TCM becomes the main component of complementary and alternative medicine (CAM) and gains increasing attention and popularity in the world [1]. Syndrome (*Zheng*), also called pattern, is the key concept in TCM theory [2]. It is a summary of the cause, nature, and location of the pathological change at a certain stage of disease [3] and is the foundation for making diagnosis and prescription. Chinese Medicine practitioners make the differentiation on the basis of all symptoms and signs collected by the four classic diagnostic methods, that is, observation, inquiry, smelling/listening, and palpation. Patients with same disease can present in different syndromes. Oppositely, patients with different diseases can present in same syndrome [4]. Due to the complexity and diversity of syndrome, TCM experts have attempted to quantize the description and standardize the terminology of syndrome since 1980s [5, 6]. Certain practice guidelines for difference syndromes of a specific disease are established as a reference for making diagnosis and prescribing treatment.

Constipation is a common problem in advanced cancer patients, which affects an estimated 32% to 87% of patients [7–10] and is only superseded by pain and anorexia [7]. Constipation is also a distressing symptom, and if untreated, can give rise to nausea, vomiting, abdominal distension, urinary

retention, anal fissures, and even bowel obstruction [11]. However, the "best" treatment for constipation on both efficacy and safety remains an unresolved issue [12, 13]. According to the TCM theory, constipation can be broadly divided into excessive and deficient patterns based on the underlying aetiology. The excessive pattern is characterized by excess heat or pathological accumulation of *Qi* (stagnation), while the deficient pattern is characterized by the dryness from insufficient fluid lubrication in the form of blood (*Yin*) or lack of propulsion power from the deficiency of *Qi* or *Yang* [4, 14, 15]. Five common TCM syndromes can be summarized from the TCM references of Internal Medicine of Chinese Medicine [4], Criteria of Diagnosis and Therapeutic Effect of Diseases and Syndromes in Traditional Chinese Medicine [5], and Clinical Handbook of Internal Medicine [14, 15]. They are the intestine with excess heat, intestinal *Qi* stagnation, spleen deficiency with weakness of *Qi*, spleen-kidney *Yang* deficiency, and *Yin* deficiency with intestinal dryness. Each of them has its unique treatment principle and prescription. However, none of these references is specified on advanced cancer patients. The constitutions of these patients are different from general patients only with constipation; therefore, an epidemiological study on syndrome distribution is important for the establishment of practice guidelines in palliative cancer care.

In this present study, the TCM syndromes distribution and their common symptoms and signs among constipated advanced cancer patients are first determined. Besides, the impacts of patient demographics and opioids prescribed on TCM syndromes are also investigated. The results are important for tailor-made Chinese herbal formulation for the management of constipation for advanced cancer patients, and launching large-scale clinical study in future. In parallel, the bowel habits and the use of relieving measures are examined and reported in a separated article [16].

2. Materials and Methods

This study was a cross-sectional survey carried out in the palliative care units of Caritas Medical Centre and Our Lady of Maryknoll Hospital, which ran a comprehensive range of specialist palliative care services including inpatient, outpatient, home care, and day care services. Participants were interviewed by a registered Chinese medicine practitioner who possessed a degree in Chinese Medicine and with at least three-year working experience on clinical practice and TCM-related scientific researches. The whole study was conducted in accordance with the Declaration of Helsinki. Ethics approval was obtained from the Kowloon West Cluster Research Ethics Committee, Hong Kong Hospital Authority, and the study protocol was registered at ClinicalTrials.gov (NCT01399294).

2.1. Patients. All advanced cancer patients (aged 18 or above) under the care of palliative care unit of Caritas Medical Centre and Our Lady of Maryknoll Hospital who had constipation during the period from May 1, 2010 to July 31, 2010 were invited to participate in the study. Patients were recruited only from those who reported (1) on medications (laxatives, enemas, Chinese herbal medicine (CHM), and/or health supplements) for facilitating bowel movement; (2) constipation as two points or more by the constipation visual analogue scale (CVAS) (0: none to 7: most severe) [17]. Patients who were unable to communicate, cognitive impaired, put on colostomy bag, clinically diagnosed gastrointestinal obstruction, or at end-of-life (EOL) were excluded. Written or verbal informed consents were obtained before starting the interview, and all subjects were free to withdraw at any time from the study.

2.2. Questionnaire. The questionnaire was designed by the research team with Traditional Chinese Medicine professionals and Palliative Medicine specialists, comprising of three parts written in Chinese (See Appendix 1 available online at doi:10.1155/2012/739642). The first part consisted of patient demographics. Patient's age, gender, primary cancer, functional status as measured by Palliative Performance Scale (PPS), biochemical parameters from blood tests, and prescription of opioid were recorded by the Palliative Medicine specialists. The second part was about patients' perception of bowel function. The bowel habit, such as frequency, stool type, rectal measure, and laxatives/enemas required, was enquired. Besides, the severity of constipation was evaluated by the constipation visual analogue scale (CVAS). It was an 8-point ordinal rating scale, where 0-1 indicated no constipation, 2–4 indicated constipation, and 5–7 indicated severe constipation [18]. Specific analysis and interpretations about the correlation between the patient demographics and their bowel habits were reported with details in a separate paper [16].

The third part consisted of the TCM syndrome patterns as diagnosed by a registered Chinese Medicine practitioner. Five syndromes, that is, excess heat, *Qi* stagnation, *Qi* deficiency, *Yang* deficiency, and *Yin* deficiency, were simplified from the TCM references [4, 5, 14, 15]. The former two were in excessive pattern, while the latter three were in deficient pattern. Typical symptoms and signs of each syndrome were listed in a designated table. The Chinese Medicine practitioner collected data with the four classic diagnostic methods, completed the table, and diagnosed the syndrome of patient instantaneously.

2.3. Statistical Analyses. The data were entered into the Statistical Package for Social Sciences programme (SPSS 13.0), while the completed questionnaires were kept in a locked cabinet inside the clinic. Continuous variables were calculated using analysis of variance (ANOVA), and the chi-square test was used for analysing categorical data. All statistical tests were two-sided, and a P value of <0.05 was considered significant.

3. Results

A total of 228 advanced cancer patients were recruited. Three patients were excluded: two refused to participate and one could not complete questionnaire during interview. Thirty-nine participants, who could not write, only gave their verbal informed consent. For the 225 cases further analyzed, 127

Table 1: Patient demographic data.

	Deficiency ($n = 127$)	Excess ($n = 7$)	Complex ($n = 91$)	P value
Gender ratio male: female	1 : 0.84	1 : 0.75	1 : 0.98	0.837
Age in years (mean ± SD)	75.82 ± 11.21	67.14 ± 9.17	72.57 ± 12.85	0.039
PPS (0–100) (mean ± SD)	55.69 ± 16.18	71.67 ± 17.22	60.23 ± 15.49	0.016
Primary cancer	Number of patients (% within group)			
Lung	38 (29.9%)	3 (42.9%)	24 (26.4%)	
Colorectal	11 (8.7%)	1 (14.3%)	16 (17.6%)	
Hepatobiliary	16 (12.6%)	1 (14.3%)	9 (9.9%)	
Prostate	9 (7.1%)	0 (0%)	6 (6.6%)	
Stomach	11 (8.7%)	0 (0%)	3 (3.3%)	
Breast	7 (5.5%)	0 (0%)	5 (5.5%)	
Gynaecological	7 (5.5%)	0 (0%)	3 (3.3%)	
Pancreas	3 (2.4%)	0 (0%)	6 (6.6%)	
Urinary system	4 (3.1%)	1 (14.3%)	4 (4.4%)	0.898
Nasopharyngeal	3 (2.4%)	0 (0%)	1 (1.1%)	
Thyroid	3 (2.4%)	0 (0%)	1 (1.1%)	
Haematological	3 (2.4%)	0 (0%)	1 (1.1%)	
Oesophagus	2 (1.6%)	0 (0%)	1 (1.1%)	
Brain	2 (1.6%)	0 (0%)	1 (1.1%)	
Head and neck	2 (1.6%)	0 (0%)	0 (0%)	
Others	3 (2.4%)	0 (0%)	4 (4.4%)	
Unknown/missing data	3 (2.4%)	1 (14.3%)	6 (6.6%)	
	Biochemical parameters (mean ± SD)			
Haemoglobin mg/dL	10.20 ± 2.02	11.63 ± 1.33	11.00 ± 1.94	0.008
Urea mmol/L	5.90 ± 3.49	5.15 ± 3.54	7.01 ± 5.02	0.140
Creatinine μmol/L	90.71 ± 47.19	70.50 ± 58.06	97.10 ± 62.50	0.425
Alkaline phosphatase IU/L	200.59 ± 256.56	187.67 ± 151.49	234.82 ± 275.38	0.711
Alanine aminotransferase U/L	36.08 ± 69.48	40.50 ± 15.98	88.64 ± 449.30	0.445
Serum calcium mmol/L	2.26 ± 0.27	2.15 ± 0.25	2.22 ± 0.17	0.243
Serum albumin mg/L	26.74 ± 6.27	23.00	27.44 ± 6.46	0.712

Deficiency: deficient pattern; excess: excessive pattern; complex: deficiency-excess complex.
PPS: palliative performance scale.
Biochemical parameters were determined for those patients with blood tests within three months.

patients (56.4%) and 7 patients (3.1%) were in deficient and excessive patterns, respectively, while 91 patients (40.4%) were in deficiency-excess complex. Deficiency-excess complex was a pathological state in which both deficiency and excess syndromes existed in the disease process [3]. The distributions of the five syndromes were: *Qi* deficiency (93.3%), *Qi* stagnation (40.0%), blood (*Yin*) deficiency (28.9%), *Yang* deficiency (22.2%), and excess heat (5.8%).

3.1. Demographic Data. Among the total of 225 patients, there were 119 males and 106 females. The three most common primary cancers were lung, colorectal, and hepatobiliary, accounting for 52.9% of the total. Patients in deficient pattern had the highest mean age at 75.82 years (SD = 11.21) and lowest PPS score at 55.69 years (SD = 16.18), while those in excessive pattern had the lowest mean age at 67.14 years (SD = 9.17) and highest PPS score at 71.67 (SD = 17.22), with P value < 0.05. However, there were no significant differences in gender and type of primary cancer. For the biochemical parameters, the level of blood haemoglobin, but not for urea, creatinine, alkaline phosphatase, alanine aminotransferase, calcium, and albumin, had significant differences among three groups. Patients in excessive pattern had the highest level of blood haemoglobin at 11.63 mg/dL (SD = 1.33), while those in deficient pattern had the lowest level at 10.20 mg/dL (SD = 2.02), with P value = 0.008 (Table 1).

3.2. Manifestations and Distributions for Patients in the Five Syndromes. The syndromes of excess heat, *Qi* stagnation, *Qi* deficiency, blood (*Yin*) deficiency and *Yang* deficiency were differentiated by the Chinese Medicine practitioner, and coexistence was allowed for patients with symptoms and signs complicated from more than one syndrome. The dominant manifestations of patients in each syndrome, with prevalence ≥50%, were listed in Table 2. Dry mouth, fatigue, and fine pulse were the common manifestations for patients with these five syndromes. Besides, the pattern of *Qi* deficiency was the fundamental syndrome among advanced

Table 2: Dominant symptoms and signs of the five syndromes.

Excessive pattern:
Excess heat: 13 patients (5.8%)
Dry mouth 12/13 (92.3%), fatigue 10/13 (76.9%), phlegm production 7/13 (53.8%), slimy fur 8/13 (61.5%), white fur 8/13 (61.5%), fine pulse 7/13 (53.8%), and string-like pulse 9/13 (69.2%)
Qi Stagnation: 90 patients (40.0%)
Dry mouth 69/90 (76.7%), fatigue 81/90 (90.0%), mind disquieted/susceptible to fright 49/90 (54.4%), belching/nausea/vomiting 61/90 (67.8%), abdominal distension/pain 58/90 (64.4%), anorexia 46/90 (51.1%), inadequate pushing force 56/90 (62.2%), pale red tongue 51/90 (56.7%), white fur 60/90 (66.7%), fine pulse 64/90 (71.1%), and string-like pulse 47/90 (52.2%)
Deficient patterns:
Qi deficiency: 210 patients (93.3%)
Dry mouth 148/210 (70.5%), fatigue 200/210 (95.2%), mind disquieted/susceptible to fright 114/210 (54.3%), inadequate pushing force 110/210 (52.4%), pale red tongue 116/210 (55.2%), white fur 139/210 (66.2%), and fine pulse 147/210 (70.0%)
Blood (*Yin*) deficiency: 65 patients (28.9%)
Dry mouth 57/65 (87.7%), fatigue 62/65 (95.4%), mind disquieted/susceptible to fright 45/65 (69.2%), anorexia 43/65 (66.2%), inadequate pushing force 38/65 (58.5%), red tongue 51/65 (78.5%), scanty fur/peeling fur/peeled fur 48/65(73.8%), and fine pulse 45/65 (69.2%)
Yang deficiency: 50 patients (22.2%)
Dry mouth 39/50 (78.0%), fatigue 48/50 (96%), mind disquieted/susceptible to fright 32/50 (64.0%), cold intolerance 41/50 (82.0%), pale red tongue 25/50 (50%), white fur 29/50 (58.0%), and fine pulse 33/50 (66.0%)

Dominant symptoms and signs were defined as prevalent for more than or equal to 50% of each syndrome.

cancer patients with constipation, with prevalence of 93.3%. For further analyzing its combination with other syndromes, one-third were in pure *Qi* deficiency, another one-third were coexistence with *Qi* stagnation or blood (*Yin*) deficiency, and the rest were in different combinations between the five syndromes (Table 3).

3.3. Manifestations for Patients in the Three Patterns. The prevalence of symptoms and signs for patients in deficient pattern, excessive pattern, and deficiency-excess complex was determined. The manifestations of pale/sallow complexion, fatigue, mind disquieted/susceptible to fright and anorexia among patients were significantly higher in deficient pattern than that in excess, with P value < 0.05. On the contrary, bitter taste, belching/nausea/vomiting, stuffiness and fullness of chest, abdominal distension/pain, water intention, insomnia, and vacuous pulse were more common in excessive pattern than that in deficiency, with P value < 0.05. For the description of constipation symptoms, patients in deficient pattern reported significantly higher prevalence of inadequate pushing force (42.5%), sense of incomplete defecation (15.7%), and difficulty in defecation (24.4%) when comparing with the group in excess (Table 4).

3.4. Patients' Severity of Constipation and Prescription of Opioid. The severity of constipation was significantly different among the three groups of patients with the most severe in the group of deficiency-excess complex (3.91 ± 1.57 points) and least in the deficient pattern (3.19 ± 1.85 points) ($P = 0.012$). About 50% patients in deficient pattern and deficiency-excess complex were in constipation, while patients in excessive pattern showed a discrete distribution of severity of constipation, for which 42.9% were in non-constipation and severe constipation, respectively. For the prescription of opioids, there were no significant difference on Syndrome distribution for whether patients were prescribed morphine, methadone, fentanyl, tramadol, dihydrocodeine, dextropropoxyphene or codeine, with P value > 0.05 (Table 5).

4. Discussion

From the results of this study, more than 90% of patients presented in deficient pattern, while 40% were in deficiency-excess complex. It illustrated that the healthy *Qi* (a collective designation for all normal functions of the human body and the abilities to maintain health [3]) of advanced cancer patients was greatly damaged, and many of them were complicated by excessive pattern. The excess condition could be caused by the accumulation of pathological factors, such as *Qi*, blood, phlegm, food, and dampness. Only a small number of patients were in pure excessive pattern. Therefore, the treatment principle of advanced cancer patients with constipation should reinforce the deficiency and eliminate the excess condition simultaneously. For the distribution of five common syndromes, two-thirds of patients present in *Qi* deficiency, or its combination with *Qi* stagnation and blood (*Yin*) deficiency, respectively. A designated TCM formula targeting on replenishing the deficiency of *Qi*, redirecting the flow of *Qi* stagnation and moistening the dryness caused by the blood (*Yin*) deficiency should be effective for the management of constipation in palliative care.

For analyzing the prevalence of symptoms and signs, there are many coincidences between different syndrome patterns. For example, dry mouth, fatigue, and fine pulse were the common manifestations of the five syndromes (Table 2). Even there were significant differences between deficient and excessive patterns, a large proportion of cases

Table 3: The combination of deficiency of *Qi* with other syndromes.

Patients with *Qi* deficiency	Number of patients (%) 210/225 (93.3%)
Qi deficiency (pure)	70/210 (33.3%)
Coexistence with *Qi* deficiency	
Qi stagnation	39/210 (18.6%)
Blood (*Yin*) deficiency	31/210 (14.8%)
Yang deficiency	18/210 (8.6%)
Excess heat	5/210 (2.4%)
Qi stagnation and blood (*Yin)* deficiency	16/210 (7.6%)
Qi stagnation and *Yang* deficiency	14/210 (6.7%)
Qi stagnation and excess heat	4/210 (1.9%)
Deficiency of blood (*Yin*) and *Yang*	6/210 (2.9%)
Qi stagnation and deficiency of blood (*Yin*) and *Yang*	6/210 (2.9%)
Qi stagnation, excess heat, and deficiency of blood (*Yin*) and *Yang*	1/210 (0.5%)

Table 4: Prevalence of symptoms and signs among three patterns.

	Deficiency ($n = 127$)	Excess ($n = 7$)	Complex ($n = 91$)	P value
Pale/sallow complexion	59 (46.5%)	0 (0%)	28 (30.8%)	0.007
Bitter taste	28 (22.0%)	2 (28.6%)	37 (40.7%)	0.012
Fatigue	122 (96.1%)	4 (57.1%)	82 (90.1%)	<0.001
Mind disquieted/susceptible to fright	66 (52.0%)	0 (0%)	50 (54.9%)	0.019
Belching/nausea/vomiting	18 (14.2%)	4 (57.1%)	57 (62.6%)	<0.001
Stuffiness and fullness of chest	5 (3.9%)	1 (14.3%)	40 (44.0%)	<0.001
Abdominal distension/pain	9 (7.1%)	1 (14.3%)	57 (62.6%)	<0.001
Water retention (with pleural/abdominal fluid)	0 (0%)	1 (14.3%)	13 (14.3%)	<0.001
Anorexia	47 (37.0%)	1 (14.3%)	46 (50.5%)	0.044
Insomnia	36 (28.3%)	4 (57.1%)	40 (44.0%)	0.029
Vacuous pulse	9 (7.1%)	3 (42.9%)	5 (5.5%)	0.001
Description of constipation symptoms				
Inadequate pushing force during defecation	54 (42.5%)	1 (14.3%)	58 (63.7%)	0.001
Incomplete defecation	20 (15.7%)	0 (0%)	27 (29.7%)	0.017
Difficult defecation	31 (24.4%)	1 (14.3%)	37 (40.7%)	0.024

Deficiency: deficient pattern; excess: excessive pattern; complex: deficiency-excess complex.
Only the symptoms and signs with significant differences among three syndrome patterns were listed in the table.

Table 5: The relationship between severity of constipation and opioids intake with the pattern distribution.

	Deficiency ($n = 127$)	Excess ($n = 7$)	Complex ($n = 91$)	P value
Severity (mean ± SD)	3.19 ± 1.85	3.29 ± 2.21	3.91 ± 1.57	0.012
No constipation	32 (25.2%)	3 (42.9%)	9 (9.9%)	0.013
Constipation	63 (49.6%)	1 (14.35%)	50 (54.9%)	
Severe constipation	32 (25.2%)	3 (42.9%)	32 (35.2%)	
With opioids	73 (57.5%)	4 (57.1%)	60 (65.9%)	0.442
With strong opioids	29 (22.8%)	2 (28.6%)	26 (28.6%)	0.618
With weak opioids	49 (38.6%)	2 (28.6%)	36 (39.6%)	0.847

Deficiency: deficient pattern; excess: excessive pattern; complex: deficiency-excess complex.
Severity of constipation was evaluated with an 8-point ordinal rating scale, where 0-1 indicated no constipation, 2–4 indicated constipation, and 5–7 indicated severe constipation.
Strong opioids included morphine, methadone, and fentanyl, while weak opioids included tramadol, dihydrocodeine, dextropropoxyphene, and codeine.

was actually in deficiency-excess complex (Table 4). It is not only because the specificity of symptom and sign for a particular syndrome pattern is relatively low, but also human body is an organic and complex whole, for which coexistence and transition of syndrome patterns are ordinary. Therefore, syndrome pattern should be differentiated comprehensively from a series of symptoms and signs. The dominant manifestations listed in Table 2 showed the norm of each symptom, which can be a reference for other TCM studies on advanced cancer.

In the past decades, the essence of syndrome patterns is determined with modern medical examination in terms of system biology [19]. For example, Chu et al. used serum proteomes to distinguish the essential hypertension patients with abundant phlegm-dampness from the healthy persons and the essential hypertension patients with non-phlegm-dampness [20]. In this study, patients in deficient pattern was significantly in higher mean age, and lower functional status (PPS) and level of blood haemoglobin when comparing with that in excessive pattern, while their Complex was at intermediate ($P < 0.05$). These objective assessment measures in conventional Western medicine may be as an auxiliary for the differentiation of syndrome patterns, although further investigations are necessary to develop certain guidelines. Furthermore, the impact of prescribed opioids on syndrome patterns cannot be concluded in this study. The influence of confounding by the opioid dose, duration of opioids intake, polypharmacy and polytreatment may be probably present.

Syndrome is the quintessence of TCM theory. However, consensus on its diagnosis is still limited; the diagnostic consistency among Chinese Medicine practitioners can be as low as 30% [21]. These not only make syndrome difficult to interpret and repeat on researches, but also impede the generalization of TCM to the world. We believe that standardizing the terminology, quantizing the description of syndrome, and validating TCM with evidence-based approaches are urgently needed for the development of TCM in future. Moreover, TCM has its vantage on analyzing diseases from a macroscopic point of view and human-oriented mind. Subjective measures, such as inadequate pushing force, sense of incomplete defecation and difficulty in defecation used in this study, are important for making diagnosis and prescription in TCM. On the contrary, conventional medicine is more dependent on objective measures and scientific assessment tools. Up till recently, patient's own perception of difficult defecation in the clinical assessment of constipation in palliative care and in treatment evaluation is emphasized [22]. We foresee that there are many opportunities for the incorporation of traditional medicine to convention medicine on diagnosis and treatment in both clinical practice and scientific researches.

Two aspects of this study should be reported as potential limitations in drawing broad conclusions. First, the whole study only involved 225 cases from two palliative care units, and there were 13 and seven patients in the groups of excess Heat and pure excessive pattern, respectively. When working with these small sample sizes, the results obtained from statistics may be underpowered to detect important effects or associations [23]. The distribution of syndrome patterns may not be able to generalize for all palliative cancer patients in Hong Kong. Second, variations in diagnosis do exist among CM practitioners [24]. Therefore, the diagnosis of syndrome should be made from more than one CM practitioner, and disagreements are resolved by discussions.

5. Conclusion

Advanced cancer patients were subject to be in deficient pattern, and many of them were complicated by excessive pattern. *Qi* deficiency and its combination of *Qi* stagnation and blood (*Yin*) deficiency were the most common syndromes for patients with constipation. Furthermore, age, functional status, and level of blood haemoglobin were factors related to the type of TCM syndrome. A TCM prescription with the functions on replenishing the deficiency, redirecting the flow of *Qi* stagnation and moistening the dryness caused by the blood (*Yin*) deficiency can be made for the treatment of advance cancer patients with constipation. Robust trials are urgently needed for further justifying its efficacy and safety in evidence-based approaches.

Conflict of Interests

The authors declare that there are no competing financial or other interests exist.

Acknowledgments

This study was fully supported by the Yan Chai Hospital-Hong Kong Baptist University Clinical Centre for Training and Research in Chinese Medicine (West Kowloon). The authors would also like to thank the Hong Kong Hospital Authority for providing administrative support.

References

[1] T. L. Lee, "Complementary and alternative medicine, and traditional Chinese medicine: time for critical engagement," *Annals of the Academy of Medicine Singapore*, vol. 35, no. 11, pp. 749–752, 2006.

[2] A. P. Lu, H. W. Jia, C. Xiao, and Q. P. Lu, "Theory of traditional chinese medicine and therapeutic method of diseases," *World Journal of Gastroenterology*, vol. 10, no. 13, pp. 1854–1856, 2004.

[3] World Health Organization Western Pacific Region, "WHO International Standard Terminologies on Traditional Medicine in the Western Pacific Region," 2007, http://www.wpro.who.int/publications/PUB_9789290612487/en/index.html.

[4] B. Y. Zhang, J. H. Dong, and Z. Y. Zhou, *Internal Medicine of Chinese Medicine*, Shanghai Scientific and Technical Publishers, Shanghai, China, 1985.

[5] The State Administration of traditional Chinese Medicine of the People's Republic of China, *Criteria of diagnosis and therapeutic effect of diseases and syndromes in traditional Chinese medicine*, Nanjing University Press, Beijing, China, 1994.

[6] X. Y. Zheng, *Guidelines for Clinical Research on New Drug of Chinese Medicine (trial implementation)*, China Medical Science and Technology Press, Beijing, China, 2002.

[7] J. Potter, F. Hami, T. Bryan, and C. Quigley, "Symptoms in 400 patients referred to palliative care services: prevalence and patterns," *Palliative Medicine*, vol. 17, no. 4, pp. 310–314, 2003.
[8] E. B. Curtis, R. Krech, and T. D. Walsh, "Common symptoms in patients with advanced cancer," *Journal of palliative care*, vol. 7, no. 2, pp. 25–29, 1991.
[9] M. T. Fallon, "Constipation in cancer patients: prevalence, pathogenesis, and cost- related issues," *European Journal of Pain*, vol. 3, pp. 3–7, 1999.
[10] I. Mancini and E. Bruera, "Constipation in advanced cancer patients," *Supportive Care in Cancer*, vol. 6, no. 4, pp. 356–364, 1998.
[11] K. E. Clemens and E. Klaschik, "Management of constipation in palliative care patients," *Current Opinion in Supportive and Palliative Care*, vol. 2, no. 1, pp. 22–27, 2008.
[12] C. L. Miles, D. Fellowes, M. L. Goodman, and S. Wilkinson, "Laxatives for the management of constipation in palliative care patients," *Cochrane Database of Systematic Reviews*, no. 4, Article ID CD003448, 2006.
[13] B. Candy, L. Jones, M. L. Goodman, R. Drake, and A. Tookman, "Laxatives or methylnaltrexone for the management of constipation in palliative care patients," *Cochrane Database of Systematic Reviews*, vol. 1, Article ID CD003448, 2011.
[14] W. Maclean and J. Lyttleton, *Clinical Handbook of Internal Medicine: The Treatment of Disease with Traditional Chinese Medicine*, University of Western Sydney, Sydney, Australia, 2002.
[15] B. Peng, X. Fang, and Y. Wang, *Traditional Chinese Internal Medicine*, People's Medical Publishing House, Beijing, China, 2007.
[16] C. W. Shooing, A. O. Kwok, Z. X. Bian, and D. M. Tse, "A cross sectional study of constipation and laxatives use in advanced cancer patients: insights for revision of current practice," *Support Care Cancer*. In press.
[17] S. J. Lewis and K. W. Heaton, "Stool form scale as a useful guide to intestinal transit time," *Scandinavian Journal of Gastroenterology*, vol. 32, no. 9, pp. 920–924, 1997.
[18] M. Goodman, J. Low, and S. Wilkinson, "Constipation management in palliative care: a survey of practices in the United Kingdom," *Journal of Pain and Symptom Management*, vol. 29, no. 3, pp. 238–244, 2005.
[19] L. Liu, S. Z. Guo, and W. Wang, "The present situation and development tendency of syndrome of traditional Chinese medicine," *Zhong Hua Zhong Yi Yao Za Zhi*, vol. 23, pp. 661–663, 2008.
[20] Y. G. Chu, J. Shi, Y. H. Hu et al., "Serum proteomes of hypertension patients with abundant phlegm-dampness," *Journal of Chinese Integrative Medicine*, vol. 7, no. 7, pp. 629–635, 2009.
[21] R. Q. Chen, C. M. Wong, K. J. Cao, and T. H. Lam, "An evidence-based validation of traditional Chinese medicine syndromes," *Complementary Therapies in Medicine*, vol. 18, no. 5, pp. 199–205, 2010.
[22] L. Brown, I. Lawrie, V. B. D'Sa, S. Wilcox, and M. Bennett, "Constipation: patient perceptions compared to diagnostic tools," *Palliative Medicine*, vol. 20, no. 7, pp. 717–718, 2006.
[23] M. Noordzij, F. W. Dekker, C. Zoccali, and K. J. Jager, "Sample size calculations," *Nephron - Clinical Practice*, vol. 118, no. 4, pp. c319–c323, 2011.
[24] J. J. Y. Sung, W. K. Leung, J. Y. L. Ching et al., "Agreements among traditional Chinese medicine practitioners in the diagnosis and treatment of irritable bowel syndrome," *Alimentary Pharmacology and Therapeutics*, vol. 20, no. 10, pp. 1205–1210, 2004.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 480715, 9 pages
doi:10.1155/2012/480715

Research Article

Traditional Chinese Medicine *ZHENG* Identification Provides a Novel Stratification Approach in Patients with Allergic Rhinitis

Kai-Li Liang,[1,2] Rong-San Jiang,[1,2] Chia-Lin Lee,[3] Pei-Jung Chiang,[4,5] Jui-Shan Lin,[5] and Yi-Chang Su[5]

[1] *Department of Otolaryngology, Taichung Veterans General Hospital, Taichung 40705, Taiwan*
[2] *School of Medicine, Chung Shan Medical University, Taichung 40201, Taiwan*
[3] *Department of Endocrinology and Metabolism, Taichung Veterans General Hospital, Taichung 40705, Taiwan*
[4] *Department of Traditional Chinese Medicine, Taichung Veterans General Hospital, Taichung 40705, Taiwan*
[5] *Graduate Institute of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung 40421, Taiwan*

Correspondence should be addressed to Yi-Chang Su, juishan.lin@msa.hinet.net

Received 13 January 2012; Revised 4 April 2012; Accepted 6 April 2012

Academic Editor: Shi-Bing Su

Background. We aimed to apply the *ZHENG* identification to provide an easy and useful tool to stratify the patients with allergic rhinitis (AR) through exploring the correlation between the quantified scores of AR symptoms and the TCM *ZHENGs*. *Methods*. A total of 114 AR patients were enrolled in this observational study. All participants received the examinations of anterior rhinoscopy and acoustic rhinometry. Their blood samples were collected for measurement of total serum immunoglobulin E (IgE), blood eosinophil count (Eos), and serum eosinophil cationic protein (ECP). They also received two questionnaire to assess the severity scores of AR symptoms and quantified TCM *ZHENG* scores. Multiple linear regression analysis was used to determine explanatory factors for the score of AR manifestations. *Results*. IgE and ECP level, duration of AR, the 2 derived TCM*ZHENG* scores of "*Yin-Xu* − *Yang-Xu*", and "*Qi-Xu* + *Blood-Xu*" were 5 explanatory variables to predict the severity scores of AR symptoms. The patients who had higher scores of "*Yin-Xu* − *Yang-Xu*" or "*Qi-Xu* + *Blood-Xu*" tended to manifest as "sneezer and runner" or "blockers," respectively. *Conclusions*. The TCM *ZHENG* scores correlated with the severity scores of AR symptoms and provided an easy and useful tool to stratify the AR patients.

1. Introduction

Allergic rhinitis (AR) is a common disease with a prevalence of at least 10% to 20% in western nations [1, 2]. Many patients suffering from AR seek help from complementary medical treatment, such as traditional Chinese medicine (TCM) [3–5]. Some well-designed controlled studies showed benefits of TCM treatment in allergic diseases [6–10]. Patients having the same disease in western medicine may have different TCM *ZHENG* diagnosis and therefore are prescribed different TCM herbal treatment.

ZHENG (syndrome), a basic unit and key concept in TCM theory, is the diagnosis made or concluded after careful analysis of all symptoms and signs. This process to achieve the diagnosis is based on the physiology and pathology of TCM, and is named "differentiation of *ZHENG*" or "*ZHENG* identification" [11]. This characteristic of TCM diagnosis denotes that the diseased person and the integrity of the human body are more focused than the person's disease. The purpose of collecting and analyzing symptoms and signs is to evaluate the overall maladjustment of the human body [12]. Guided with the *ZHENG* identification, the treatment modalities are individualized and mainly based on the *ZHENG* which each patient is diagnosed with.

Currently, there are no agreed ways to predict the severity of allergic rhinitis manifestations. Either nasal airway assessments, laboratory parameters, or physical findings usually show a low degree of correlation or a nonsignificant correlation with patients' symptoms [12–17]. Since the TCM *ZHENG* is diagnosed according to each patient's clinical manifestations, it reflects more subtle individual differences in patients with the same disease, which may be a tool to

predict the severity of AR symptoms or categorize the AR patients. The purpose of this study was to probe the correlation between the severity of AR symptoms and the TCM *ZHENG*, and also try to adopt the TCM *ZHENG* identification to provide an easy and useful tool to stratify the AR patients.

2. Participants and Methods

2.1. Study Design and Subjects. This was an explorative, cross-sectional, and observational clinical trial. Patients diagnosed with AR were enrolled from the outpatient of the Otorhinolaryngology Department of Taichung Veterans General Hospital from 2004 to 2005. The diagnosis of AR was made by the specialist physician according to the clinical manifestations, history, and positive skin testing of a local screening panel (house dust, cotton, ragweed, *Candida, Alternaria, Aspergillus, Cladosporium*, and *Penicillium*). A written informed consent was obtained from each participant. Exclusion criteria for this trial were those who (1) took antihistamine, decongestant, or had used topical steroids within 2 weeks, (2) were under 12 years old, (3) were pregnant, (4) were ongoing immunotherapy, or (5) were with severe physical or mental illness. The study protocols were approved by the Institutional Review Board of the Taichung Veterans General Hospital (IRB TCVGH no. 930116/279).

2.2. Patient Assessments

2.2.1. Physical Exam and Inflammatory Maker. One specialist physician (R. S. Jiang) performed the anterior rhinoscopy for all eligible patients and graded their rhinoscopic findings. The edematous degree of inferior turbinate and the amount of nasal discharge were graded from 0 (none) to 3 (severe). Nasal minimal cross-sectional area (MCA) was assessed by acoustic rhinometry in each patient. The blood samples were collected from the patients for analysis of the inflammatory marker, including: total serum immunoglobulin E (IgE), blood eosinophil count (Eos), and serum eosinophil cationic protein (ECP).

2.2.2. Scoring of AR Symptoms Severity. Each enrolled patient completed a self-report questionnaire assessment to assess the severity of allergic symptoms within the latest 2 weeks: this questionnaire, which also included questions on age, gender, family history, comobility (allergic asthma, atopic dermatitis, or urticaria), and duration of AR, was specific to the severity of allergic symptoms including nasal obstruction, sneezing, rhinorrhea, itchy nose, and itchy eye. These symptoms were graded from 0 to 3 according to the severity within previous 2 weeks (0 = no symptom; 1 = mild symptom, no impact on daily life; 2 = moderate symptom, impact on daily life; 3 = severe symptom, impact on daily life).

2.2.3. Scoring of TCM ZHENGs . In TCM, a disease is a common product of both pathogenetic factors and maladjustments in the body. The body must have the capacity to regulate itself in order to maintain homeostasis and adapt to the environmental stimulus. If the body's regulation ability fails to maintain homeostasis, then diseases may develop [12]. Therefore, the signs and symptoms expressed by patients are analyzed to identify the type of internal maladjustments (e.g., hyporesponse or hyperresponse). This diagnostic process is called "TCM *ZHENG* Identification." The diagnosis of TCM *ZHENG* is the summary of a specific functional state of the human body [12]. There are many *ZHENGs* in TCM, either simple *ZHENG* or combined ones [11].

In our study, the 4 basic TCM *ZHENGs*: "*Yin-Xu*, *Yang-Xu*, *Qi-Xu*, and *Blood-Xu*" were chosen to be measured in the AR patients. It was because *Internal Classis*, an important TCM literature, points out that: "*Qi, Blood, Yin,* and *Yang*" are 4 basic important elements to maintain the body's normal function; the physiological equilibrium and the circulation of *Qi* and *Blood* all change in response to the environmental variations. These responses help preserve the dynamic equilibrium of the body's *Yin* and *Yang* [18]. Once the functional status maintained by these 4 elements fails to keep its normal capacity, the body will manifest related signs and symptoms. Then, the 4 basic TCM *ZHENGs*: "*Yin-Xu*, *Yang-Xu*, *Qi-Xu*, and *Blood-Xu*" will be diagnosed (Figure 1).

In order to integrate TCM with modern medicine, each enrolled patient completed a self-report questionnaire assessment to score the 4 basic TCM *ZHENGs* (*Yin-Xu*, *Yang-Xu*, *Qi-Xu*, and *Blood-Xu*). The measurement of this questionnaire provided a quantified and comparable parameter to explore the correlation between TCM *ZHENGs* and the severity score of AR symptoms. An easy-applied and standardized TCM *ZHENG* diagnosis instrument was developed by our research team through 2 rounds of TCM experts' meetings. After several discussions, according to the TCM theory and considering its use in western medical setting, this instrument was designed to measure the 4 basic TCM *ZHENGs* by answering the 24 questions which described the signs and symptoms of the 4 *ZHENGs*. These signs and symptoms in the previous 2 weeks were self-reported and assessed by a 4-point frequency and intensity scale (each was graded from 0 to 3). With higher scores of frequency and intensity, a more pronounced pathological status of each TCM *ZHENG* was indicated (the TCM *ZHENG* Questionnaire and the TCM *ZHENG* measured by each question are listed in the appendix). The Cronbach α coefficients of this questionnaire were 0.70, 0.64, 0.77, and 0.76 for the four TCM *ZHENG* domains of "*Yin-Xu*, *Yang-Xu*, *Qi-Xu,* and *Blood-Xu*," respectively. The scores of "*Yin-Xu*, *Yang-Xu*, *Qi-Xu,* and *Blood-Xu*" represented the decreased level of *Yin*, *Yang*, *Qi,* and *Blood* to maintain normal function of the body (Figure 1).

Furthermore, in TCM physiology, since "*Qi and Blood*" and "*Yin* and *Yang*" both work synergically to keep the equilibrium of the body, some items in the questionnaire were designed to measure more than one TCM *ZHENG*. Therefore, collinearity existed between the measurement results of "*Qi-Xu* and *Blood-Xu*" *and* "*Yin-Xu* and *Yang-Xu*." To solve this problem, two derivative parameters were constructed based on not only the TCM theory about the pathological mechanism but also on the consideration of statistical modification: (1) "*Yin-Xu* − *Yang-Xu*": this derived from the score

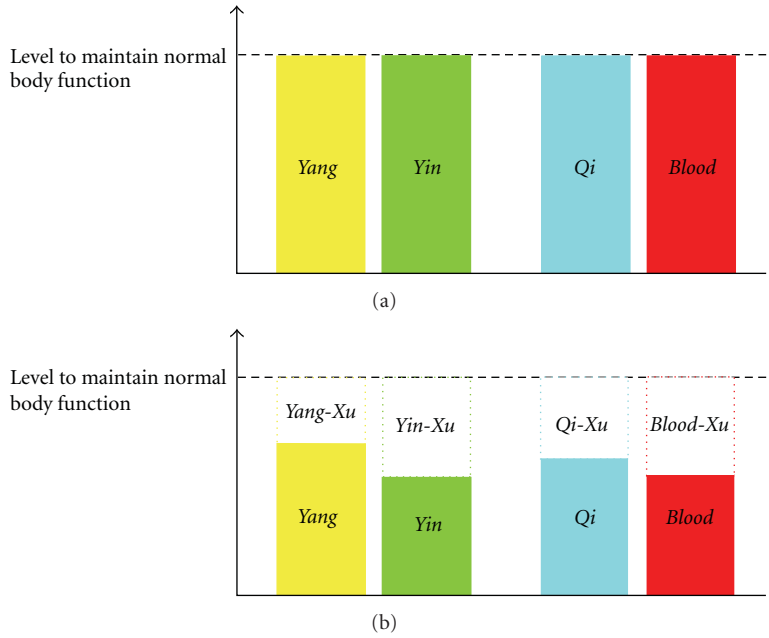


Figure 1: Illustration of the TCM *ZHENGs* and their derivative parameter. (a) Level of *Yang, Yin, Qi,* and *Blood* in normal physiological functional status. (b) Level of *Yang, Yin, Qi,* and *Blood* in pathological functional status.

of *Yang-Xu ZHENG* subtracted from the score of *Yin-Xu ZHENG*, and denoted the TCM *ZHENG* "*Yin-deficiency with Yang-hyperactivity.*" Physiologically, the normal body function is maintained upon the harmonious cooperation and mutual restrain of *Yin* and *Yang* [19]. Pathologically, once the decreased *Yin* is unable to restrain *Yang*, some signs and symptoms of *Yang-hyperactivity* will manifest, since the level of *Yin* and *Yang* is expected to be equal in normal circumstance. So, the derived parameter score of "*Yin-Xu* − *Yang-Xu*" was aimed to quantify this pathological status (Figure 1(b)).

(2) "*Qi-Xu* + *Blood-Xu*": this derived from the score of *Qi-Xu ZHENG* added to the score of *Blood-Xu ZHENG*, and denoted the TCM *ZHENG* "*dual deficiency of Qi and Blood.*" In TCM physiology, *Blood* is transported by the driving force of *Qi* and they are both produced from the "middle energizer." Pathologically, once the production of *Qi* and *Blood* or the efficacy of the transportation of *Qi* is decreased, a person may express signs and symptoms of "*Qi-Xu*" and/or "*Blood-Xu*." So, the derived parameter score of "*Qi-Xu* + *Blood-Xu*" was aimed to quantify the whole body's pathological deficiency status of *Qi* and *Blood* (Figure 1(b)).

2.3. Statistical Analysis. Statistical analyses were conducted using the Statistical Package for the Social Science (SPSS Inc., Chicago, IL, USA) version 12.0. The demographic characteristics of the observed patients were described by frequency, percentage, mean, and standard deviation. The correlation among the study variables was examined by Spearmen's rank correlation. A test for linearity was used to evaluate the trend of the TCM *ZHENG* scores with the severity scores of AR symptoms. Multiple linear regression analysis with the total symptom score as the dependent variable was carried out to determine the variables independently associated with the severity of AR. Binary logistic regression was used to link the symptoms which impacted daily life (symptom score = 2 or 3) and TCM *ZHENG* score. Two-tailed P value <0.05 was considered statistically significant.

3. Results

A total of 114 AR patients were enrolled in our study. Both the questionnaire assessment and anterior rhinoscopy were done for all the 114 patients; examination of acoustic rhinometry for 111, measurements of IgE for 109, measurement of ECP for 106, and measurement of Eos for 100. The basic characteristics of the patients are listed in Table 1.

3.1. Correlation with the Severity Score of AR Symptoms

3.1.1. Demographic Characteristics. There was no significant correlation between the AR patients' age and the total or each AR symptom score; neither the correlation between the smoking habit and the symptom score. Patients who had other allergic diseases (allergic asthma, urticaria, or atopic dermatitis) or family members (parents, grandparents, or siblings) with allergic diseases (allergic asthma, allergic rhinitis, urticaria, or atopic dermatitis) did not have higher symptom scores either. However, the duration of AR showed significant correlation with both the total symptom scores and the score of itchy eye (Table 2).

3.1.2. Rhinoscopic Findings and Nasal Airway Assessment. There was no correlation noted between the scores of rhinoscopic findings and the AR symptom scores. The sum of

Table 1: Basic characteristic of the observed patients ($n = 114$).

	Number (percentage)	Mean ± SE
Age (yr)		27.80 ± 1.19
Gender		
Male	63 (55.3%)	
Female	51 (44.7%)	
Smoking	17 (14.9%)	
Family history [a]	79 (69.3%)	
Comobility[b]	28 (24.6%)	
Duration of AR (months)		93.75 ± 6.86
Rhinoscopic findings	Turbinate swelling	1.86 ± 0.06
	Nasal discharge	0.71 ± 0.07
	Total scores	2.57 ± 0.09
Sum of MCA2 (cm^2)		0.87 ± 0.03
Inflammatory markers	IgE (kU/L)	407.60 ± 66.01
	ECP (pg/mL)	18.89 ± 2.01
	Eos ($/mm^3$)	279.35 ± 19.65
Symptom scores	Nasal obstruction	2.04 ± 0.08
	Sneezing	1.86 ± 0.08
	Rhinorrhea	2.11 ± 0.08
	Itchy nose	1.49 ± 0.08
	Itchy eye	1.30 ± 0.09
	Total symptom scores	8.81 ± 0.29
Scores of *ZHENG*	*Yin-Xu*	6.25 ± 0.33
	Yang-Xu	5.39 ± 0.30
	Qi-Xu	7.25 ± 0.38
	Blood-Xu	7.96 ± 0.40
	Yin-Xu − *Yang-Xu*[c]	0.86 ± 0.35
	Qi-Xu + *Blood-Xu*[d]	15.21 ± 0.73

[a] Parents, grandparents, or siblings had allergic rhinitis, allergic asthma, atopic dermatitis, or urticaria.
[b] Patients had allergic asthma, atopic dermatitis, or urticaria.
[c] Score of *Yang-Xu ZHENG* subtracted from score *Yin-Xu* of *ZHENG*.
[d] Score of *Qi-Xu ZHENG* added to score of *Blood-Xu ZHENG*.
AR: allergic rhinitis; MCA2 = the second minimal cross-sectional area.

second nasal minimum cross-sectional area (MCA2) measured by acoustic rhinometry did not correlate with the symptom scores either (Table 2).

3.1.3. Inflammatory Makers. When correlation was analyzed between each two variables, IgE and Eos did not correlate to the total nasal symptom scores. When it goes to the individual nasal symptoms, the ECP level had significant correlation with the score of rhinorrhea ($r = 0.206$ and $P = 0.034$) and moderate correlation with sneezing ($r = 0.164$ and $P = 0.093$). The IgE level had moderate correlation with sneezing ($r = 0.157$ and $P = 0.102$) (Table 2).

3.1.4. Scores of TCM ZHENGs. The score of "*Yin-Xu*" *ZHENG* correlated significantly with the total and each AR symptom score. The score of "*Yang-Xu*" *ZHENG* did not correlate with any symptom score. The scores of "*Qi-Xu*" and "*Blood-Xu*" *ZHENG* correlated significantly with the total symptom scores, the scores of nasal obstruction, and itchy nose and eye.

The derivative parameter "*Yin-Xu* − *Yang-Xu*" correlated significantly with the total and each AR symptom score, except nasal obstruction. Another derivative parameter "*Qi-Xu* + *Blood-Xu*" correlated significantly with the total symptoms scores, the scores of nasal obstruction, and itchy nose and eye (Table 2).

3.2. Explanatory Factors for Severity Score of AR Symptoms. Then, multiple linear regression analysis was performed to determine explanatory (predictive) factors for the severity scores of AR symptoms. Beside the correlated variables noted in the above bivariate correlation analysis, since age and sex were important demographic factors, they were put into the multiple linear regression model. Simultaneously, IgE was also added considering it being checked regularly in the clinical practice for AR patients.

Table 2: Correlations of study variables.

Symptom score	Total	Nasal obstruction	Sneezing	Rhinorrhea	Itchy nose	Itchy eyes
Age	0.064	0.103	0.067	0.011	0.047	0.011
Smoking	−0.115	−0.095	−0.050	−0.128	−0.040	−0.085
Comobility[a]	0.104	0.063	0.132	0.119	0.060	0.026
Duration of AR	0.243*	0.109	0.133	0.123	0.172	0.312*
Rhinoscopic findings	0.117	0.110	0.014	0.108	−0.110	0.107
MCA2	−0.109	−0.063	−0.085	−0.039	−0.156	−0.070
IgE	0.081	−0.180	0.157	0.064	0.029	0.049
ECP	0.116	−0.022	0.164	0.206*	0.119	−0.018
Eos	0.120	−0.600	0.126	0.156	0.101	0.037
Yin-Xu	0.357*	0.206*	0.188*	0.200*	0.352*	0.340*
Yang-Xu	0.730	0.128	−0.810	−0.180	0.0780	0.131
Qi-Xu	0.256*	0.199*	0.054	0.012	0.342*	0.304*
Blood-Xu	0.267*	0.194*	0.097	0.074	0.281*	0.332*
Yin-Xu − Yang-Xu	0.282*	0.082	0.259*	0.238*	0.245*	0.196*
Qi-Xu + Blood-Xu	0.266*	0.194*	0.065	0.036	0.325*	0.339*

Data presented with coefficient.
*P value < 0.05.
[a]Patients had allergic asthma, atopic dermatitis, or urticaria.

In the beginning of the multiple linear regression analysis, we faced the problem of collinearity when the 4 basic TCM *ZHENG* scores were used as explanatory variables for regression analysis. The collinearity was solved by using the score of the derivative parameter "*Yin-Xu − Yang-Xu*" and "*Qi-Xu + Blood-Xu*" instead of the 4 basic TCM *ZHENGs* for the regression model.

Finally, we found 5 independent predictors: IgE level ($P = 0.039$), ECP level ($P = 0.017$), duration of AR ($P = 0.016$), the scores of "*Yin-Xu − Yang-Xu*" ($P = 0.004$), and the score of "*Qi-Xu + Blood-Xu*" ($P = 0.015$) (using enter regression model, $R^2 = 0.280$, $P < 0.001$, Table 3).

3.3. Correlation between Predictive Factors and Each Symptom. Furthermore, we went on to exam the correlation between the above 5 predictors and each AR symptom using binary logistic regression. Before the analysis, the AR symptom scores were processed in advance as follows: (1) the original symptom scores graded from 0 to 1 were recategorized into "0," which meant no impact on daily life; (2) the original scores graded from 2 to 3 were recategorized into "1," which meant the symptoms had impact on daily life.

When binary logistic regression was used to link these predictors with the AR symptoms which impacted daily life, we found that nasal obstruction which impacted daily life was correlated with higher scores of "*Qi-Xu + Blood-Xu*" (OR = 1.081, 95% CI = 1.009 to 1.158). The symptoms of rhinorrhea and itchy nose which impacted daily life were correlated with higher scores of "*Yin-Xu − Yang-Xu*" (OR = 1.165, 95% CI = 1.018 to 1.334, and OR = 1.147, 95% CI = 1.012 to 1.300, resp.). The symptom of itchy eye which impacted daily life (the score of itchy eye = 2 or 3) was correlated with higher scores of "*Yin-Xu − Yang-Xu*" (OR = 1.164, 95% CI = 1.015 to 1.335) and longer duration of AR (OR = 1.015, 95% CI = 1.006 to 1.024) (Table 4).

Table 3: Factors predicting severity scores of symptom in patients with allergic rhinitis.

Variable	Regression coefficient	SE	t
Age	0.017	0.024	0.701
Male sex	−0.995	0.589	−1.689
Duration of AR	0.01*	0.004	2.451
IgE	0.0008*	0.000	2.091
ECP	0.033*	0.014	2.418
Yin-Xu − Yang-Xu	0.232*	0.078	2.957
Qi-Xu + Blood-Xu	0.092*	0.037	2.480

Model: multiple linear regression, use enter regression; $R^2 = 0.280$, $P < 0.001$.
*$P < 0.05$.

4. Discussion

This explorative, cross-sectional, and observational clinical study adopted and integrated both the diagnostic method of western medicine and TCM in patients with AR. AR is defined as a symptomatic disorder of the nose induced after allergen exposure by an IgE-mediated inflammation. The update treatment guidelines initiated by the World Health Organization recommend classification of allergic rhinitis into "intermittent" (IAR) or "persistent" (PER) allergic rhinitis, instead of previous classification of "seasonal" or "perennial" allergic rhinitis [1, 2]. It is believed that the new classification shows better adherence to real life. In this study, we enrolled patients with history of typical symptoms of allergic rhinitis including nasal obstruction, sneezing, rhinorrhea, itchy nose and eyes. The IgE-mediated etiology of the enrolled rhinitis patients has confirmed with positive skin testing of a local screening panel. Therefore we enrolled a group of rhinitis patients with same underlying etiology

Table 4: Link of allergic symptoms which impacted daily life (symptom score = 2 or 3) with predictive factors.

	Nasal obstruction		Sneezing		Rhinorrhea		Itchy nose		Itchy eyes	
	Model of regression: enter		Model of regression: enter		Model of regression: enter		Model of regression: enter		Model of regression: enter	
	$R^2 = 0.122$, $P = 0.244$		$R^2 = 0.150$, $P = 0.101$		$R^2 = 0.210$, $P = 0.022$		$R^2 = 0.161$, $P = 0.063$		$R^2 = 0.323$, $P < 0.001$	
	Accuracy of model = 74.0%		Accuracy of model = 67.3%		Accuracy of model = 68.3%		Accuracy of model = 61.5%		Accuracy of model = 71.2%	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Age	1.009	0.971–1.049	1.014	0.976–1.054	1.011	0.970–1.053	1.012	0.976–1.049	0.994	0.953–1.036
Male sex	1.425	0.521–3.894	1.313	0.514–3.353	1.393	0.503–3.860	1.470	0.604–3.579	2.096	0.796–5.522
Duration of AR	1.002	0.995–1.008	1.003	0.996–1.010	1.007	0.999–1.015	1.02	0.995–1.008	1.015*	1.006–1.024
IgE	1.001	0.999–1.002	1.001	1.000–1.003	1.001	1.000–1.002	1.000	1.000–1.001	1.000	1.000–1.001
ECP	1.015	0.987–1.043	1.021	0.992–1.051	1.026	0.992–1.061	1.009	0.988–1.030	1.017	0.994–1.040
Yin-Xu − Yang-Xu	1.003	0.878–1.147	1.111	0.982–1.256	1.165*	1.018–1.334	1.147*	1.012–1.300	1.164*	1.015–1.335
Qi-Xu + Blood-Xu	1.081*	1.009–1.158	1.020	0.961–1.083	1.028	0.961–1.098	1.056	0.996–1.119	1.055	0.992–1.123

Analyzed by binary logistic regression.
OR: odds ratio
95% CI: 95% confidence interval
*$P < 0.05$.

Table 5

	0	1	2	3	TCM *ZHENG measured*
(1) I have become fretful and irritated about everything.	☐	☐	☐	☐	*Yin-Xu*
(2) I have heartburn.	☐	☐	☐	☐	*Yin-Xu*
(3) I have been suffering from insomnia.	☐	☐	☐	☐	*Yin-Xu Blood-Xu*
(4) It takes a long time for me to fall asleep.	☐	☐	☐	☐	*Yin-Xu Blood-Xu*
(5) My hands and feet are warm.	☐	☐	☐	☐	*Yin-Xu*
(6) I have night sweat even if it's not hot.	☐	☐	☐	☐	*Yin-Xu*
(7) I'm always thirsty. My throat feels completely dried out soon after I drink.	☐	☐	☐	☐	*Yin-Xu*
(8) I have to get up to pee at night.	☐	☐	☐	☐	*Yang-Xu*
(9) I urinate a lot, and the color of my urine is faint.	☐	☐	☐	☐	*Yang-Xu*
(10) I feel cold when others feel cool and comfortable.	☐	☐	☐	☐	*Yang-Xu*
(11) I wear more layers because I feel cold.	☐	☐	☐	☐	*Yang-Xu*
(12) I like to stay in a warm place and like to huddle up to feel warm.	☐	☐	☐	☐	*Yang-Xu*
(13) I have loose stools.	☐	☐	☐	☐	*Yang-Xu*
(14) I feel very tired after mild activity.	☐	☐	☐	☐	*Qi-Xu*
(15) I feel dizzy when getting up quickly.	☐	☐	☐	☐	*Qi-Xu Blood-Xu*
(16) I do not like to talk because I soon feel tired after saying a few words.	☐	☐	☐	☐	*Qi-Xu*
(17) I still feel sleepy after a long sleep.	☐	☐	☐	☐	*Qi-Xu*
(18) I feel lightheaded.	☐	☐	☐	☐	*Qi-Xu Blood-Xu*
(19) I sweat even if it is cool.	☐	☐	☐	☐	*Qi-Xu*
(20) I get out of breath when I walk a little.	☐	☐	☐	☐	*Qi-Xu*
(21) I feel palpations even when still or peaceful.	☐	☐	☐	☐	*Blood-Xu*
(22) I am neither nearsighted nor farsighted (or has already been corrected), but I still have blurred vision.	☐	☐	☐	☐	*Blood-Xu*
(23) My body and limbs feel numb when I keep still.	☐	☐	☐	☐	*Blood-Xu*
(24) My ears ring when it is quiet.	☐	☐	☐	☐	*Blood-Xu*

(IgE-mediated allergic rhinitis). We excluded the patients who took antihistamine, decongestant or had used topical steroids within 2 weeks, and who were ongoing immunotherapy. The above exclusion criteria were for reducing the drug effects affecting our assessment.

TCM doctors diagnose the *ZHENG* based on the TCM theory after inquiry and physical examination. However, the theory of TCM is complicated and not easily realized by western medicine (WM) physicians and investigators. WM doctors are often skeptical about the validity of TCM clinical diagnosis [20, 21]. We designed the "TCM *ZHENG* Questionnaire" as a simplified mathematic model of TCM inquiry and provided an easy-applied, standardized diagnosis tool. Questions in the questionnaire were designed to survey patients' physical conditions based on the four basic TCM *ZHENGs:* "*Yin-Xu*, *Yang-Xu*, *Qi-Xu*, and *Blood-Xu*." Higher scores in the questionnaire meant more pronounced pathological statuses. The TCM physical findings of patients' pulse, tongue, nails, lips, and face were not included in this study because of the difficulty in standardization.

Investigations of the predictors of the severity of allergic rhinitis manifestations are few and conflicting. Several studies have proved the relationship between IgE, ECP and Eos, and atopic diseases [22–25]. However, using inflammatory markers as predictors for AR's severity has not been established [13, 14, 26, 27]. Winther et al. [14] conducted a study and investigated the relationship between laboratory parameters and the severity of AR. They found that certain laboratory parameters were significantly correlated with disease severity, but could account for only a minor part of the seasonal variation of the symptom scores. In this study, we found only the duration of AR and the TCM *ZHENG* scores to be associated with the severity of AR by analysis of bivariate correlation. The inflammatory parameters correlated with the total symptom scores after adjusting the TCM *ZHENG* scores, meaning the TCM *ZHENG* scores were a confounding factor to the inflammatory parameters.

Acoustic rhinometry is a geographic measurement of the nasal cavity by using reflections of sound wave. The acoustic rhinometry is safe and its validity has been proven by comparison with measures obtained by computerized tomography or magnetic resonance imaging scanning [28–31]. However, the subjective reporting of nasal obstruction may not correlate well with acoustic rhinometry measures [17, 32]. This could be because the sensation of nasal obstruction can be influenced by changes in the ostiomeatal complex and existence of nasal discharge rather than purely reflecting nasal cavity size [15]. We found the result of acoustic rhinometry had no significant correlation with the total symptom scores or the scores of nasal obstruction.

The TCM *ZHENG* ("*Yin-Xu − Yang-Xu*" and "*Qi-Xu + Blood-Xu*"), duration of AR, IgE, and ECP level were found to be good predictors for the severity scores of AR in our study. However, the R^2 value was only 0.28, indicating some other factors could contribute to the severity of allergic rhinitis. We believe that environmental factors, or mucociliary function may play a role causing the severity of AR manifestations.

Khanna and Shah [33] reported a new classification of patients with allergic rhinitis, according to the ARIA report [1], as "sneezer and runner" and "blocker" was mandatory. Their study demonstrated that the two groups had distinct clinical profiles. We also found that these two groups had different TCM *ZHENG* scores: "blockers" (the symptom scores of nasal obstruction = 2 or 3) having significant higher scores of "*Qi-Xu + Blood-Xu*", while "sneezer and runner" (the symptom scores of rhinorrhea, itchy nose or eye = 2 or 3) having higher scores of "*Yin-Xu − Yang-Xu*". From the TCM pathological point of views, these findings were very reasonable and closely fitted to the TCM theory. Since the nasal obstruction may be caused by the deficiency of *Qi* and/or *Blood*; while the rhinorrhea or itchy nose or eye are the manifestations of *Yin-deficiency with Yang-hyperactivity*. These results showed that the TCM *ZHENG* diagnosis correlated with the modern western medicine, and the TCM *ZHENG* diagnosis could reflect subtle differences among the patients with AR.

To our knowledge, this was the first study which adopted the TCM diagnostic questionnaires to quantify disease-specific severity and to categorize the patients with AR. Our results revealed that the TCM diagnostic questionnaires can be used similarly to disease specific quality of life standardized questionnaires such as the SF-36 (a general QOL questionnaire) or the RQLQ (a disease-specific QOL) instrument. Our research team had conducted several clinical studies which adopted both the TCM diagnostic questionnaires and the quality of life standardized questionnaires. It was found that the results measured by the 2 questionnaires were comparable in several aspects. Our team will report these interesting findings and new application of TCM diagnosis continuously.

5. Conclusion

The TCM *ZHENG* score, the duration of AR, and the IgE and ECP level were found to be independently and significantly explanatory of the severity of AR manifestations. The TCM *ZHENG* diagnosis correlated with the modern western diagnosis and may provide a novel approach to stratify the AR patients. These findings may provide a new applied field of TCM *ZHENG* diagnosis.

Appendix

A. Traditional Chinese Medicine ZHENG Questionnaire

In Table 5 you will find a list of symptoms associated with the traditional Chinese medicine *ZHENG*. We would appreciate you answering the following questions to the best of your ability. Please rate your problems as they have been over *the past two weeks* (all questions are graded as 0 = never; 1 = sometimes; 2 = often; 3 = always).

Acknowledgments

This study was supported by the Committee on Chinese Medicine and Pharmacy, Department of Health, Executive Yuan, Taiwan (Grant no. CCMP92-RD-020). They also thank the Biostatistics Task Force of Taichung Veterans General Hospital for the assistance with the statistical analysis.

References

[1] J. Bousquet, P. Van Cauwenberge, and N. Khaltaev, "Allergic rhinitis and its impact on asthma.," *Journal of Allergy and Clinical Immunology*, vol. 108, no. 5, pp. S147–S334, 2001.

[2] J. Bousquet, N. Khaltaev, A. A. Cruz et al., "Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA^{2}LEN and AllerGen)," *Allergy*, vol. 63, supplement 86, pp. 8–160, 2008.

[3] J. H. Krouse and H. J. Krouse, "Patient use of traditional and complementary therapies in treating rhinosinusitis before consulting an otolaryngologist," *Laryngoscope*, vol. 109, no. 8, pp. 1223–1227, 1999.

[4] L. X. Man, "Complementary and alternative medicine for allergic rhinitis," *Current Opinion in Otolaryngology and Head and Neck Surgery*, vol. 17, no. 3, pp. 226–231, 2009.

[5] T. Schäfer, "Epidemiology of complementary alternative medicine for asthma and allergy in Europe and Germany," *Annals of Allergy, Asthma and Immunology*, vol. 93, no. 2, pp. S5–S10, 2004.

[6] B. Brinkhaus, J. Hummelsberger, R. Kohnen et al., "Acupuncture and Chinese herbal medicine in the treatment of patients with seasonal allergic rhinitis: a randomized-controlled clinical trial," *Allergy: European Journal of Allergy and Clinical Immunology*, vol. 59, no. 9, pp. 953–960, 2004.

[7] C. C. Xue, R. English, J. J. Zhang, C. Da Costa, and C. G. Li, "Effect of acupuncture in the treatment of seasonal allergic rhinitis: a randomized controlled clinical trial," *American Journal of Chinese Medicine*, vol. 30, no. 1, pp. 1–11, 2002.

[8] C. C. L. Xue, F. C. K. Thien, J. J. S. Zhang, C. Da Costa, and C. G. Li, "Treatment for seasonal allergic rhinitis by Chinese herbal medicine: a randomized placebo controlled trial," *Alternative Therapies in Health and Medicine*, vol. 9, no. 5, pp. 80–87, 2003.

[9] W. H. Hsu, T. J. Ho, C. Y. Huang et al., "Chinese medicine acupoint herbal patching for allergic rhinitis: a randomized controlled clinical trial," *American Journal of Chinese Medicine*, vol. 38, no. 4, pp. 661–673, 2010.

[10] G. Hu, R. S. Walls, D. Bass et al., "The Chinese herbal formulation Biminne in management of perennial allergic rhinitis: a randomized, double-blind, placebo-controlled, 12-week clinical trial," *Annals of Allergy, Asthma and Immunology*, vol. 88, no. 5, pp. 478–487, 2002.

[11] A. P. Lu, H. W. Jia, C. Xiao, and Q. P. Lu, "Theory of traditional chinese medicine and therapeutic method of diseases," *World Journal of Gastroenterology*, vol. 10, no. 13, pp. 1854–1856, 2004.

[12] W. Y. Jiang, "Therapeutic wisdom in traditional Chinese medicine: a perspective from modern science," *Trends in Pharmacological Sciences*, vol. 26, no. 11, pp. 558–563, 2005.

[13] P. Y. Hsu, Y. H. Yang, Y. T. Lin, and B. L. Chiang, "Serum eosinophil cationic protein level and disease activity in childhood rhinitis," *Asian Pacific Journal of Allergy and Immunology*, vol. 22, no. 1, pp. 19–24, 2004.

[14] L. Winther, L. Moseholm, C. M. Reimert, P. S. Skov, and L. K. Poulsen, "Basophil histamine release, IgE, eosinophil counts, ECP, and EPX are related to the severity of symptoms in seasonal allergic rhinitis," *Allergy*, vol. 54, no. 5, pp. 436–445, 1999.

[15] R. A. Nathan, R. Eccles, P. H. Howarth, S. K. Steinsvåg, and A. Togias, "Objective monitoring of nasal patency and nasal physiology in rhinitis," *Journal of Allergy and Clinical Immunology*, vol. 115, no. 3, pp. S442–S459, 2005.

[16] R. Castano, C. Trudeau, and H. Ghezzo, "Correlation between acoustic rhinometry and subjective nasal patency during nasal challenge test in subjects with suspected occupational rhinitis; a prospective controlled study," *Clinical Otolaryngology*, vol. 35, no. 6, pp. 462–467, 2010.

[17] R. F. André, H. D. Vuyk, A. Ahmed, K. Graamans, and G. J. Nolst Trenité, "Correlation between subjective and objective evaluation of the nasal airway. A systematic review of the highest level of evidence," *Clinical Otolaryngology*, vol. 34, no. 6, pp. 518–525, 2009.

[18] Z. Liu and L. Liu, "Holism in CM," in *Essentials of Chinese Medicine*, vol. 1, pp. 36–38, Springer, New York, NY, USA, 2009.

[19] Z. Liu and L. Liu, "Theory of Yin-Yang," in *Essentials of Chinese Medicine*, vol. 1, pp. 11–17, Springer, New York, NY, USA, 2009.

[20] C. J. Hogeboom, K. J. Sherman, and D. C. Cherkin, "Variation in diagnosis and treatment of chronic low back pain by traditional Chinese medicine acupuncturists," *Complementary Therapies in Medicine*, vol. 9, no. 3, pp. 154–166, 2001.

[21] G. G. Zhang, W. Lee, B. Bausell, L. Lao, B. Handwerger, and B. Berman, "Variability in the Traditional Chinese Medicine (TCM) diagnoses and herbal prescriptions provided by three TCM practitioners for 40 patients with rheumatoid arthritis," *Journal of Alternative and Complementary Medicine*, vol. 11, no. 3, pp. 415–421, 2005.

[22] Y. G. Jung, K. H. Kim, H. Y. Kim, H. J. Dhong, and S. K. Chung, "Predictive capabilities of serum eosinophil cationic protein, percentage of eosinophils and total immunoglobulin E in allergic rhinitis without bronchial asthma," *Journal of International Medical Research*, vol. 39, no. 6, pp. 2209–2216, 2011.

[23] L. G. Sensi, F. Marcucci, E. Migali, and G. Coniglio, "Eosinophil cationic protein and specific IgE in serum and nasal mucosa of patients with grass-pollen-allergic rhinitis and asthma," *Allergy*, vol. 56, no. 3, pp. 231–236, 2001.

[24] M. F. Kramer, G. Burow, E. Pfrogner, and G. Rasp, "In vitro diagnosis of chronic nasal inflammation," *Clinical and Experimental Allergy*, vol. 34, no. 7, pp. 1086–1092, 2004.

[25] A. Sin, E. Terzioğlu, A. Kokuludağ, F. Sebik, and T. Kabakçi, "Serum eosinophil cationic protein (ECP) levels in patients with seasonal allergic rhinitis and allergic asthma," *Allergy and Asthma Proceedings*, vol. 19, no. 2, pp. 69–73, 1998.

[26] A. C. Ferguson, R. Vaughan, H. Brown, and C. Curtis, "Evaluation of serum eosinophilic cationic protein as a marker of disease activity in chronic asthma," *Journal of Allergy and Clinical Immunology*, vol. 95, no. 1, pp. 23–28, 1995.

[27] D. Wang, P. Clement, J. Smitz, M. De Waele, and M. P. Derde, "Correlations between complaints, inflammatory cells and mediator concentrations in nasal secretions after nasal allergen challenge and during natural allergen exposure," *International Archives of Allergy and Immunology*, vol. 106, no. 3, pp. 278–285, 1995.

[28] O. Hilberg, "Objective measurement of nasal airway dimensions using acoustic rhinometry: methodological and clinical aspects," *Allergy*, vol. 57, no. 70, pp. 5–39, 2002.

[29] H. Terheyden, S. Maune, J. Mertens, and O. Hilberg, "Acoustic rhinometry: validation by three-dimensionally reconstructed computer tomographic scans," *Journal of Applied Physiology*, vol. 89, no. 3, pp. 1013–1021, 2000.

[30] B. Mamikoglu, S. Houser, I. Akbar, B. Ng, and J. P. Corey, "Acoustic rhinometry and computed tomography scans for the diagnosis of nasal septal deviation, with clinical correlation," *Otolaryngology - Head and Neck Surgery*, vol. 123, no. 1, pp. 61–68, 2000.

[31] J. Numminen, P. Dastidar, T. Heinonen, T. Karhuketo, and M. Rautiainen, "Reliability of acoustic rhinometry," *Respiratory Medicine*, vol. 97, no. 4, pp. 421–427, 2003.

[32] R. Roithmann, P. Cole, J. Chapnik, S. M. Barreto, J. P. Szalai, and N. Zamel, "Acoustic rhinometry, rhinomanometry, and the sensation of nasal patency: a correlative study," *Journal of Otolaryngology*, vol. 23, no. 6, pp. 454–458, 1994.

[33] P. Khanna and A. Shah, "Categorization of patients with allergic rhinitis: a comparative profile of "sneezers and runners" and 'blockers'," *Annals of Allergy, Asthma and Immunology*, vol. 94, no. 1, pp. 60–64, 2005.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 968083, 11 pages
doi:10.1155/2012/968083

Research Article

Application of Metabolomics in Traditional Chinese Medicine Differentiation of Deficiency and Excess Syndromes in Patients with Diabetes Mellitus

Tao Wu,[1,2] Ming Yang,[3] Hua-Feng Wei,[4] Song-Hua He,[4] Shun-Chun Wang,[5] and Guang Ji[2]

[1] *Center of Chinese Medicine Therapy and Systems Biology, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*
[2] *Institute of Digestive Disease, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200032, China*
[3] *Department of Medicament, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200032, China*
[4] *Department of Internal Medicine, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200032, China*
[5] *Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*

Correspondence should be addressed to Shun-Chun Wang, wsc@shutcm.edu.cn and Guang Ji, jiliver@vip.sina.com

Received 18 February 2012; Revised 9 April 2012; Accepted 16 April 2012

Academic Editor: Wei Jia

Metabolic profiling is widely used as a probe in diagnosing diseases. In this study, the metabolic profiling of urinary carbohydrates was investigated using gas chromatography/mass spectrometry (GC/MS) and multivariate statistical analysis. The kernel-based orthogonal projections to latent structures (K-OPLS) model were established and validated to distinguish between subjects with and without diabetes mellitus (DM). The model was combined with subwindow permutation analysis (SPA) in order to extract novel biomarker information. Furthermore, the K-OPLS model visually represented the alterations in urinary carbohydrate profiles of excess and deficiency syndromes in patients with diabetes. The combination of GC/MS and K-OPLS/SPA analysis allowed the urinary carbohydrate metabolic characterization of DM patients with different traditional Chinese medicine (TCM) syndromes, including biomarkers different from non-DM patients. The method presented in this study might be a complement or an alternative to TCM syndrome research.

1. Introduction

Diabetes mellitus (DM) is a complex metabolic disorder characterized by chronic hyperglycemia, hypoinsulinemia, and ketosis. In 2000, around 171 million people were affected with DM. By 2030, this number is estimated to increase to 366 million [1]. Current statistics shows that over 10% of the world's aged population (60 years and above) suffers from this disease, and 90% of these patients have type 2 diabetes mellitus (T_2DM) [2]. Diabetes always causes high morbidity and mortality rates due to chronic microvascular complications (e.g., retinopathy, nephropathy, or neuropathy) and macrovascular complications (e.g., ischemic cardiac problems, cerebral vascular accidents, and peripheral vascular disorders) [3].

In ancient China, DM was recognized as *xiaokezheng*, a disease with symptomatic polydipsia. Traditional Chinese medicine (TCM) has a long history of treatments for *xiaokezheng* [4]. According to TCM theory, Yin (things associated with the physical form of an object), Yang (things associated with energetic qualities), Qi (life force that animates the forms of the world), and Xue (dense form of body fluids that have been acted upon and energized by Qi) [5] are in an unbalanced state when people are suffering from a disease. Similarly, patients with DM could be classified as having deficiency syndrome or excess syndrome, which refers to the organs' insufficiency or excess in Qi, Xue, Yin, and Yang.

Metabolic profiling is defined as the quantitative measurement of the dynamic multiparametric response of a living system to pathophysiological stimuli or genetic modification [6]. The objective of metabolomics is to gain new insight into the pathophysiology of a disease and identify individual metabolites or profiles of metabolites as potential biomarkers that can distinguish between normal and

pathological states [7]. Metabolomics has been used in the diagnosis and evaluation of diabetic patients [8] because of its effectiveness in evaluating systemic responses to any subtle metabolic perturbation. In addition, it has also been used in the identification of potential biomarkers [9].

Recent animal and human metabolomic studies have investigated the metabolic effects of oral glucose challenge [10–12], insulin resistance [13–18], type 1 [19, 20] or T_2DM [20–28]. Previous studies investigated the metabolic profiling of plasma phospholipids in T_2DM using liquid chromatography/mass chromatography (LC/MS) coupled with multivariate statistical analysis [29]. Methods based on plasma fatty acid profiles analyzed via GC/MS were also developed to investigate the differences between T_2DM patients and healthy volunteers [30]. A multianalytical platform method using GC/MS and ultra performance liquid chromatography-mass spectrometry (UPLC/MS) was developed to obtain the global metabolite profiles of DM in rat models [31]. An imbalance between carbohydrate and lipid metabolisms is involved in the etiology and pathophysiology of diabetes. Therefore, a metabolic analysis is necessary to visualize the alteration of globally circulating metabolites in a person suffering from diabetes. In the present study, a metabolic profiling was performed using GC/MS of urinary carbohydrates in subjects with and without DM.

Partial least square linear discriminant analysis (PLSLDA) is currently the common method used in supervised linear modeling in the field of metabolomics. However, the relationship between the disease and metabolic data displays nonlinear characteristics in some cases. Therefore, nonlinear modeling has been applied in metabolomics [32, 33]. Recently, the "kernel trick" has been efficient in dealing with nonlinear problems. Kernel-based orthogonal projections to latent structures (K-OPLS) [34, 35] can considerably improve the predictive performance in situations where a strong nonlinear relationship exists. Model population analysis (MPA) was developed based on the idea of statistically analyzing the outputs of Monte Carlo Sampling (MCS)-derived "population" of models. The MPA-based method is expected to provide some comprehensive insights into the data because it allows the statistical analysis of some interesting outputs of several models. One typical MPA-based method can be used to identify important variables by examining the distribution of prediction errors of all the submodels [36]. Subwindow permutation analysis (SPA) was used in the present study to reveal informative metabolites by incorporating the Monte Carlo technique and strictly implementing the idea of MPA [37, 38].

Several diabetes-related studies have been reported in recent years. However, the metabolic profiles involved in the pathological processes of diabetes are yet to be addressed. Thus, the identification of biomarkers is needed for the adequate screening and diagnosis of diabetes. Syndrome differentiation is an important element in TCM theories and is the basis for the treatments of all diseases, including DM. Therefore, the TCM syndromes of patients with DM are necessary to characterize. However, previous studies have not revealed the differences among the urinary carbohydrate metabolites in the TCM syndromes of these patients. In the present work, we conducted a comparative analysis of 366 subjects using GC/MS combined with K-OPLS/SPA analysis to (1) compare the urinary carbohydrate profiles of subjects with and without DM, (2) compare the relationship between urine carbohydrate levels and TCM syndromes in subjects with DM, and (3) determine the characteristics and differences in TCM syndrome distribution between excess and deficiency syndromes.

2. Materials and Methods

2.1. Chemicals. Carbohydrate standards (C_4 sugar 1, inositol C, talose, mannose, inositol D, glucose, inositol A, arabinose, xylose, and C_4 sugar 2) were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (HPLC grade), methanol (HPLC grade), and methylimidazole were purchased from Fisher/Aldrich (NJ, USA). Sodium borohydride ($NaBH_4$), dimethyl sulfoxide, trifluoracetic acid, acetic acid, acetic anhydride, and chloroform (analytical grade) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Water was obtained from a Milli-Q ultra-pure water system (Millipore, Billerica, USA).

2.2. Clinical Research Design. DM patients from the Tianlin Community Health Service Center, Shanghai city of P.R. China August 2009 to May 2010 were prospectively included in the study. All 366 samples included 308 patients with DM (241 deficiency and 67 excess samples) and 58 patients without DM as the comparison group.

Patients were required to abstain from eating greasy and sweet food before the study to avoid an interference with the metabolism of the human body. Study protocol was approved by the Ethics Committee of the Hospital, and a written informed consent was obtained from each respondent. Each blood sample collected in a fasting condition was immediately centrifuged at $3000\times$ g for 10 min, and the plasma was transferred into a clean tube. All urine samples collected in fasting condition and plasma samples were stored at -80°C until analysis.

2.3. Inclusion and Syndrome Differentiation Criteria. Based on the criteria formulated by the World Health Organization in 1999, DM is characterized by a fasting plasma glucose (FPG) of $\geq$7.0 mmol/L, a postload plasma glucose (2h PG) of $\geq$11.1 mmol/L, or a history of oral hypoglycemic or insulin use, or both [39]. TCM syndromes, including deficiency and excess syndromes, were differentiated according to the guidelines [40]. The information gathered from inspection, auscultation, and inquiring was obtained on the day of admission. Manifestations and other diagnostic information were determined independently by three experienced physicians to ensure an objective evaluation. If the three were in accordance, the subject will be included in the study. Otherwise, he/she will be excluded.

2.4. Exclusion Criteria. Patients suffering from other serious diseases involving major organs or infective diseases were excluded from the study. Moreover, those who cannot or

are not willing to complete the study or those who had psychiatric disorders or intellectual dysfunctions were also excluded.

2.5. Clinical and Laboratory Assessment. Clinical data including date of birth, height, weight, body mass index (BMI), waist and hip circumference, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were determined by a senior physician. Obesity is characterized by a BMI of $\geq 25.0\,kg/m^2$ according to the Asian guidelines [41]. Serum levels of alanine aminotransferase (ALT), FPG, glycated hemoglobin (HbA1c), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein cholesterol (VLDL-C) in fasting condition, and 2h PG were measured using an automatic biochemical analyzer (Hitachi7180, Tokyo, Japan).

2.6. Sample Preparation of Urine for GC/MS. A 200 μL sample of urine from each group was blended with 20 μL of ammonia and 1 μL of 0.5 mol/L $NaBH_4$/dimethyl sulfoxide (DMSO). Acetic acid (100 μL) was added dropwise to reduce the abundance of $NaBH_4$ after the reduction reaction (120 min at 40°C). Acetylation (10 min at 40°C) was performed after adding 200 μL of 1 methylimidazole and 1 mL of acetic anhydride. Subsequently, 2 mL of water was mixed with the extracts for 10 min at 40°C, and the mixtures were extracted with 2 mL of chloroform. The samples were centrifuged (4000× g for 10 min), and the supernatant was discarded. The samples were washed with 5 mL of water to remove the chloroform layer. The remaining layer was added with 1 g of sodium sulfate and taken for GC/MS. Allose (20 μL) was used as an internal standard to be added into each 200 μL sample.

2.7. GC/MS Conditions. GC/MS was performed using a Finnigan gas chromatograph (ThermoFinnigan, USA) coupled with a mass spectrometer (TRACE DSQ). A TR-5ms capillary column (60 m × 0.25 mm × 0.25 μm, Thermo) was used in the gas chromatographic system. The inlet temperature was 250°C. Column temperature was increased from an initial 140°C to 198°C (2°C per min for 4 min). It was then programmed from 198°C to 214°C (4°C per min), 214°C to 217°C (1°C per min for 4 min), and 217°C to 250°C (3°C per min for 5 min). Inlet temperature was maintained at 250°C. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The GC/MS was injected with 1 μL aliquots. The mass spectrometer was operated in electron impact and full-scan monitoring modes (m/z 40–450) with 0.2 s/scan velocity. Source temperature, electron energy, and solvent delay were set at 250°C, 70 eV, and 10 min, respectively.

2.8. Data Analysis and Software. All data were processed by the Xcalibur software (ThermoFinnigan, USA), and the detected peaks were aligned using hand integral methods. The ion peak area for each detected peak was normalized by NIST 05 Standard mass spectral databases in the NIST MS search 2.0 (NIST, Gaithersburg, MD, USA) software. Semiquantitative concentrations of urinary monosaccharides were obtained through the ratio of the peak area to the standard. The K-OPLS package (available at http://kopls.sourceforge.net/download.shtml) and Statistic toolbox of the MATLAB (version 7.1, Mathwork Inc.) software were used in the statistical treatment of the data and application of various multivariate methods. Parts of the source codes used in implementing SPA in MATLAB were freely available at http://code.google.com/p/spa2010/downloads/list.

Data are shown as mean ± standard deviations (SD). In addition, significance was expressed through independent t-tests for continuous variables and Pearson Chi-square tests for categorical variables using the SPSS 17.0 software (SPSS, Chicago, Ill, USA). Fisher's exact tests were calculated when the expected frequencies were less than 5 in any cell. A P value of <0.05 was considered to indicate statistical significance.

2.9. K-OPLS Models for Classification. Based on our previous work [42] and related literature [34, 43], the K-OPLS model was employed in the present study to build a classifier, with σ as the parameter for the Gaussian kernel function. The kernel matrix K was centered to model estimation. The K-OPLS algorithm modeled the kernel matrix K through a set of predictive and Y-orthogonal components. Thus, the predictive score matrix and the Y-orthogonal score vector were estimated. After the estimation step of each Y-orthogonal component, K was deflated using the Y-orthogonal variation, followed by a subsequent updating of the predictive score matrix and further estimation of Y-orthogonal components. The kernel function parameter (σ) and the number of Y-orthogonal components (Ao) of the K-OPLS model were optimized using 10-fold cross-validation. All the samples were randomly partitioned into 10 equally sized folds according to their categories. Subsequently, 10 iterations of calibration and validation were performed. As a result, onefold of the data was held out for validation, whereas the remaining nine folds were used for calibration. Details on the model are provided in the previous work.

2.10. Revealing Informative Metabolites through Statistical Assessment of Variable Importance. Previous studies [37, 44] indicated that the SPA method used for uncovering informative metabolites is constructed based on the prediction error distribution of the K-OPLS models, which are based on the subdatasets obtained through Monte Carlo sampling in both sample and variable space.

In the equation $DMEAN_j = MEAN_{j,B} - MEAN_{j,A}$, $MEAN_{j,A}$ and $MEAN_{j,B}$ denote the mean prediction errors calculated by the normal K-OPLS and the latter permuted K-OPLS models of the jth metabolite, respectively. If $DMEAN_j > 0$, the inclusion of the jth metabolite in the K-OPLS model may improve the predictive performance. This type of metabolite is deemed as a candidate of informative metabolites in the present study. By contrast, if $DMEAN_j <$

0, the inclusion of this metabolite into a model may most probably reduce the predictive performance. Therefore, this type of metabolite is considered uninformative/interfering.

With these preparations, the informative metabolites were identified in the following successive steps. (1) All the metabolites with $\mathrm{DMEAN}_j < 0$ were removed. (2) The Mann-Whitney U test was used in the remaining metabolites to check the significance of the difference between the two distributions. (3) The metabolites were ranked using the P value. The metabolites with P values smaller than the predefined threshold (e.g., 0.01) were considered informative metabolites, whereas those with P values larger than the threshold were considered uninformative metabolites. The P values calculated in this manner are conditional in all other metabolites because both normal prediction errors and permuted prediction errors are dependent on all other metabolites included in all the subwindows [37, 44]. Usually, the more important a metabolite is, the higher the score assigned to it. In this case, a so-called Conditional Synergetic Score (COSS) is defined as the minus logarithm-transformed P value:

$$\mathrm{COSS}_i = \begin{cases} -\log_{10}(P_i), & \mathrm{DMEN}_i > 0 \\ -\log_{10}(P_i + 1), & \mathrm{DMEN}_i \leq 0. \end{cases} \quad (1)$$

Clearly, the more significant a metabolite is, the higher the score it will get. Particularly, a metabolite with $P < 0.01$ will have a COSS > 2. Thus, the informative metabolites revealed via SPA may be considered the most probable biomarker candidates.

3. Results

3.1. Clinical Characteristics of Excess and Deficiency Syndromes in Patients with DM. Clinical characteristics of the 366 subjects are summarized in Table 1. Among the 366 subjects, 308 (84.1%) were diagnosed with DM, 67 (21.8%) of which had excess syndrome. The patients with deficiency syndromes were significantly more likely to be older than those with excess syndromes in the DM group ($P < 0.01$). However, other statistical significances were not found. The systolic blood pressure, serum fasting and post-load glucose levels, and glycated hemoglobin were significantly higher in subjects with DM compared with those without DM ($P < 0.001$). However, opposite results were found for incorporative hyperlipidemia ($P < 0.001$).

3.2. GC/MS Profiles of Urine Samples. Based on the previously developed method and related literature [45], the GC/MS parameters were optimized for the Thermo GC/MS system used in the present study. This system allowed the detection of several peaks from the GC/MS chromatogram within 50 min of analysis cycle. The typical total-ion chromatograms from the GC/MS of urine samples from DM patients are shown in Figure 1. Ten urinary carbohydrate metabolites were identified in patients with and without DM using standards, and their peak areas were integrated for further multivariate analysis.

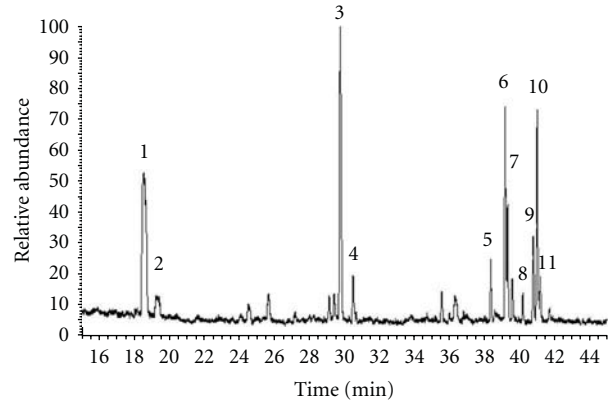


Figure 1: GC/MS profiles of carbohydrate metabolites from urine of the DM patients: (1) C_4 sugar 1, (2) C_4 sugar 2, (3) arabinose, (4) xylose, (5) inositol A, (6) allose (the internal standard), (7) inositol C, (8) talose, (9) mannose, (10) glucose, and (11) inositol D.

3.3. Classification of the K-OPLS Models. All the samples were used to build models. In the present study, K-OPLS was performed using the Gaussian kernel function. σ and Ao were optimized using 10-fold cross-validation. Accuracy of classification of cross-validation (ACCV) was calculated for each combination of σ and Ao. These parameters were optimized by generating models with σ and Ao values of 0.1 to 10 and 1 to 10, respectively.

Figure 1 shows the results after cross-validation. ACCV was the largest at $\sigma = 0.5$ and Ao $= 1$ for DM and non-DM as well as for excess and deficiency syndrome groups. These optimal parameters were selected to model for these two groups, respectively (Figures 2(a) and 2(b)).

Tenfold cross-validation was applied to evaluate the predictive abilities of the constructed K-OPLS-DA models. The primary data were divided into 10 sets. One set was the "test set," and the others were the "training sets," which were repeatedly calculated 10 times to obtain the components. Table 2 shows the Q2Y, R2Y, and R2X used in evaluating all the calibration models of the two groups. R2X and R2Y were defined as the explained variation of the input (metabolic data) and output variables (disease category data), respectively. Q2Y denoted the prediction statistics over cross-validation for the classification task [46]. The values of these parameters approaching 1.0 indicate a stable model with a predictive reliability [47]. High coefficient values of R2Y and Q2Y represent good prediction [48]. As displayed by the score plots of K-OPLS (Figure 3(a)), the two sample groups can be separated into distinct clusters to indicate the changes in the metabolic response of the DM and non-DM urine samples. The samples in the excess and deficiency groups were also clearly separated (Figure 3(b)). The R2X, R2Y, and Q2Y of the former model were 0.591, 1, and 0.853, respectively, whereas those of the latter model were 0.543, 1, and 0.783, respectively (Table 2). These results indicated that the models had a good ability of explaining and predicting the variations in the X and Y matrices.

Table 1: Clinical and biological characteristics of excess and deficiency syndromes in patients with DM.

	Subjects with DM (n = 308)				Subjects without DM (n = 58)	
	Total	Excess	Deficiency	P^a	Total	P^b
Gender (male/female, n)	308(116/192)	67(27/40)	241(89/152)	0.718	58(22/36)	0.969
Age (year)	70.32 ± 9.08	65.09 ± 9.71	71.77 ± 8.36	<0.001	67.84 ± 10.84	0.066
BMI (kg/m^2)	25.28 ± 2.96	24.92 ± 3.01	25.38 ± 2.92	0.261	26.10 ± 3.11	0.056
Waist circumference (cm)	91.01 ± 8.42	89.88 ± 8.70	91.32 ± 8.33	0.213	91.55 ± 8.22	0.654
Hip circumference (cm)	101.24 ± 7.58	100.50 ± 7.81	101.44 ± 7.52	0.358	101.53 ± 6.33	0.781
Waist-to-hip ratio (WHR)	0.90 ± 0.06	0.89 ± 0.05	0.90 ± 0.06	0.439	0.90 ± 0.07	0.743
Obese (BMI ≥ 25)	51.3%(158/308)	49.3%(33/67)	51.9%(125/241)	0.810	60.3%(35/58)	0.262
Hypertension	93.2%(287/308)	89.6%(60/67)	94.2%(227/241)	0.290	98.3%(57/58)	0.232
Hyperlipidemia	39.3%(121/308)	37.3%(25/67)	39.8%(96/241)	0.816	81.0%(47/58)	<0.001
Coronary heart disease	21.8%(67/308)	23.9%(16/67)	21.2%(51/241)	0.757	25.9%(15/58)	0.605
Cerebrovascular accident	0.07%(22/308)	0.06%(4/67)	0.07%(8/241)	0.878	0.07%(4/58)	1.000
Hyperuricemia	0.07%(23/308)	0.06%(4/67)	0.08%(19/241)	0.791	0.09%(5/58)	0.973
Fatty liver disease	75.3%(232/308)	67.2%(45/67)	77.6%(187/241)	0.112	87.9%(51/58)	0.053
SBP (mmHg)	138.68 ± 14.37	137.34 ± 13.71	139.05 ± 14.55	0.391	133.00 ± 14.22	0.006
DBP (mmHg)	78.70 ± 9.44	79.97 ± 9.23	78.35 ± 9.48	0.209	79.10 ± 8.84	0.764
FPG (mmol/L)	7.43 ± 1.95	7.56 ± 2.21	7.39 ± 1.88	0.994	5.78 ± 1.41	<0.0001
2h PG (mmol/L)	11.25 ± 3.70	11.21 ± 3.54	11.26 ± 3.75	0.918	7.69 ± 3.30	<0.0001
HbA1c (%)	7.25 ± 1.35	7.13 ± 1.31	7.28 ± 1.36	0.461	6.17 ± 0.96	<0.0001
TG (mmol/L)	1.53 ± 0.92	1.45 ± 0.79	1.56 ± 0.96	0.908	1.87 ± 1.31	0.068
HDL cholesterol (mmol/L)	1.34 ± 0.36	1.31 ± 0.26	1.35 ± 0.39	0.473	1.39 ± 0.39	0.363
AST (U/L)	25.21 ± 12.83	27.14 ± 13.49	24.68 ± 12.61	0.171	26.38 ± 13.93	0.537
VLDL cholesterol (mmol/L)	2.56 ± 0.56	2.55 ± 0.59	2.56 ± 0.55	0.951	2.48 ± 0.57	0.322

[a] P value refers to the comparison between excess versus deficiency syndromes within the DM group. [b] P value refers to the comparison between subjects with and without DM using chi-square test or t-test analysis.

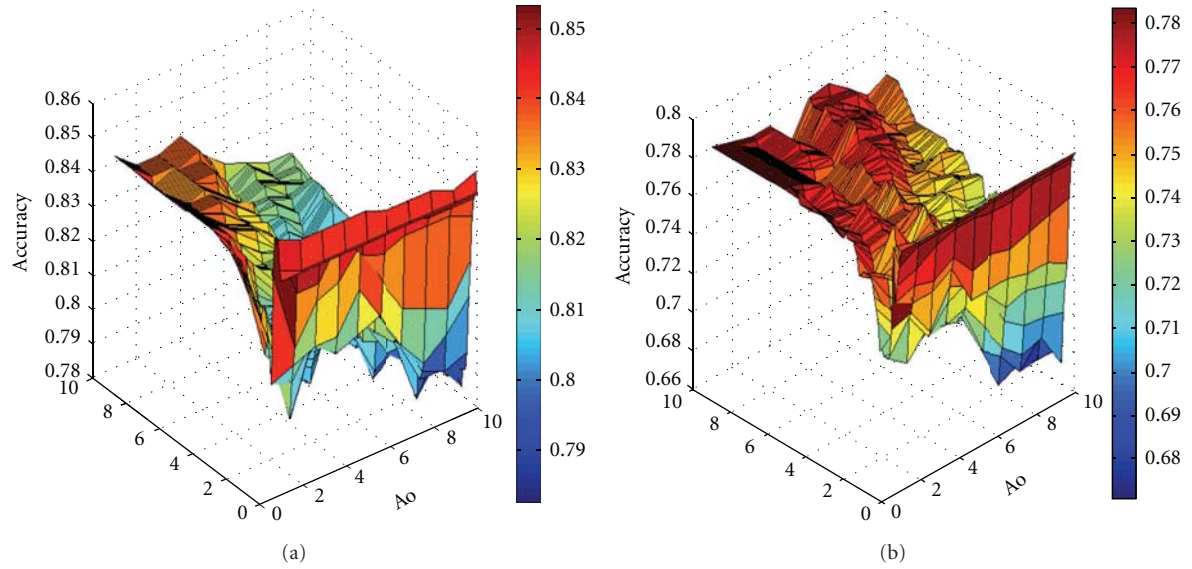


Figure 2: Accuracy of classification of cross-validation (ACCV) produced from each combination of σ and Ao parameters after cross-validation. ACCV was the largest when σ = 0.5 and Ao = 1 for (a) DM and non-DM subjects as well as for (b) excess and deficiency groups.

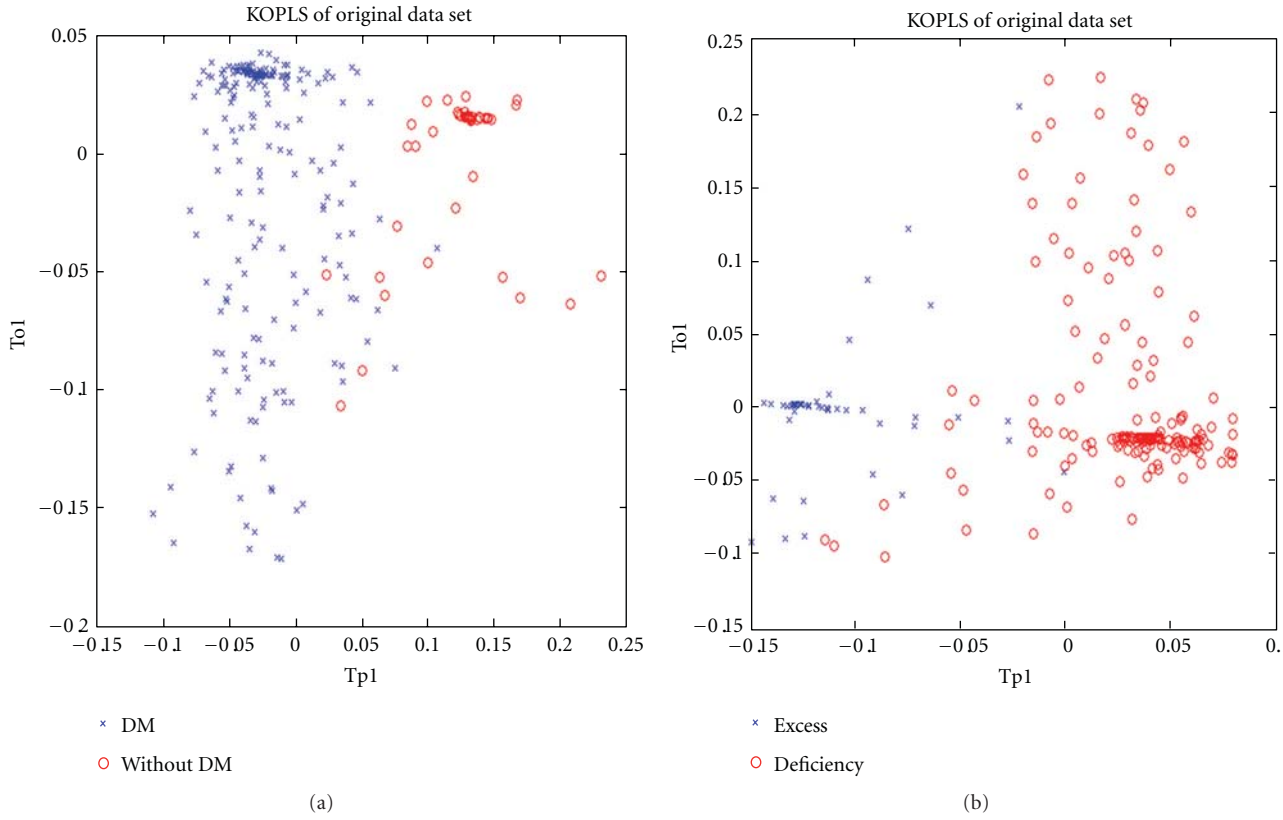


Figure 3: First predictive and Y-orthogonal score components, depicting how the Y-orthogonal variation was captured by the K-OPLS model. (a) Changes in the metabolic response in the urine of DM and non-DM patients. (b) Clear separation of the excess and deficiency groups.

Table 2: Results of prediction of the K-OPLS models.

Models	σ	Ao	R2X	R2Y	Q2Y
DM and non-DM	0.5	1	0.591	1.000	0.853
Excess and deficiency	0.5	1	0.543	1.000	0.783

3.4. Differential Metabolites from SPA Based on the K-OPLS Models. For this data, the number of Monte Carlo Simulation (N), ratio of calibration samples to the total samples (R), and number of variables to be sampled in each Monte Carlo Simulation (Q) of SPA were set to 1000, 0.8, and 8, respectively.

Each metabolite was first standardized with zero mean and unit variance before further analysis. With this setup, the SPA was applied to the data, and the P value of each metabolite was computed through the Mann-Whitney U test (Figures 4(a) and 4(b)). The corresponding COSS for each metabolite is shown in Figures 4(c) and 4(d).

The two plots of DM and non-DM data obviously suggest that metabolites, including C_4 sugar 1, inositol C, mannose, inositol D, glucose, and C_4 sugar 2, were of small P values (smaller than 0.01) and COSS $>$ 2. These six metabolites may possibly be formative metabolites or biomarkers. Thus, they should be included in further analysis. The remaining four metabolites were of high P values and COSS $<$ 2. The first six significant metabolites were selected to have the best metabolite patterns, which collectively showed high prediction abilities in the clinical outcome. Combined with the t-test results ($P < 0.05$), the four metabolites were as follows: C_4 sugar 1, inositol D, glucose, and C_4 sugar 2. Similarly, the variables C_4 sugar 1, C_4 sugar 2, inositol C, talose, and xylose were found to have $P < 0.01$ and COSS > 2 in the excess and deficiency group data. However, based on the t-test results, only xylose and C_4 sugar 2 were statistically significant in the two groups.

4. Discussion

TCM is a medical system with at least 3000 years of uninterrupted clinical practice. It has the advantage of collecting macroscopic information of a patient for diagnosis, with syndrome as the core of diagnosis and therapy in TCM [49]. Nowadays, the diagnosis of syndromes in TCM mainly relies on four examinations (inspection, listening and smelling examinations, inquiry, and palpation). Outcomes of TCM diagnoses may lack consistency among TCM doctors [50, 51]. Thus, the accuracy is relatively low. The use of objective indices in syndrome diagnosis in TCM may significantly improve accuracy.

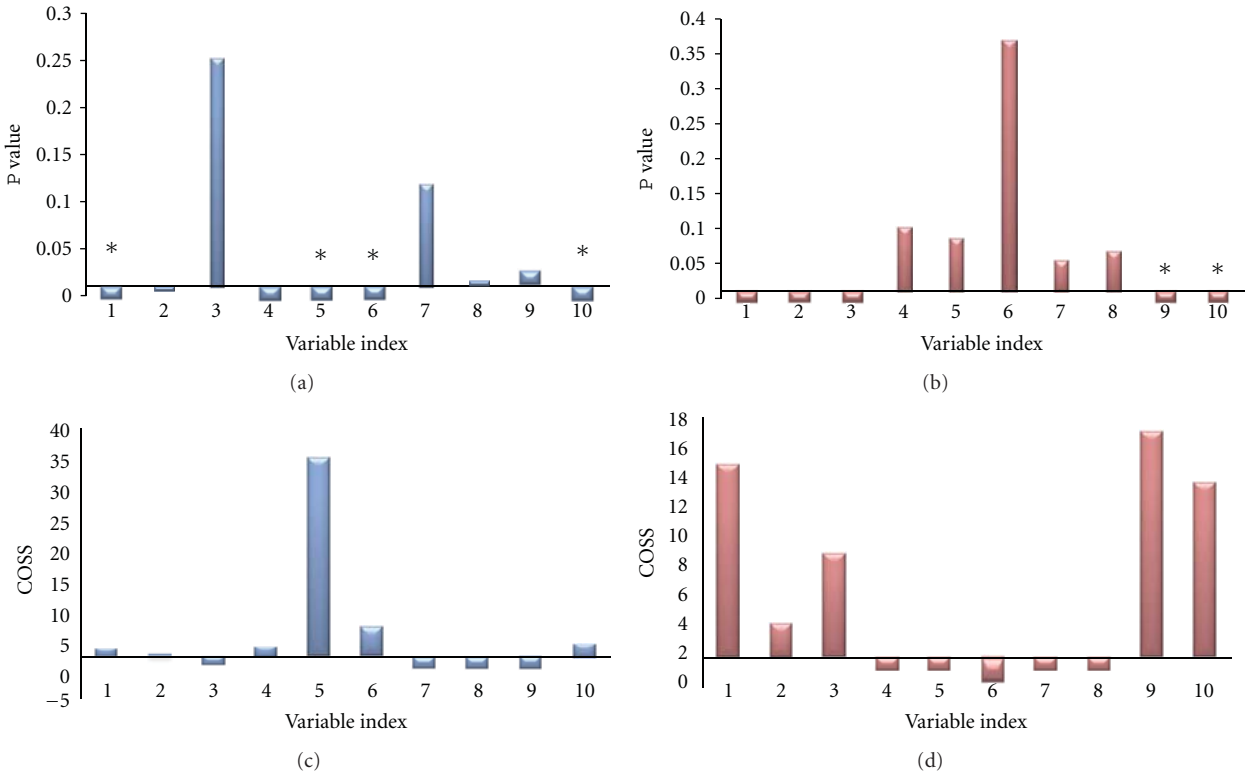


Figure 4: The computed P values and COSS through SPA for DM and non-DM group data (a and c) and excess and deficiency group data (b and d). The variable index consists of the following: (1) C_4 sugar 1, (2) inositol C, (3) talose, (4) mannose, (5) inositol D, (6) glucose, (7) inositol A, (8) arabinose, (9) xylose, and (10) C_4 sugar 2. *Represents $P < 0.05$ from the t-test between groups.

Until now, syndromes in TCM have always been studied in a specific disease or biomedical condition. In addition, several studies have demonstrated that syndromes are significantly associated with diseases [49, 52, 53]. However, the biological basis of a syndrome in the context of a disease is rarely studied. The issue is significantly critical because it not only establishes a diagnostic avenue in a microcosmic level but also divides the disease into several subtypes and provides a basis for individual therapy. The establishment of a diagnostic method in the microcosmic level is an urgent and major problem in TCM [54].

DM is characterized by two major defects: a dysregulation in pancreatic hormone secretion and a decrease in insulin action on target tissues (insulin resistance). These abnormalities are related to several defects in insulin-signaling mechanisms and several steps in regulating glucose metabolism (transport and key enzymes of glycogen synthesis or mitochondrial oxidation) [55]. The development of strategies to diagnose, prevent, or delay the progression of DM has gained increasing interest because of its high morbidity and mortality rates. TCM has played an important role in lowering blood glucose and controlling the development of DM. Many studies have shown that TCM, such as Radix Astragali, Radix Rehmanniae, and Radix Trichosanthis, also has hypoglycemic effects [56]. Thus, the present study was designed to determine whether metabolomics is useful and powerful enough to differentiate between the deficiency and excess syndromes of TCM using DM as a model.

The systolic blood pressure, serum concentrations of fasting and post-load glucose, and glycated hemoglobin were significantly higher in subjects with DM than in those without DM. This result is in accordance with the characteristics of diabetes. By contrast, no clear difference was found between the two groups. This result reflects that the two subject groups had relative backgrounds in terms of age, sex, waist circumference, hip circumference, WHR, diastolic blood pressure, TG, ALT, VLDL, and HDL levels, except for the incidence of incorporative hyperlipidemia.

The deficiency syndrome patients were older than the excess. This finding is in agreement with the TCM theory that Qi, Xue, Yin, and Yang are more insufficient in older than in younger people. However, other differences including biochemical values were not found between the two groups. This result implies that the TCM syndromes are difficult to differentiate using the clinical biochemical indicators. Therefore, TCM syndromes should be distinguished using other methods.

Considering the intrinsic relationship between TCM theory and systems biology, some researchers began to discuss the prospective application of metabolomics to TCM theory. Metabolic profiling has been recently exploited in the pathophysiological studies of diseases [57–60]. However,

only a few reports concerning the metabolomics approach in TCM research have been found in the current literature [61, 62]. In the present study, a GC/MS-based metabolomic approach was used for determining the biochemical profiles of different TCM syndrome types in DM. Moreover, the method was also used in testing whether the metabolomics approach is powerful enough to differentiate TCM syndrome types.

With the development of metabolomics, the data-mining technique has become increasingly mature. Its advantages are very applicable to the complex correlativity study of TCM syndromes and metabolites. However, the relationship between disease and metabolic data displayed nonlinear characteristics in the present study. Therefore, good models were not performed using the PLSLDA or OPLSDA method, such as R2X < 0.3 or Q2Y < 0.1. The nonlinear classification model K-OPLS had later shown stronger classification ability than the PLSLDA and OPLSDA linear classifiers.

In the present study, we first discovered that the comprehensive differences of metabolic intermediates between subjects with and without DM focused mainly on those involved in glucose metabolism. The study identified ten carbohydrate compositions, including C_4 sugar 1, inositol C, talose, mannose, inositol D, glucose, inositol A, arabinose, xylose, and C_4 sugar 2. Based on the results of K-OPLS/SPA, six and five possible markers with $P < 0.01$ and COSS > 2 were found in DM and non-DM subjects and excess and deficiency groups, respectively. T-test was also used to compute the P value for each metabolite. Clearly, the results of t-test were not comparable with those of SPA. Two or three of them had no significant difference between groups based on the t-test ($P > 0.05$), further suggesting that the conditional P value calculated via SPA was much more informative. The main reason may be that the variable importance computed using SPA can reflect the synergetic effect to some extent [44]. Therefore, one metabolite may not be alone in a disease status but interacts with other metabolites.

Consequently, four intermediates including inositol D, C_4 sugar 2, glucose, and C_4 sugar 1 produced during glycolysis were elevated in the DM group samples. The high prediction performance of the four metabolites indicates that they are possible biomarker candidates for DM. Furthermore, two potential biomarkers, xylose and C_4 sugar 2, were discovered in the two syndromes using K-OPLS/SPA and t-test. These potential biomarkers can be identified by the MS database and corresponding standards.

Metabolites are endogenous and exogenous molecules that play a role in cellular regulatory and biological systems. Glucose is the major source of energy production and macromolecule biosynthesis in maintaining the normal state of the body. Highly active glycolysis and an impaired Krebs cycle guarantee enough metabolic intermediates by avoiding thorough oxidation of glucose. This phenomenon is essential for the synthesis of macromolecules, such as lipid, protein, and nuclear acid, during cell division [63–65]. The circulating glucose is filtrated by the glomerulus and absorbed by the renal tubules. Therefore, healthy human urine should not contain any sugar. Hyperglycemia, other metabolic disorders, and chronic complications due to an absolute lack of insulin and/or a reduction of the biological effects of insulin may cause the appearance of corresponding sugars in urinary metabolites. For example, 4-carbon sugars are the intermediate products of glucose metabolism. Inositol, a water-soluble vitamin, can play insulin-like roles on a metabolic enzyme. Mannose is a sugar monomer of the aldohexose series of carbohydrates and a C-2 epimer of glucose. It cannot be metabolized well in vivo. Hence, 90% of mannose will be discharged through the urine within 30 min to 60 min, and 99% of mannose in residual urine will be excreted in the next 8 h. Arabinose is a monosaccharide containing five carbon atoms and is decomposed into glucose and fructose by intestinal sucrose. Sucrose is involved in amino and nucleotide sugar metabolisms. Xylose is the connection unit between the sugar chain and serine or threonine as a combined form in vivo. Talose, also called hydrolysis of lactose, has an unknown significance so far. Therefore, the above components were present in the urine of DM patients. This finding indicates the presence of significant glucose metabolism disorders in diabetes.

Metabolic profiling can sensitively reflect all physiological and pathological changes. Moreover, it can elucidate the "syndrome" concept in TCM complex physiological systems. Using all metabolites in the evaluation of the human health status is more accurate and comprehensive than using a single index [66, 67]. The present study indicated that xylose and C_4 sugar 2 were higher in the excess than in the deficiency group. Therefore, the holistic application of metabolic profiling in studying the syndrome essence of TCM is reasonable. In summary, these potential biomarkers reflected the deregulation of glucose metabolism in diabetic individuals, which might help in DM diagnosis and TCM syndrome differentiation.

5. Conclusions

This research strongly supported that metabolic profiling analysis combined with K-OPLS and SPA is a powerful tool in revealing metabolic differences between various groups, obtaining valuable information to probe molecular mechanisms, and discovering the scientific connotation of TCM theory. Larger randomized trials with an appropriate methodology, including the study of diabetic patients with different TCM syndromes, are required to confirm the results of the present study.

Authors Contribution

T. Wu and M. Yang have equally contributed to this paper.

Acknowledgments

The authors are grateful to the anonymous reviewers and the editors for their helpful comments and suggestions, which substantially improved the quality of this paper. The authors are indebted to all the patients who participated in this study.

This study was supported by the National Natural Science Foundation of China (30872360), the Leading Academic Discipline Project and Innovative Research Team in Universities from Shanghai Municipal Education Commission (J50305 & E3008), and the Natural Science Foundation of Shanghai (11ZR1436900). There is no conflict of interests involved in this paper.

References

[1] S. Wild, G. Roglic, A. Green, R. Sicree, and H. King, "Global prevalence of diabetes: estimates for the year 2000 and projections for 2030," *Diabetes Care*, vol. 27, no. 5, pp. 1047–1053, 2004.

[2] L. Yi, J. He, Y. Liang, D. Yuan, H. Gao, and H. Zhou, "Simultaneously quantitative measurement of comprehensive profiles of esterified and non-esterified fatty acid in plasma of type 2 diabetic patients," *Chemistry and Physics of Lipids*, vol. 150, no. 2, pp. 204–216, 2007.

[3] C. Castell, "Epidemiology and classification for diabetes mellitus," *Revista de Enfermería*, vol. 33, no. 2, pp. 9–15, 2010.

[4] G. Ning, J. Hong, Y. Bi et al., "Progress in diabetes research in China," *Journal of Diabetes*, vol. 1, no. 3, pp. 163–172, 2009.

[5] L. M. Wang, X. Zhao, X. L. Wu et al., "Diagnosis analysis of 4 TCM patterns in sub-optimal health status: a structural equation modeling approach," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 970985, 6 pages, 2012.

[6] J. K. Nicholson, J. Connelly, J. C. Lindon, and E. Holmes, "Metabonomics: a platform for studying drug toxicity and gene function," *Nature Reviews Drug Discovery*, vol. 1, no. 2, pp. 153–161, 2002.

[7] N. Malandrino and R. J. Smith, "Personalized medicine in diabetes," *Clinical Chemistry*, vol. 57, no. 2, pp. 231–240, 2011.

[8] J. L. Griffin and A. Vidal-Puig, "Current challenges in metabolomics for diabetes research: a vital functional genomic tool or just a ploy for gaining funding?" *Physiological Genomics*, vol. 34, no. 1, pp. 1–5, 2008.

[9] M. van Doorn, J. Vogels, A. Tas et al., "Evaluation of metabolite profiles as biomarkers for the pharmacological effects of thiazolidinediones in type 2 diabetes mellitus patients and healthy volunteers," *British Journal of Clinical Pharmacology*, vol. 63, no. 5, pp. 562–574, 2007.

[10] O. Shaham, R. Wei, T. J. Wang et al., "Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity," *Molecular Systems Biology*, vol. 4, article 214, 2008.

[11] S. Wopereis, C. M. Rubingh, M. J. van Erk et al., "Metabolic profiling of the response to an oral glucose tolerance test detects subtle metabolic changes," *PLoS ONE*, vol. 4, no. 2, Article ID e4525, 2009.

[12] X. Zhao, A. Peter, J. Fritsche et al., "Changes of the plasma metabolome during an oral glucose tolerance test: is there more than glucose to look at?" *American Journal of Physiology - Endocrinology and Metabolism*, vol. 296, no. 2, pp. E384–E393, 2009.

[13] J. Chen, X. Zhao, J. Fritsche et al., "Practical approach for the identification and isomer elucidation of biomarkers detected in a metabonomic study for the discovery of individuals at risk for diabetes by integrating the chromatographic and mass spectrometric information," *Analytical Chemistry*, vol. 80, no. 4, pp. 1280–1289, 2008.

[14] R. S. Plumb, K. A. Johnson, P. Rainville et al., "The detection of phenotypic differences in the metabolic plasma profile of three strains of Zucker rats at 20 weeks of age using ultra-performance liquid chromatography/orthogonal acceleration time-of-flight mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 20, no. 19, pp. 2800–2806, 2006.

[15] J. Shearer, G. Duggan, A. Weljie, D. S. Hittel, D. H. Wasserman, and H. J. Vogel, "Metabolomic profiling of dietary-induced insulin resistance in the high fat-fed C57BL/6J mouse," *Diabetes, Obesity and Metabolism*, vol. 10, no. 10, pp. 950–958, 2008.

[16] A. A. Toye, M. E. Dumas, C. Blancher et al., "Subtle metabolic and liver gene transcriptional changes underlie diet-induced fatty liver susceptibility in insulin-resistant mice," *Diabetologia*, vol. 50, no. 9, pp. 1867–1879, 2007.

[17] R. E. Williams, E. M. Lenz, M. Rantalainen, and I. D. Wilson, "The comparative metabonomics of age-related changes in the urinary composition of male Wistar-derived and Zucker (fa/fa) obese rats," *Molecular BioSystems*, vol. 2, no. 3-4, pp. 193–202, 2006.

[18] R. Williams, E. M. Lenz, A. J. Wilson et al., "A multi-analytical platform approach to the metabonomic analysis of plasma from normal and Zucker (fa/fa) obese rats," *Molecular BioSystems*, vol. 2, no. 3-4, pp. 174–183, 2006.

[19] V. P. Mäkinen, P. Soininen, C. Forsblom et al., "1H NMR metabonomics approach to the disease continuum of diabetic complications and premature death," *Molecular Systems Biology*, vol. 4, article no. 167, 2008.

[20] S. Zhang, G. A. Nagana Gowda, V. Asiago, N. Shanaiah, C. Barbas, and D. Raftery, "Correlative and quantitative 1H NMR-based metabolomics reveals specific metabolic pathway disturbances in diabetic rats," *Analytical Biochemistry*, vol. 383, no. 1, pp. 76–84, 2008.

[21] G. T. Gipson, K. S. Tatsuoka, R. J. Ball et al., "Multi-platform investigation of the metabolome in a leptin receptor defective murine model of type 2 diabetes," *Molecular BioSystems*, vol. 4, no. 10, pp. 1015–1023, 2008.

[22] T. Huo, S. Cai, X. Lu, Y. Sha, M. Yu, and F. Li, "Metabonomic study of biochemical changes in the serum of type 2 diabetes mellitus patients after the treatment of metformin hydrochloride," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 49, no. 4, pp. 976–982, 2009.

[23] R. M. Salek, M. L. Maguire, E. Bentley et al., "A metabolomic comparison of urinary changes in type 2 diabetes in mouse, rat, and human," *Physiological Genomics*, vol. 29, no. 2, pp. 99–108, 2007.

[24] M. van Doorn, J. Vogels, A. Tas et al., "Evaluation of metabolite profiles as biomarkers for the pharmacological effects of thiazolidinediones in type 2 diabetes mellitus patients and healthy volunteers," *British Journal of Clinical Pharmacology*, vol. 63, no. 5, pp. 562–574, 2007.

[25] X. Zhang, Y. Wang, F. Hao et al., "Human serum metabonomic analysis reveals progression axes for glucose intolerance and insulin resistance statuses," *Journal of Proteome Research*, vol. 8, no. 11, pp. 5188–5195, 2009.

[26] Y. Bao, T. Zhao, X. Wang et al., "Metabonomic variations in the drug-treated type 2 diabetes mellitus patients and healthy volunteers," *Journal of Proteome Research*, vol. 8, no. 4, pp. 1623–1630, 2009.

[27] H. Li, Y. Ni, M. Su et al., "Pharmacometabonomic phenotyping reveals different responses to xenobiotic intervention in rats," *Journal of Proteome Research*, vol. 6, no. 4, pp. 1364–1370, 2007.

[28] J. R. Bain, R. D. Stevens, B. R. Wenner, O. Ilkayeva, D. M. Muoio, and C. B. Newgard, "Metabolomics applied to diabetes research: moving from information to knowledge," *Diabetes*, vol. 58, no. 11, pp. 2429–2443, 2009.

[29] C. Wang, H. Kong, Y. Guan et al., "Plasma phospholipid metabolic profiling and biomarkers of type 2 diabetes mellitus based on high-performance liquid chromatography/electrospray mass spectrometry and multivariate statistical analysis," *Analytical Chemistry*, vol. 77, no. 13, pp. 4108–4116, 2005.

[30] L. Z. Yi, J. He, Y. Z. Liang, D. L. Yuan, and F. T. Chau, "Plasma fatty acid metabolic profiling and biomarkers of type 2 diabetes mellitus based on GC/MS and PLS-LDA," *FEBS Letters*, vol. 580, no. 30, pp. 6837–6845, 2006.

[31] R. Williams, E. M. Lenz, A. J. Wilson et al., "A multi-analytical platform approach to the metabonomic analysis of plasma from normal and Zucker (fa/fa) obese rats," *Molecular BioSystems*, vol. 2, no. 3-4, pp. 174–183, 2006.

[32] R. Goodacre, D. Broadhurst, A. K. Smilde et al., "Proposed minimum reporting standards for data analysis in metabolomics," *Metabolomics*, vol. 3, no. 3, pp. 231–241, 2007.

[33] X. Lin, Q. Wang, P. Yin et al., "A method for handling metabonomics data from liquid chromatography/mass spectrometry: combinational use of support vector machine recursive feature elimination, genetic algorithm and random forest for feature selection," *Metabolomics*, vol. 7, pp. 549–558, 2011.

[34] M. Rantalainen, M. Bylesjö, O. Cloarec, J. K. Nicholson, E. Holmes, and J. Trygg, "Kernel-based orthogonal projections to latent structures (K-OPLS)," *Journal of Chemometrics*, vol. 21, no. 7–9, pp. 376–385, 2007.

[35] M. Bylesjö, M. Rantalainen, J. K. Nicholson, E. Holmes, and J. Trygg, "K-OPLS package: kernel-based orthogonal projections to latent structures for prediction and interpretation in feature space," *BMC Bioinformatics*, vol. 9, article 106, 2008.

[36] D. S. Cao, Y. Z. Liang, Q. S. Xu, H. D. Li, and X. Chen, "A new strategy of outlier detection for QSAR/QSPR," *Journal of Computational Chemistry*, vol. 31, no. 3, pp. 592–602, 2010.

[37] H. D. Li, Y. Z. Liang, Q. S. Xu, and D. S. Cao, "Model population analysis for variable selection," *Journal of Chemometrics*, vol. 24, no. 7-8, pp. 418–423, 2010.

[38] X. Li, Z. Xu, X. Lu et al., "Comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry for metabonomics: biomarker discovery for diabetes mellitus," *Analytica Chimica Acta*, vol. 633, no. 2, pp. 257–262, 2009.

[39] World Health Organisation, "Definition, diagnosis, and classification of diabetes mellitus and its complications," Report of a WHO Consultation. Part 1: Diagnosis and Classification of Diabetes Mellitus, World Health Organisation, Geneva, Switzerland, 1999.

[40] Y. Y. Zheng, *Guiding Principle of Clinical Research on New Drugs of Traditional Chinese Medicine*, Chinese Medicine Science and Technology, Beijing, China, 2002.

[41] V. Choo, "WHO reassesses appropriate body-mass index for Asian populations," *The Lancet*, vol. 360, no. 9328, article 235, 2002.

[42] M. Yang, J. L. Chen, X. F. Shi, and H. J. Niu, "Rapid determination of aesculin, aesculetin and fraxetin in cortex fraxini extract solutions based on ultraviolet spectroscopy," *E-Journal of Chemistry*, vol. 8, supplement 1, pp. S225–S236, 2011.

[43] P. Filzmoser, B. Liebmann, and K. Varmuza, "Repeated double cross validation," *Journal of Chemometrics*, vol. 23, no. 4, pp. 160–171, 2009.

[44] H. D. Li, M. M. Zeng, B. B. Tan, Y. Z. Liang, Q. S. Xu, and D. S. Cao, "Recipe for revealing informative metabolites based on model population analysis," *Metabolomics*, vol. 6, no. 3, pp. 353–361, 2010.

[45] J. H. Xie, M. Y. Xie, S. P. Nie, M. Y. Shen, Y. X. Wang, and C. Li, "Isolation, chemical composition and antioxidant activities of a water-soluble polysaccharide from *Cyclocarya paliurus* (Batal.) Iljinskaja," *Food Chemistry*, vol. 119, no. 4, pp. 1626–1632, 2010.

[46] K. Yuan, H. Kong, Y. Guan, J. Yang, and G. Xu, "A GC-based metabonomics investigation of type 2 diabetes by organic acids metabolic profile," *Journal of Chromatography B*, vol. 850, no. 1-2, pp. 236–240, 2007.

[47] Y. Ni, Y. Wang, and S. Kokot, "Multicomponent kinetic spectrophotometric determination of pefloxacin and norfloxacin in pharmaceutical preparations and human plasma samples with the aid of chemometrics," *Spectrochimica Acta A*, vol. 70, no. 5, pp. 1049–1059, 2008.

[48] J. Girard, "Contribution of free fatty acids to impairment of insulin secretion and action. Mechanism of β-cell lipotoxicity," *Medecine/Sciences*, vol. 21, pp. 19–25, 2005.

[49] H. H. Zhao, J. X. Chen, N. Hou et al., "Discovery of diagnosis pattern of coronary heart disease with Qi Deficiency syndrome by the T-test-based adaboost algorithm," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 408650, 7 pages, 2011.

[50] J. J. Y. Sung, W. K. Leung, J. Y. L. Ching et al., "Agreements among traditional Chinese medicine practitioners in the diagnosis and treatment of irritable bowel syndrome," *Alimentary Pharmacology and Therapeutics*, vol. 20, no. 10, pp. 1205–1210, 2004.

[51] G. G. Zhang, B. Bausell, L. Lao, W. L. Lee, B. Handwerger, and B. Berman, "The variability of TCM pattern diagnosis and herbal prescription on rheumatoid arthritis patients," *Alternative Therapies in Health and Medicine*, vol. 10, no. 1, pp. 58–63, 2004.

[52] S. Li, Z. Q. Zhang, L. J. Wu, X. G. Zhang, Y. D. Li, and Y. Y. Wang, "Understanding ZHENG in traditional Chinese medicine in the context of neuro-endocrine-immune network," *IET Systems Biology*, vol. 1, no. 1, pp. 51–60, 2007.

[53] G. L. Kang, S. Li, and J. F. Zhang, "Entropy-based model for interpreting life systems in traditional Chinese medicine," *Evidence-based Complementary and Alternative Medicine*, vol. 5, no. 3, pp. 273–279, 2008.

[54] P. H. Chiu, H. Y. Hsieh, and S. C. Wang, "Prescriptions of traditional chinese medicine are specific to cancer types and adjustable to temperature changes," *PLoS One*, vol. 7, no. 2, Article ID e31648, 2012.

[55] J. Trygg and S. Wold, "Orthogonal projections to latent structures (O-PLS)," *Journal of Chemometrics*, vol. 16, no. 3, pp. 119–128, 2002.

[56] W. Xie and L. Du, "Diabetes is an inflammatory disease: evidence from traditional Chinese medicines," *Diabetes, Obesity and Metabolism*, vol. 13, no. 4, pp. 289–301, 2011.

[57] M. Coen, E. Holmes, J. C. Lindon, and J. K. Nicholson, "NMR-based metabolic profiling and metabonomic approaches to problems in molecular toxicology," *Chemical Research in Toxicology*, vol. 21, no. 1, pp. 9–27, 2008.

[58] J. C. Lindon, E. Holmes, M. E. Bollard, E. G. Stanley, and J. K. Nicholson, "Metabonomics technologies and their applications in physiological monitoring, drug safety assessment and disease diagnosis," *Biomarkers*, vol. 9, no. 1, pp. 1–31, 2004.

[59] J. K. Nicholson and J. C. Lindon, "Systems biology: metabonomics," *Nature*, vol. 455, no. 7216, pp. 1054–1056, 2008.

[60] H. Schmid, A. Henger, and M. Kretzler, "Molecular approaches to chronic kidney disease," *Current Opinion in Nephrology and Hypertension*, vol. 15, no. 2, pp. 123–129, 2006.

[61] Y. Qiu, M. Chen, M. Su et al., "Metabolic profiling reveals therapeutic effects of *Herba Cistanches* in an animal model of hydrocortisone-induced "kidney-deficiency syndrome"," *Chinese Medicine*, vol. 3, article 3, 2008.

[62] M. Chen, L. Zhao, and W. Jia, "Metabonomic study on the biochemical profiles of a hydrocortisone-induced animal model," *Journal of Proteome Research*, vol. 4, no. 6, pp. 2391–2396, 2005.

[63] R. J. Deberardinis, N. Sayed, D. Ditsworth, and C. B. Thompson, "Brick by brick: metabolism and tumor cell growth," *Current Opinion in Genetics and Development*, vol. 18, no. 1, pp. 54–61, 2008.

[64] X. Tong, F. Zhao, and C. B. Thompson, "The molecular determinants of de novo nucleotide biosynthesis in cancer cells," *Current Opinion in Genetics and Development*, vol. 19, no. 1, pp. 32–37, 2009.

[65] E. Hervouet, H. Simonnet, and C. Godinot, "Mitochondria and reactive oxygen species in renal cancer," *Biochimie*, vol. 89, no. 9, pp. 1080–1088, 2007.

[66] L. D. Zhao, X. D. Wo, Y. Li et al., "Effects of warming and tonifying kidney, yang drugs on liver mitochondria proteome of rats with kidney yang deficiency," *Chinese Journal of TCM and Pharmacy*, vol. 22, no. 2, pp. 102–107, 2007.

[67] R. Goodacre, "Metabolic profiling: pathways in discovery," *Drug Discovery Today*, vol. 9, no. 6, pp. 260–261, 2004.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 546230, 8 pages
doi:10.1155/2012/546230

Research Article

Clinical Data Mining of Phenotypic Network in Angina Pectoris of Coronary Heart Disease

Jianxin Chen,[1] Peng Lu,[2] Xiaohan Zuo,[2] Qi Shi,[1] Huihui Zhao,[1] Liangtao Luo,[1] Jianqiang Yi,[2] Chenglong Zheng,[1] Yi Yang,[1] and Wei Wang[1]

[1] *Beijing University of Chinese Medicine, 11 Bei San Huan Dong Lu, ChaoYang District, Beijing 100029, China*
[2] *Institute of Automation, Chinese Academy of Sciences, 95 Dong Lu, Zhong-guan-cun, Hai Dian District, Beijing 100190, China*

Correspondence should be addressed to Wei Wang, wangwei@bucm.edu.cn

Received 14 January 2012; Revised 13 March 2012; Accepted 13 March 2012

Academic Editor: Shao Li

Coronary heart disease (CHD) is the leading causes of morbidity and mortality in China. The diagnosis of CHD in Traditional Chinese Medicine (TCM) was mainly based on experience in the past. In this paper, we proposed four MI-based association algorithms to analyze phenotype networks of CHD, and established scale of syndromes to automatically generate the diagnosis of patients based on their phenotypes. We also compared the change of core syndromes that CHD were combined with other diseases, and presented the different phenotype spectra.

1. Introduction

Coronary heart disease (CHD) is the leading causes of morbidity and mortality in China [1].

Angina pectoris (AP) is one of the most common types of CHD. Its treatment in modern medicine mainly includes nitrates, β-blockers, Ca^{2+} channel blockers, and coronary angioplasty or coronary artery bypass graft surgery. However, its side effects could be ignored. Traditional Chinese Medicine (TCM) presented a complementary and alternative avenue to treating AP of CHD. It uses a holistic concept to balance whole body, not like western medicine whose treatment of AP places heavily on healing of the heart organ.

TCM has a history of more than 1000 years to fight with CHD. The Chinese ancients used words "thoracic obstruction (Xiongbi in Mandarin)" to describe phenotypes of CHD and piled thousands of formula to treat CHD. The key concept of TCM is syndrome, which is the core of TCM diagnosis and therapy theory. A syndrome is composed of a set of phenotypes, Wu et al. [2] proposed a computational framework called CIPHER that integrates information from phenotypes and genes, and the preferable results confirmed the biological significance of phenotypes. Li et al. [3] investigated the key pathological principle, ZHENG, in the context of the neuroendocrine immune (NEI) system and reported their important finding about predominant parts in the Cold/Hot ZHENG network, the connections between these two networks, and interaction pathways the genes related to ZHENG-related diseases were mainly present in. All of these were subsequently verified by experiments on a rate model of collagen-induced arthritis. Their excellent work demonstrated the thousand-year-old concept of ZHENG might have a molecular basis with NEI as background for the first time.

The past decades of CHD syndrome-related research effort place heavily on blood stasis syndrome (BSS). Most of them were to investigate biological basis of blood stasis syndrome in the context of CHD, for example, proteomic study of BSS [4], animal model establishment of BSS in the context of myocardial infarction [5], the association between BSS and clinical biological index [6], or the action mechanism of formula on treating BSS [7]. Despite these progresses made in complementary and alternative research of CHD, the standardization and modernization of syndromes in the context of CHD are still far from need of worldwide clinical applications. The correct diagnosis of syndromes in the context of CHD plays a key role in modernization of syndromes. However, due to complex pathopoiesis factors of CHD and relatively simple statistical data analysis methods, a diagnostic scale of syndromes in CHD was hard to establish.

Traditionally, a syndrome scale was build according to three steps. The first was to determine phenotype pool of the syndrome. Then, the score or weight of each phenotype was computed. The final step was to determine a diagnostic threshold of the syndrome. Among these, the first step is most important. Till now, the most used method to determine phenotype pool was subjective, for example, by using TCM experts' questionnaire, which is hard to enhance diagnosis accuracy of syndromes. The complex data analysis methods for establishing diagnostic scale of syndromes were urgent.

In this paper, we presented mutual-information- (MI-) based complex system computational methods to objectively determine phenotype pools of syndromes. We carried out a large sample cohort of CHD subjects. Four MI-based association algorithms were compared to retrieve phenotype pairs with significant association. The phenotype networks were established accordingly. A validation algorithm was presented to choose a better algorithm, and thus phenotype pool of each syndrome in the context of CHD was determined. We also investigate different phenotype spectra of CHD when combined with hypertension, diabetes, hyperlipemia, and chronic heart failure.

2. Materials and Methods

2.1. AP of CHD Cohort. 2050 AP subjects aged between 45 and 75 were collected from 7 clinical centers located in 7 provinces in China from the same demographic area and at the same time from November 2008 to November 2010. Stable AP was strictly diagnosed according to *ACC/AHA/ACP-ASIM Guidelines for the Management of Patients with Chronic Stable Angina* [8]. Unstable AP was diagnosed as per *Diagnosis and Treatment Recommendation of Unstable Angina Pectoris* published by Chinese Society of Cardiology [8]. The exclusion criteria were composed of four conditions. (1) Patients with acute myocardial infarction, myocarditis, pericardial disease, cardiac neurosis, intercostal neuralgia, menopausal syndrome, and severe chest pain caused by cervical spondylosis were excluded; (2) patients with AP caused by other diseases such as rheumatic fever, syphilis, congenital coronary abnormalities, hypertrophic cardiomyopathy, aortic stenosis, or regurgitation were excluded; (3) patients with combined diseases such as stroke, pulmonary infection, nephritis, renal failure, urinary tract infections, rheumatism, severe arrhythmia, cancer, liver, kidney, hematopoietic system, primary and other serious diseases, uncontrolled hypertension or systolic blood pressure $\geqslant$180 mmHg or diastolic blood pressure $\geqslant$110 mmHg after blood pressure control were also excluded; (4) pregnancy or breast-feeding women, patients with allergy (included in the state except when the nonallergic), or the mentally ill were excluded from the cohort.

The study protocol was approved by both the ethics committee of Dongzhimen Hospital affiliated to Beijing University of Chinese Medicine and the local ethics committee of the collaborative hospitals. All subjects who included in the study provided written informed consent.

Table 1: Basic statistics of 2050 cohort of AP.

	Frequency	Percentage
Male/female	1361/689	66.4%/33.6%
Hypertension	1374	67%
Diabetes	552	26.9%
Hyperlipemia	420	20.5%
Chronic heart failure	520	25.4%

2.2. Phenotype Information Determination and Collection. Besides demographic information, characteristics of disease history, medication information, as well as main symptoms and signs in western medicine, 107 phenotypic variables composed of symptoms, signs, tongue, and pulse information were also carefully investigated. They were collected by watching, listening, inquiring, and pulse feeling. The inclusion of the 107 variables was determined by a combination of three avenues. Firstly, literatures with AP and Traditional Chinese Medicine were fully collected from publicly accessed databases. All phenotypic variables were manually acquired from the literatures. Synonym and phenotype with similar clinical meaning were combined, forming a candidate pool of TCM phenotype terms for AP of CHD. Alternatively, two rounds of TCM experts questionnaire were carried out to screen a compact set of phenotype variables based on an idea that clinical experts consensus on the phenotype information of diseases could reduce the complexity of phenotype and increase the objectivity of the determination of phenotype to be clinically investigated. Finally, a preliminary clinical epidemiology of 100 AP cases was performed to investigate frequency of each phenotype. A cut of 5% was used to determine a final version of phenotypes of AP.

2.3. Data Analysis. Frequency of each phenotype was computed and descending ranked. Association between phenotypes was calculated by revised mutual information [9]. Four computational algorithms were used or presented to retrieve several numbers of associations to construct phenotype network for AP. A validation strategy was presented to evaluate each network and screen a better algorithm for building such network. The subnetwork of AP combined with hypertension, diabetes hyperlipemia, or chronic heart failure was constructed, respectively. The difference between each subnetwork was significantly understood to investigate phenotype spectra of AP when combined with distinctive diseases. Pajek 2.0 was used to build complex phenotype networks [10].

3. Results and Discussion

3.1. Basic Statistics. Table 1 listed the basic information of demography and combined diseases of the study cohort. The average age of the AP subjects was 62.95 $\pm$ 10.56. Hypertension occupied more than 67% of AP cohort, indicating that it is a key risk factor to AP by the retrospective epidemiology. Nearly two in three AP patients are male. As shown in Figure 1, eight phenotypes appeared in more

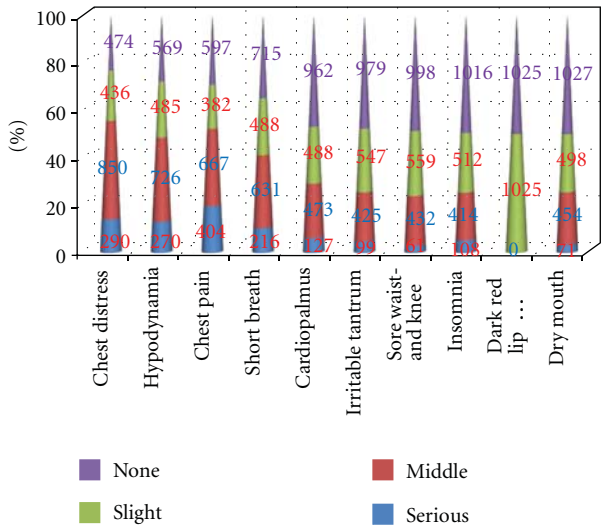


Figure 1: The initial 10 phenotypes and their frequencies of four classes, that is, serious, middle, slight, and none. Eight phenotypes occurred at more than 50% of subjects.

than 50% subjects. The most frequent phenotype in AP subject was chest distress, which is a typical symptom of AP. It is surprising that hypodynamia is with slightly higher frequency than chest pain. The latter is an anther typical phenotype following with AP. However, this situation is solvable by mean of viewpoint of TCM. Hypodynamia is a characteristic symptom of Qi deficiency syndrome in TCM, which is considered as key pathology of AP.

Mutual information is good at quantitatively describing association between categorical variables. As depicted in Table 2, the top 10 phenotype pair and their association were given. A phenotype with an asterisk in the right cornu superius means that it is in the list of top 10 phenotypes of AP. It is found that phenotype with high-frequency phenotype was prone to associated with the other high-frequency phenotype. However, they only occupied 50% of top 10 phenotype pairs with highest MI, which indicated that MI could balance between frequency and association. A phenotype pair with high MI association not only showed a high value of cooccurrence but also described a high frequency of co-nonoccurrence. The latter usually makes two totally adverse and useless phenotypes highly associated (data not shown here). Thus, the revised MI was used here to prevent negative association from positive association pairs.

The inherent drawback of MI algorithm is that it ignores frequency of the features, so it is inclined to select lower-frequency features such as co-nonoccurrence phenotype pairs. For this reason, we proposed a revised MI that takes use the "positive occurrence frequency" to control the growth of co-nonoccurrence pairs in MI computation. The positive occurrence frequency is defined as the frequency of cooccurrence of phenotype pairs. The positive occurrence frequency of strong correlation phenotypes is bigger (close to 1), and, in theory, the positive occurrence frequency of adverse phenotypes should be 0, for that it is impossible for

Table 2: The top 10 phenotype pairs with largest revised mutual information in AP.

Phenotype pair		Revised mutual information
Chest distress*	Short breath*	0.29219
Periorbital edema	Edema of lower limbs	0.262114
Short breath*	Hypodynamia*	0.219433
Chest pain*	Chest distress*	0.219238
Cough	white phlegm	0.215073
Sighing	Depression	0.202779
Short breath*	Cardiopalmus*	0.190918
Amnesia	dizziness	0.181577
Anorexia	Tastelessness in the mouth	0.158838
Chest distress*	Cardiopalmus*	0.14342

one patient to get two adverse phenotypes at the same time. So we redefine the MI as

$$\Delta\mu\left(X_i, X_j\right) = \begin{cases} H(X_i) + H\left(X_j\right) - H\left(X_i, X_j\right), & \mathrm{Po}(i,j) \geq \delta, \\ H(X_i) + H\left(X_j\right) - b^* H\left(X_i, X_j\right), & \mathrm{Po}(i,j) \geq \delta, \end{cases} \quad (1)$$

where $\mathrm{Po}(i, j)$ is the positive occurrence frequency of feature i and j, δ is preassigned positive quantity, we call it POF threshold in this paper. When $\delta = 0$, the revised version of MI is the traditional form of MI, so the revised MI is an extended version of traditional MI. b is a real number and is greater than 1, it can be seen as a penalty coefficient.

It is this better merit of MI that its four extensions would be used to establish phenotype network of AP and to further investigate the association between subnetworks and syndrome in TCM.

3.2. Complex Phenotype Network. The four MI-based algorithms only presented information on various computational methods of associations between phenotypes. Significant association algorithm was defined to determine number of associated phenotypes where the network was established. A phenotype pair that composed of P_A and P_B was defined as significant association as follows: $P_A \in R(P_B)$ and $P_B \in R(P_A)$. Where $R(P_A)$ and $R(P_B)$ denoted the top N associated phenotypes of the phenotype P_A and P_B, respectively. The number N was determined by presenting a concept of information utilization, which was defined as ratio of maximal number of phenotypes in discovered pattern to N. Here, $N = 6$ was found to achieve a high information utilization with 83.33% (equal to 5/6). 107 phenotypes were retrieved their $R(P_i, i = 1, 2, \ldots, 107)$ according to revised MI, respectively, resulting a number of 120 significant association pairs were computed. The other three MI-based algorithms were presented as follows.

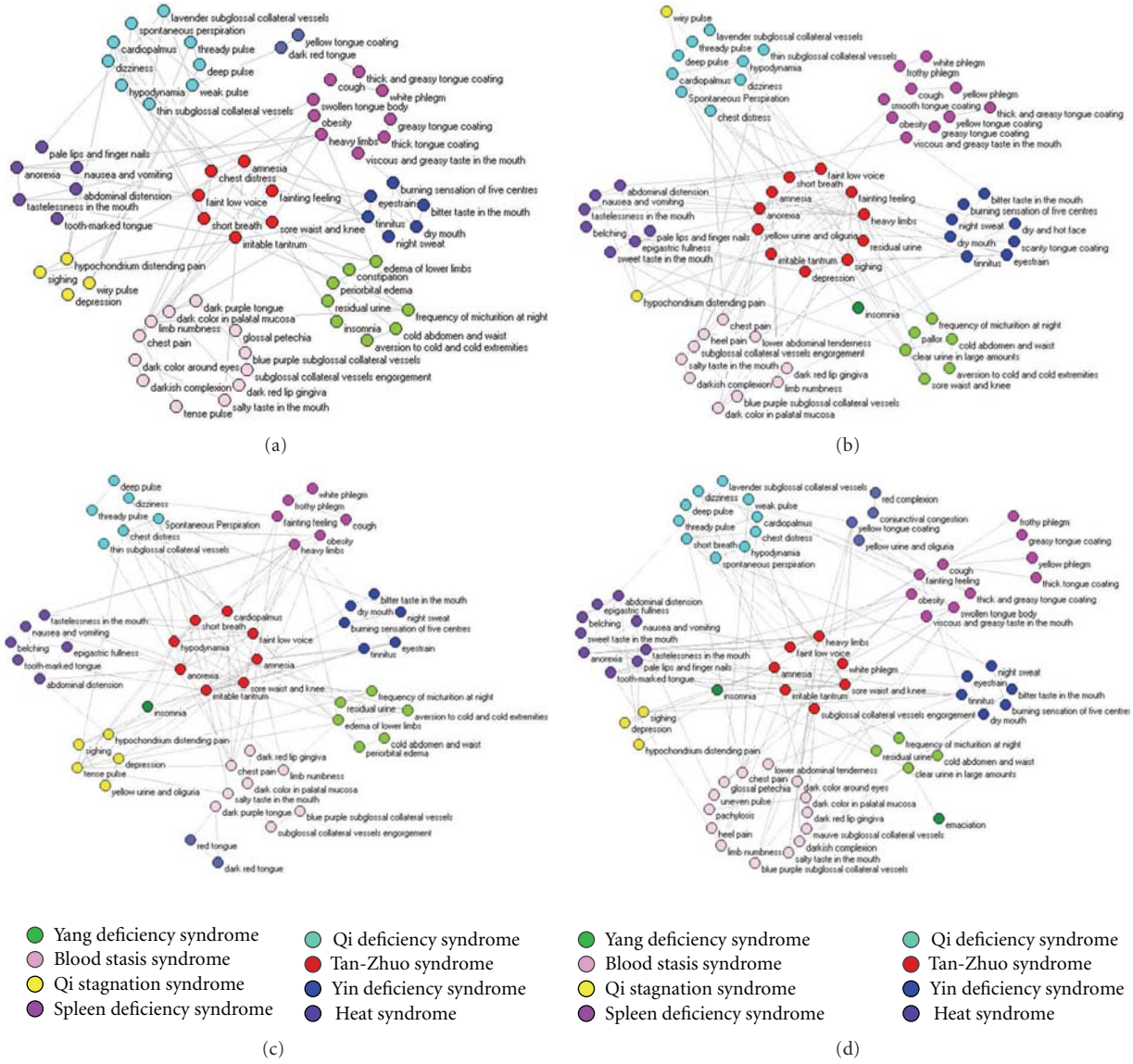


Figure 2: The phenotype networks for AP built by the four MI-based algorithms.

(1) Revised MI-based association of a phenotype pair [8].

(2) Revised MI divided by between-phenotype distance [11]. The between-phenotype distance was defined as

$$d(x,y) = \frac{\sum_{i=1}^{2050} I(x,y,i)\,|B(x,i) - B(y,i)|}{\sum_{i=1}^{2050} I(x,y,i)}, \qquad (2)$$

where $I(x,y,i) = 1$ means phenotype x and phenotype y simultaneously appeared on the ith subject and $= 0$ otherwise. $B(x,i)$ is denoted for the none (0), slight (1), middle (2), and serious (3) of phenotype x.

(3) Revised MI divided by Euclidean distance between phenotype pair.

107 phenotypes were observed and collected from clinical data under the strict quality control. In this process, there was no intervention of subjective factors. It was objective descriptions of patients' symptoms. Mutual information (MI) from complex system was used to describe association between phenotypes. The association data was consolidated into adjacency matrix and then converted into the format that Pajek software required. Pajek software 2.0 was used to analyze the node degrees of the phenotypes. With the command of "Layout-Energy-Kamada-Kawai-Separate Components," we drew the phenotype networks according to different colors and different degrees. The principles of network adjustment were delete the isolated nodes, mediate positions of other nodes with manual operation. Nodes and edges of the network could not be deleted. Then, we exported the network figures in Bitmap format. In Figure 2, the phenotypes networks were made up of the centre network (red colors) and the surrounding networks with different colors. In Figures 2(a) to 2(d), networks with the same colors

Table 3: The frequency of diagnosed seven syndromes in the context of AP.

Syndrome	Frequency	Syndrome	Frequency	Syndrome	Frequency
Qi deficiency syndrome	1409/2050 (68.73%)	Tan-Zhuo syndrome	696/2050 (33.95%)	Spleen deficiency syndrome	210/2050 (10.24%)
Blood stasis syndrome	1375/2050 (67.07%)	Yang deficiency syndrome	391/2050 (19.07%)	—	—
Yin deficiency syndrome	775/2050 (37.80%)	Qi stagnation Syndrome	236/2050 (11.51%)	—	—

reflected the same syndromes. For example, a combination of eyestrain, tinnitus, night sweat, dry mouth, bitter taste in the mouse, and burning sensation of five centres means Yin deficiency according to TCM theory (Figure 2(a)). By using this clue, the four networks involved seven syndromes, that is, Qi deficiency syndrome, Yin deficiency syndrome, Yang deficiency syndrome, Spleen deficiency syndrome, Blood stasis syndrome, Tan-Zhuo syndrome, Qi stagnation syndrome. What is more, there were two other cases needed to be explained. Firstly, the numbers of nodes that reflected "heart syndrome" were small, and these nodes were not in the presence of all the phenotypes networks. So the heat syndrome was not classified as the main syndromes. Secondly, emaciation and insomnia were not the specific responses of syndromes in clinical process. There two phenotypes may appear in patients with different syndromes. We therefore denoted them with another color. In order to express more clearly, we had already added the legend in the revised paper.

To quantitatively confirm this finding, we took the proportion of edges between nodes from different classes (colored subnets) as a measure of the efficiency of clustering. For comparison, we generated 100 randomized networks by randomly shuffling the edges between nodes while keeping the number of edges and nodes unchanged, and we find that the actual proportion of the "between classes edges" is significantly smaller than the average ones ($P < 10^{-40}$). Actually, the P values of the four networks in Figure 2 are $6.47E-130$, $5.89E-102$, $1.74E-119$, $2.99E-41$ under 100 randomized networks, and when we expand the number of networks to 1000, the P values reduced to 0. This result confirms the fact that nodes in the networks are intended to cluster into subnetworks as we declared.

Indeed, the unsupervised clustering of phenotypes here coincide the concept of complementary and alternative medicine and a subnetwork is responsible for a syndrome in TCM. For example, a combination of chest distress, faint low voice, amnesia, short breath, fainting feeling, sore waist and knee, and irritable tantrum means Qi deficiency according to TCM theory. By using this clue, the four networks involved seven syndromes, that is, Qi deficiency syndrome, Yin deficiency syndrome, Yang deficiency syndrome, spleen deficiency syndrome, blood stasis syndrome, Tan-Zhuo syndrome, Qi stagnation syndrome. The four algorithms involved 44, 54, 64, and 69 phenotypes, respectively. This means that a phenotype was average linked with about 2-3 phenotypes. Moreover, it was also found that phenotypes in each syndrome were almost the same, but slightly different (Wilcoxon rand-sum test). A validation computational method was presented to automatically determine a better MI-based association in the four algorithms.

3.3. Computational Validation Method of Established Networks. In order to automatically validate the different phenotype spectra discovered by the four algorithms, diagnosis information of the 2050 AP should be used. An AP subject included here was clinically diagnosed by at least three TCM experts to receive herbal treatment. The syndrome data was composed of seven syndromes. Name and frequency of syndromes are shown in Table 3 in a descending order. The data was represented by a $2050 * 9$ matrix, row represents a subject, and column represents a syndrome. If an AP subject is diagnosed as one of the seven syndromes, the corresponding cell of the matrix is denoted as 1, otherwise the cell is represented as 0.

In the supervised validation strategy, three computational measures (sensitivity, specificity, and accuracy) were employed to evaluate the coincidence of the four phenotype networks with the diagnosis information given by TCM experts. The algorithm was performed by the following three procedures.

Procedure 1. For each subnetwork (marked in different color) in the large phenotype network, it was returned to the phenotype data, if at least half phenotypes in the subnetwork simultaneously appear (their values are nonzero) on a subject, the serial number of the subject is recorded. The total number of each subnetwork was summed, denoted as M.

Procedure 2. Tracking the serial number of a subnetwork to the syndrome data, a matrix with $M * 7$ was retrieved.

Procedure 3. Three computational measures were calculated. The sensitivity is the ratio of the number of subjects diagnosed by the subnetwork to counterpart diagnosed by the TCM expert. The sensitivity describes the true positive of the subnetwork. The specificity refers to the ratio of the number of subjects not diagnosed by the subnetwork to the counterpart of the TCM experts. It describes the false negative of the subnetwork. The accuracy is the ratio of the number of subjects correctly (contains true positive and false negative) by the subnetwork to the counterpart of the TCM experts.

As given in Table 4, the supervised validation strategy-based association performed better than the other three algorithms. The average accuracy of the algorithm was higher than 80%, which means that the phenotype network coveys enough information of TCM clinical essence of AP. For

Table 4: The computational performance of the four MI-based algorithms.

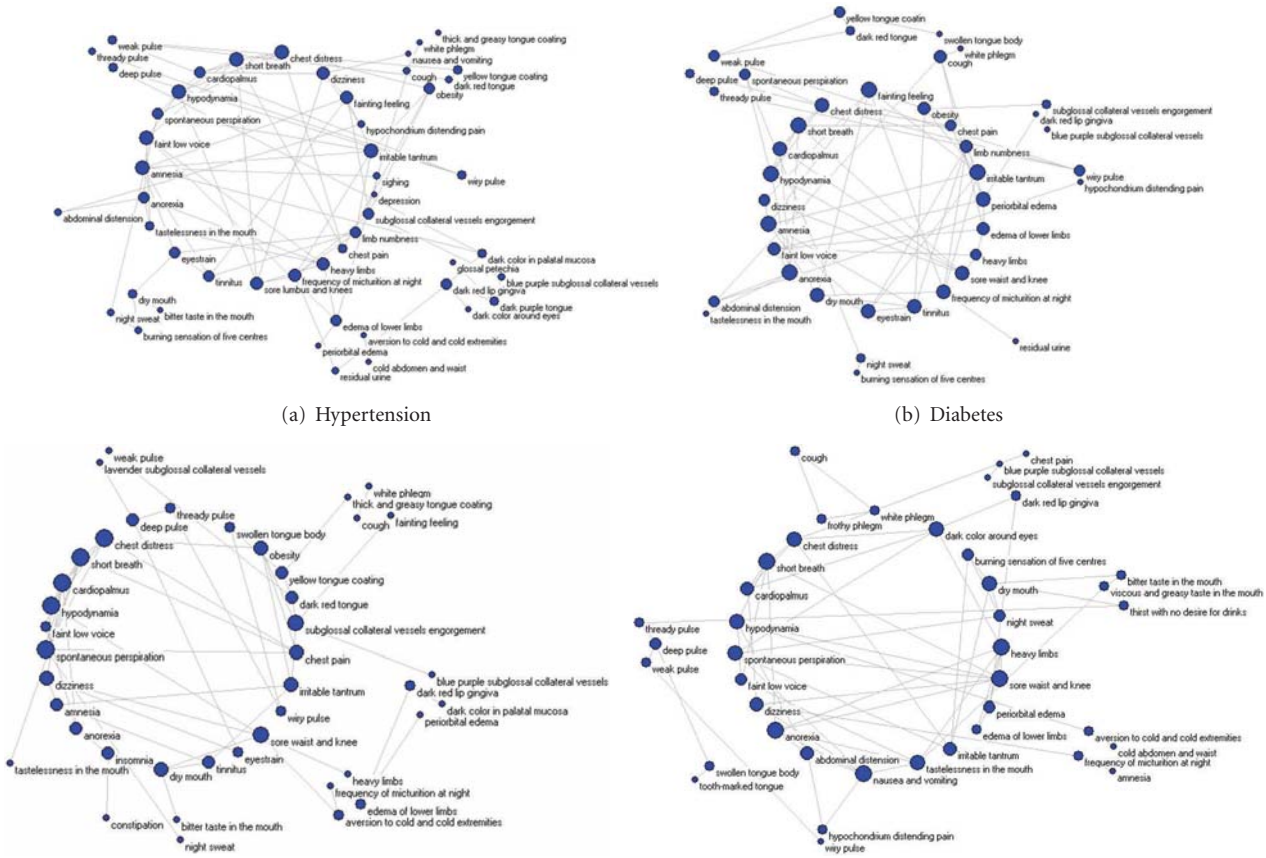
Syndrome	Algorithm	Sensitivity	Specificity	Accuracy
Qi deficiency syndrome	1	0.911497105	0.634958383	0.79804878
	2	0.819699499	0.498826291	0.686341463
	3	0.829592685	0.514757969	0.699512195
	4	0.804898649	0.473441109	0.664878049
Blood stasis syndrome	1	0.8408	0.595	0.744878049
	2	0.909171861	0.618122977	0.777560976
	3	0.828371278	0.52753304	0.695121951
	4	0.900179856	0.601279318	0.763414634
Yin deficiency syndrome	1	0.843273232	0.87434161	0.863414634
	2	0.80112835	0.845637584	0.830243902
	3	0.773049645	0.828996283	0.809756098
	4	0.812849162	0.855322339	0.840487805
Tan-Zhuo syndrome	1	0.806451613	0.877769836	0.855121951
	2	0.781701445	0.853538893	0.831707317
	3	0.806299213	0.869964664	0.850243902
	4	0.793333333	0.848275862	0.832195122
Yang deficiency syndrome	1	0.724233983	0.922531047	0.887804878
	2	0.710144928	0.914369501	0.88
	3	0.630985915	0.890962099	0.854634146
	4	0.690625	0.901734104	0.868780488
Qi stagnation syndrome	1	0.707964602	0.958333333	0.930731707
	2	0.7	0.948108108	0.923902439
	3	0.731707317	0.953387534	0.931219512
	4	0.641025641	0.940161725	0.911707317
Spleen deficiency syndrome	1	0.757575758	0.967602592	0.947317073
	2	0.773333333	0.950526316	0.937560976
	3	0.752808989	0.959401709	0.941463415
	4	0.703703704	0.949152542	0.929756098

a syndrome with high frequency in the context of AP, the algorithm achieved a high sensitivity. It obtained a high specificity for the syndrome with low frequency in AP. But the accuracy remains constantly, which indicated that the algorithm was not biased for any syndrome in AP.

3.4. Phenotype Networks for Combined Diseases. A parameter called degree of complex network was used to evaluate the phenotype networks for the four AP-combined diseases. A type of network called k-core network was used to build phenotype networks, from which different phenotype spectra among combined diseases were investigated. It was intuitively found in Figure 3 that four networks for AP combined with hypertension, diabetes, hyperlipemia, and chronic heart failure were different with each other, indicating that significant change of some phenotypes occurred in AP when combined with other diseases. In TCM theory, it means that syndromes in the context of combined diseases would significant change. Then, the treatment by Chinese herbals would accordingly change. The analysis of the difference between the four networks could guide the treatment of AP by TCM. It was found that when AP combined with hypertension the core syndromes were Blood stasis syndrome, Qi stagnation and hyperactivity of liver-Yang (or called excessive rising of liver-Yang). The last syndrome was absent from the whole network for AP (Figure 2(a)). While, in the network for diabetes, the phenotypes in the core network were hypodynamia, dizziness, tinnitus, frequency of micturition at night, tastelessness, and residual urine, which implied that Qi deficiency and Yin deficiency were core pathogenesis of AP combined with diabetes. The phenotype network in the AP combined with hyperlipemia, the core syndrome was found to be Tan-Zhuo with BSS. When AP was combined with chronic heart failure, the phenotypes turned to core syndrome with Yang deficiency with BSS. The variance in the phenotypes under the different combined diseases indicated an individual treatment strategy for AP.

4. Discussion and Conclusions

Accurate analysis of clinical syndromes is the premise of syndrome differentiation and treatment. In the clinical



(a) Hypertension (b) Diabetes

(c) Hyperlipemia (d) Chronic heart failure

Figure 3: k-core phenotype of four CHD-combined diseases.

process of TCM, the large number and complexity, multilevel relationships of phenotypes had constrained the accuracy of syndrome differentiation. In our study, the MI method firstly described the association between phenotypes much more effective and without the intervention of subjective factors. The characteristics of phenotypes were in line with that of complex networks. Not only in common with special nature on the basis of their own evolutionary mechanisms, but also closely contacted with nature and structural features. Our research showed that MI and complex networks could be applied to the distribution rules study of phenotypes. In the phenotypes networks, we could explore the diagnostic rules of syndromes with core phenotypes or phenotype groups, analysis of basic syndromes of CHD patients and summarize the different syndromes of CHD patients with different comorbidities. In addition, researching the cores of the complex network means to find the "k-core network." In k-core phenotype figures, the nodes for syndromes diagnosis had been showed clearly and intuitively. Combination of the degree values, the greater area that one node has, the more significant role it has played. In clinical diagnosis and treatment process, or during the epidemiological surveys, these core nodes (the core phenotypes) should be considered seriously.

In this paper, we did a clinical epidemiology of AP in CHD to collect 2050 subjects. Four revised mutual-information-based methods were presented to deeply understand the data, we take the positive occurrence frequency to rectify the inherent drawback of MI that prevents negative association from positive association pairs. It was found that revised MI could balance frequency and association and give a better measure of association between phenotypes. In the generation of complex phenotype network, we took a criterion that P_A and P_B composed a significant association pair if and only if P_A is one of the top N associated phenotypes of the phenotype P_B and vice versa. Compared to similar work with others that predefine the scale of the network, the algorithm proposed in this paper gives a more objective and convictive result. Pattern discovery based MI could achieve an accuracy of >80% with the diagnosis by TCM experts and discovered that there are seven syndromes considered as pathogenesis of CHD. By this algorithm and complex network analysis technique, it was found that the core pathogenesis of CHD combined with hypertension, diabetes, hyperlipemia, and chronic heart failure was Qi stagnation, Qi-Yin Deficiency, Tan-Zhuo, and Yang deficiency, respectively. The change in phenotype spectra when CHD

was combined with other diseases provides a better insight into treating CHD by TCM with an individual way.

Author's Contributions

J. Chen, P. Lu, X. Zuo, and Q. Shi contributed equally to this work.

Acknowledgments

This work was supported by the National Basic Research Program of China (973 Program) under Grant no. 2011CB505106, The National Department Public Benefit Research Foundation (200807007), The International Science and Technology Cooperation of China (2008DFA30610), and National Science Foundation of China (30902020 and 81173463).

References

[1] World Health Organization, *World Health Statistics Annual, 1993*, World Health Organization, Geneva, Switzerland, 1994.

[2] X. Wu, R. Jiang, M. Q. Zhang, and S. Li, "Network-based global inference of human disease genes," *Molecular Systems Biology*, vol. 4, article 189, pp. 1–11, 2008.

[3] S. Li, Z. Zhang, L. Wu, X. Zhang, Y. Li, and Y. Y. Wang, "Understanding ZHENG in traditional Chinese medicine in the context of neuro-endocrine-immune network," *IET Systems Biology*, vol. 1, no. 1, pp. 51–60, 2007.

[4] C. Matsumoto, T. Kojima, K. Ogawa et al., "A proteomic approach for the diagnosis of "Oketsu" (blood stasis), a pathophysiologic concept of Japanese traditional (Kampo) medicine," *Evidence-Based Complementary and Alternative Medicine*, vol. 5, no. 4, pp. 463–474, 2008.

[5] S. Guo, J. X. Chen, H. H. Zhao et al., "Building and evaluating an animal model for syndrome in traditional Chinese medicine in the context of unstable angina (Myocardial Ischemia) by supervised data mining approaches," *Journal of Biological Systems*, vol. 17, no. 4, pp. 531–546, 2009.

[6] Y. Liu, H. J. Yin, and K. J. Chen, "Research on the correlation between platelet gelsolin and blood-stasis syndrome of coronary heart disease," *Chinese Journal of Integrative Medicine*, vol. 17, no. 8, pp. 587–592, 2011.

[7] W. R. Wang, R. Lin, H. Zhang et al., "The effects of Buyang Huanwu Decoction on hemorheological disorders and energy metabolism in rats with coronary heart disease," *Journal of Ethnopharmacology*, vol. 137, no. 1, pp. 214–220, 2011.

[8] R. J. Gibbons, K. Chatterjee, J. Daley et al., "ACC/AHA/ACP-ASIM guidelines for the management of patients with chronic stable angina: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Management of Patients With Chronic Stable Angina)," *Journal of the American College of Cardiology*, vol. 33, no. 7, pp. 2092–2197, 1999.

[9] J. Chen, G. Xi, J. Chen et al., "An unsupervised pattern (syndrome in traditional Chinese medicine) discovery algorithm based on association delineated by revised mutual information in chronic renal failure data," *Journal of Biological Systems*, vol. 15, no. 4, pp. 435–451, 2007.

[10] B. Phillip, "Exploratory social network analysis with pajek," *Sociological Methods Research*, vol. 36, pp. 563–564, 2008.

[11] S. Li, B. Zhang, D. Jiang, Y. Y. Wei, and N. B. Zhang, "Herb network construction and co-module analysis for uncovering the combination rule of traditional Chinese herbal formulae," *BMC Bioinformatics*, vol. 11, supplement 11, article 6, pp. 1–12, 2010.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 409568, 9 pages
doi:10.1155/2012/409568

Research Article

Traditional Chinese Medicine Zheng in the Era of Evidence-Based Medicine: A Literature Analysis

Miao Jiang,[1] Chi Zhang,[1] Guang Zheng,[1,2] Hongtao Guo,[1] Li Li,[1] Jing Yang,[1] Cheng Lu,[1] Wei Jia,[3] and Aiping Lu[1,4,5]

[1] *Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Science, Beijing 100700, China*
[2] *School of Information Science and Engineering, Lanzhou University, Lanzhou 730000, China*
[3] *Department of Nutrition, University of North Carolina at Greensboro, North Carolina Research Campus, Kannapolis, NC 28081, USA*
[4] *School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Kowloon, Hong Kong*
[5] *E-Institute of Shanghai Municipal Education Commission, Shanghai TCM University, Shanghai 201203, China*

Correspondence should be addressed to Aiping Lu, lap64067611@126.com

Received 14 February 2012; Revised 24 March 2012; Accepted 27 March 2012

Academic Editor: Shi-Bing Su

Zheng, which is also called a syndrome or pattern, is the basic unit and a key concept of traditional Chinese medicine (TCM) theory. Zheng can be considered a further stratification of patients when it is integrated with biomedical diagnoses in clinical practice to achieve higher efficacies. In an era of evidence-based medicine, confronted with the vast and increasing volume of TCM data, there is an urgent need to explore these resources effectively using techniques of knowledge discovery in databases. The application of effective data mining in the analysis of multiple extensively integrated databases can supply new information about TCM Zheng research. In this paper, we screened the published literature on TCM Zheng-related studies in the SinoMed and PubMed databases with a novel data mining approach to obtain an overview of the Zheng research landscape in the hope of contributing to a better understanding of TCM Zheng in the era of evidence-based medicine. In our results, contrast was found in Zheng in different studies, and several determinants of Zheng were identified. The data described in this paper can be used to assess Zheng research studies based on the title and certain characteristics of the abstract. These findings will benefit modern TCM Zheng-related studies and guide future Zheng study efforts.

1. Introduction

In traditional Chinese medicine (TCM) theory, Zheng, which is also called a syndrome or pattern, is the basic unit and a key concept. TCM Zheng is the abstraction of a major disharmonious pathogenesis, which is identified from a comprehensive analysis of clinical information from four main diagnostic TCM methods: observation, listening, questioning, and pulse analyses [1]. In brief, all diagnostic and therapeutic methods in TCM are based on the differentiation of TCM Zheng, a concept that has been used in China for over 3,000 years [2, 3].

TCM Zheng can be understood as a guideline for patient classification in clinical practice from an alternate viewpoint/dimension compared to a biomedical disease diagnosis. For example, patients suffering from the same disease might be classified with different TCM Zhengs, whereas different diseases might be categorized with the same TCM Zheng. Different Zhengs may occur for one patient at the same time, and Zheng classification is dynamic because Zheng can change during the evolution of a disease. Thus, TCM Zheng classification could be considered to be a further stratification in patients with a single disease, allowing clinicians to obtain more accurate patient classifications. At present, a TCM Zheng diagnosis is integrated with a biomedical diagnosis in clinical practice, and integrative medicine emerges as an optimal approach for achieving higher efficacy [1].

However, in the era of evidence-based medicine, TCM Zheng has encountered a strong challenge from biomedical

science due to a shortage of evidence-based theoretical interpretations and solid proof of Zheng-based efficacy. Therefore, researchers have made a great deal of effort in TCM Zheng-related studies and have made considerable achievements in this field. For instance, it has been indicated that TCM Zheng classification based on symptoms can be used for further stratification of patients with rheumatoid arthritis, which can improve the efficacy of the selected biomedical intervention [4]. In addition, TCM Zheng classification would help to build up a molecular network of TCM Zheng classification in certain diseases, which would help to decipher the mechanism of TCM Zheng classification and define the potential mechanisms of herbal medicines [5, 6]. In recent years, TCM Zheng has attracted increasing attention; it has been shown that this specific patient classification method could assist in new findings for medical science if it were adopted as a significant diagnostic method in modern TCM research with regard to diagnoses, clinical trials, and new drug discoveries [7].

In the past two decades, studies in TCM Zheng have increased dramatically along with advances in medical technologies. Confronted with the large and increasing volume of TCM data, an urgent need emerges to explore these resources effectively using techniques of knowledge discovery in databases (KDD) [8]. We believe that effective data mining approach applications in the analysis of multiple extensively integrated databases (such as the TCM database SinoMed for TCM Zheng classification and the PubMed database for biomedicine) can supply new information in TCM Zheng research, including findings regarding the basic rules of Zheng distribution in certain diseases; the correlations between Zheng, disease, and herbal prescriptions; and the build-up of Zheng-Zheng and Zheng-disease correlation networks. These findings will benefit modern TCM Zheng-related studies.

In this study, we screened the published literature on TCM Zheng-related studies in the SinoMed and PubMed databases with a novel data mining approach to review the Zheng research landscape with the hope of contributing to a better understanding of TCM Zheng in the era of evidence-based medicine.

2. Materials and Methods

2.1. Materials: Source Data Collection. The majority TCM studies were found in the Chinese-language database SinoMed. Most modern TCM research studies were found in the English-language database PubMed. Thus, the TCM Zheng database was separated into two groups. The relevant studies were downloaded from PubMed (http://www.ncbi.nlm.nih.gov/PubMed/) and SinoMed (http://sinomed.imicams.ac.cn/zh/b/index.jsp).

2.1.1. Chinese Literature from the SinoMed Database. By querying the term "中医证候" (TCM Zheng) within the scope of title, keyword, and abstract, SinoMed returned a dataset containing 275,408 articles on December 11, 2011. In the procedure of data preparation, we found that there are much fewer publications (less than 5% of total records) before the year 1990, with questionable study quality comparing with recent publications, thus the data before 1990 were ignored in this study which included 11,378 records. Therefore, the dataset after 1990 contains 266,160 records.

2.1.2. English Literature from the PubMed Database. By querying the term "TCM Zheng/syndrome/pattern" on the default query search, PubMed returned a dataset of 28,103 articles on December 11, 2011.

2.2. Methods: Data Processing. In this study, TCM Zheng can be classified by several grouping policies. For each group policy, different statistical methods that were based on similar algorithms were adapted.

Because there was a delay in the literature collection process, the 2011 dataset was not completed until December 11, 2011. Therefore, not all of the data that were tagged with the year 2011 were included in all of the annual statistics in this study.

2.2.1. TCM Zheng Studies in the Chinese Literature. First, according to the carriers of TCM Zheng studies, the studies can be classified into three groups. Group one includes all TCM Zheng animal experimental studies. Group two includes all TCM Zheng clinical studies, and group three includes TCM Zheng theoretical studies and involved neither animal model nor clinical studies.

According to these three groups, the TCM Zheng statistics were focused on animal studies, clinical research, and pure TCM Zheng studies (nonanimal or nonclinical). The result is shown in Figure 1.

2.2.2. Studies of TCM Zheng and Diseases in Chinese Literature. Many studies on TCM Zheng involved biomedical diseases, so the statistics included studies that involved diseases and those that did not involve diseases.

In analyzing the Chinese literature, we filtered by title, keyword, and abstract. The count of independent Zheng studies increases by one if no disease name occurred; otherwise, the count of disease-related Zheng increases by one. The result was shown in Figure 2.

2.2.3. TCM Zheng Studies in the English Literature. Similar to Section 2.2.1, TCM Zheng studies from the PubMed database were grouped into three classes: pure Zheng studies, clinical Zheng studies, and animal Zheng studies.

The statistical method was similar to that of Section 2.2.1. The only difference was that the two methods were focused on different languages (Chinese versus English, resp.), as shown in Figure 3.

2.2.4. The Ten Most Common Diseases Associated with Chinese Zheng Studies. By limiting the TCM Zheng literature to clinical studies, we obtained the frequencies of studies related to different diseases. The 10 most commonly associated diseases were listed in Table 1.

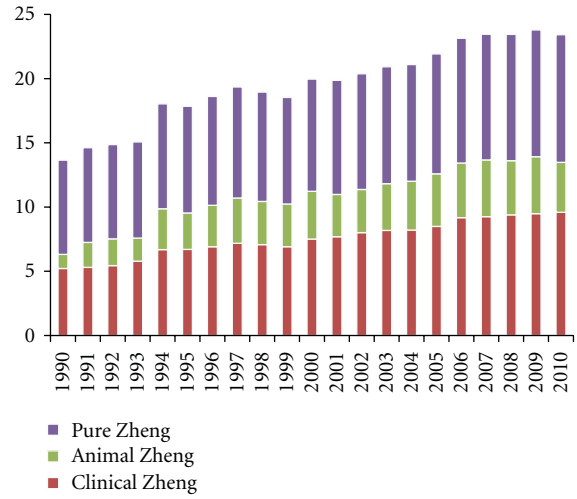


Figure 1: Annual distribution of Chinese-language articles about TCM Zheng in 3 categories (animal experimental studies, clinical studies, and pure Zheng-related studies). The data are obtained from the SinoMed database (until December 11, 2011). In the calculation, some of the annual frequencies of animal studies are 0 and 1, which are too small to be clearly shown in the column diagram. Therefore, the values are converted by the natural logarithm function "Annual Value = ln(Annual $\text{Value}_{\text{origin}}$ + 1)." Based on this function, the frequency of 0 is still 0, and the frequency of 1 (as well as other values) is ln(2).

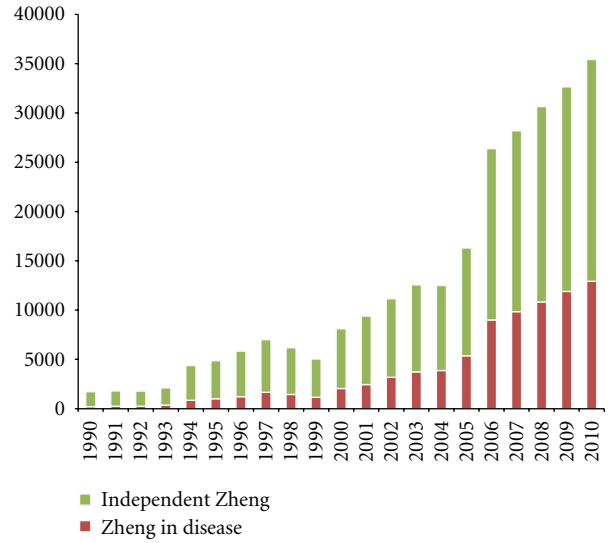


Figure 2: The annual records of Chinese-language articles about TCM Zheng classification in 2 categories. The data were obtained from the SinoMed database (until December 11, 2011). The statistics are based on scanning studies as to whether they contain a disease name in the framework of Western medicine. The count of "independent Zheng" increases by one if a study does not contain a disease name, and the count of "Zheng in disease" increases by one if the study contains one or more disease names.

2.2.5. Annual Distribution of 10 Most Common Diseases Associated with TCM Zheng. According to the results of Section 2.2.4 and Table 1, we filtered the Chinese studies that were associated with these 10 diseases and separate them into 10 datasets. By analyzing these datasets with respect to their dates of publication, we obtained their annual distributions, shown in Figure 4.

2.2.6. Zheng-Zheng Network Generated from the Chinese Literature. Based on the cooccurrence of TCM Zheng and applying the data slicing algorithm [9], we obtained the Zheng-Zheng network, shown in Figure 5.

2.2.7. The Twenty Most Common Zhengs and Their Associated Diseases. Because there was a strong connection between TCM Zheng and disease in both clinical practice and research studies, it was necessary to obtain the frequencies of different disease-Zheng association items that commonly existed in the Chinese literature.

By analyzing the literature associated with both TCM Zheng and disease names in a framework of Western medicine, we obtained a list of associated items of disease-Zheng and their frequencies. For simplicity, we list the 20 most common in Table 2.

2.2.8. Disease-Zheng Network Generated from the Chinese Literature. Because one disease could be involved with several Zhengs, it is necessary to explore the major Zhengs that are associated with each particular disease.

These statistics were focused on the cooccurrence of disease names and Zheng terms. By analyzing the Chinese literature, we obtained a disease-Zheng network. In Figure 6, we listed the 5 most common diseases and their associated Zhengs.

3. Results

In total, 266,160 Chinese-language studies on TCM Zheng were obtained from the SinoMed database, and 28,103 English-language studies were obtained from PubMed. All analyses were performed based on these studies.

3.1. Overall Literature Profiles of Zheng-Related Research. Figure 1 showed an annual increase in the number of publications in the SinoMed database. The number of articles has increased rapidly in the past 2 decades. In addition, the portion of clinical studies has increased substantially, especially after 2006. Animal experimental studies remained insignificant, and the numbers of related articles remained a small proportion of the total, indicating that animal experimentation has not been a major part of Zheng-related studies.

As a diagnostic method, TCM Zheng diagnosis can be integrated with a biomedical diagnosis in clinical practice, thus we can classify the whole studies into two categories, independent Zheng and Zheng in disease. The former indicates those studies considering only TCM Zheng classification without any biomedical disease information; the Zheng in disease studies refers to those studies aiming at the TCM Zheng research based on one or more biomedical diseases, or the integrative study on TCM Zheng and

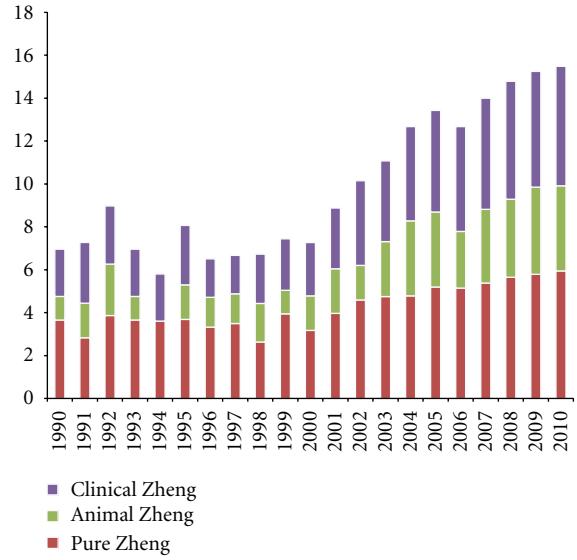


Figure 3: The annual records of English-language articles about TCM Zheng classification in 3 categories. The data were obtained from the PubMed database (until December 11, 2011). As in Figure 1, the annual values are also converted by the function "Annual Value = ln(Annual Value$_{origin}$ + 1)." Therefore, a comparison between SinoMed and PubMed can be obtained based on the same standard.

biomedical diseases. The majority of studies are independent of biomedical disease, as shown in Figure 2, confirming that TCM Zheng classification can be discussed as a different classification system independent of disease diagnosis, although the integration of Zheng and disease diagnosis is common in clinical practice. The proportion of studies that were correlated with biomedical diseases is increasing over time, especially after the year 2000. The advantage of integrating TCM Zheng with biomedical disease diagnoses has been emphasized in recent years, and a number of novel achievements have been acquired in this field.

After 2000, the annual number of articles in English-language journals on TCM Zheng in PubMed increased dramatically, but the total number was far less than the number of Chinese-language articles, as shown in Figure 3. Among these studies, the percentage of clinical studies grew rapidly, a trend that was consistent with that of Chinese-language studies. A higher proportion of animal experimental studies was reported in PubMed than in SinoMed.

The 10 most common diseases in Chinese-language TCM Zheng-related studies are summarized in Table 1, and the annual numbers are shown in Figure 4. From Table 1 and Figure 4, it can be concluded that most of the TCM Zheng-related diseases are complex chronic diseases, which implies that researchers tend to focus on these chronic diseases in TCM Zheng-related studies due to the superior efficacy of herbal prescriptions in treating these diseases. There are thousands of studies per year focusing on TCM Zheng studies of diabetes mellitus and gastritis, and both of these diseases manifest with multiple symptoms with an increasing incidence in China and can be treated with herbal medicines.

Table 1: Ten most common diseases in Chinese-language TCM Zheng-related clinical studies.

No.	Disease	Frequency
1	Heart failure	7,953
2	Rheumatoid arthritis	5,754
3	Rheumatic heart disease	4,802
4	Diabetes mellitus	4,386
5	Myocardial infarction	3,519
6	Respiratory failure	2,439
7	Arrhythmia	2,021
8	Chronic obstructive lung disease	2,009
9	Gastritis	1,414
10	Osteoarthritis	1,205

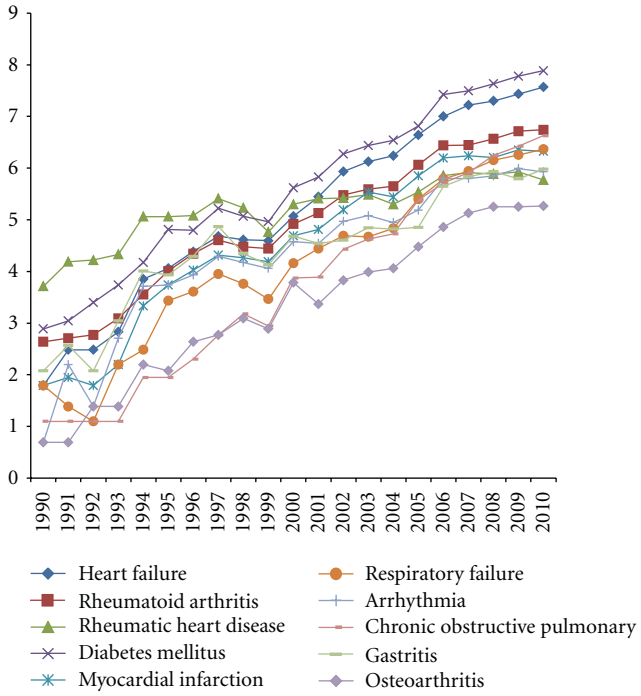


Figure 4: The ten most common diseases in Chinese-language TCM Zheng-related clinical studies annually. The data were obtained from the SinoMed database (until December 11, 2011). Each line represents the annual studies of TCM Zheng for one particular disease. The annual values are converted by the function "Annual Value = ln(Annual Value$_{origin}$ + 1)" to better display the tendency.

3.2. Basic Zhengs and the Zheng-Zheng Association Analysis. As a basic unit in a TCM diagnosis, Zheng can be shown in combination (two or more Zhengs) in a patient, and Zheng can change during the development of an illness. During the data analysis, it can be found that most disease-Zhengs studies are published in Chinese. Although there are a small amount of English publications concerning the disease-Zheng research, most of them were published in English abstract, which actually were published in Chinese, and can be collected in SinoMed database. Thus we abandoned the

English data in this analysis, for the data is too few, and also it is not appropriate in this study to combine both data together. There are 18 basic Zhengs that are filtered out in the TCM publications in SinoMed. Figure 5 illustrates those Zhengs and the Zheng-Zheng association network. Clockwise from the largest node, the first is the liver-kidney yin deficiency pattern (connecting five nodes: yin deficiency pattern, kidney yang deficiency pattern, spleen-kidney yang deficiency pattern, pattern of dual deficiency of qi and yin, and liver qi depression pattern). Six nodes of the network are connected to the second largest node kidney yin deficiency pattern. The yang deficiency pattern and pattern of dual deficiency of yin and yang are two patterns with relatively low frequencies. The upper left corner is the dampness-heat pattern and connecting node spleen-stomach dampness-heat pattern. The upper right is the qi deficiency pattern, connecting with the spleen qi deficiency pattern and lung qi deficiency pattern. The lower right is the blood stasis pattern, connecting with the pattern of qi deficiency with blood stasis and pattern of qi stagnation with blood stasis.

3.3. Disease-Zheng Association Analysis. The integration of disease diagnosis and TCM Zheng classification is a common model in clinical practice, and many studies have focused on this integration. According to Zheng-Zheng association analysis in Section 3.2, most disease-Zhengs studies are published in Chinese, and English data were abandoned for the small quantity; we then developed an approach to visualization that classifies data according to disease-zheng association analysis. Details of the top 20 frequent disease-Zheng (Zheng in a specific disease) are provided in Table 2. In the pattern distribution, the patterns with yin deficiency were the most frequent (1,794; 44.89%), and the two TCM viscera (liver and kidney, internal organs where essence and qi are formed and stored in TCM) were the most frequent (1,151; 28.80%).

To further confirm the disease-Zheng associations, 20 disease-Zheng were selected for more comprehensive analyses. Figure 6 reveals insights into the disease-Zheng association; it was built by analyzing 5 kinds of popular diseases. The constructed view shows three attributes. The first (upper left) attributes identify the relevant Zheng research on primary hypertension (PH); there are 2 TCM Zheng for PH. The second (upper right) attributes represent the 6 most influential Zheng in gastritis research. The third attribute represents the total number of shared Zheng among diabetes mellitus (DM), hepatocirrhosis, and HF. Kidney yin deficiency Zheng can be found in both DM and Hepatocirrhosis, and Qi deficiency with blood stasis Zheng can be found in both DM and HF.

Table 2: The top 20 most frequent Disease-Zheng in published studies.

No.	Disease	Zheng	Frequency
1	Diabetes mellitus	Dual deficiency of qi and yin	783
2	Diabetes mellitus	Dual deficiency of yin and yang	247
3	Diabetes mellitus	Blood stasis	237
4	Diabetes mellitus	Phlegm- dampness obstructing the lung	184
5	Diabetes mellitus	Kidney yin deficiency	150
6	Diabetes mellitus	Yin deficiency with exuberant heat	128
7	Diabetes mellitus	Qi deficiency with blood stasis	109
8	Gastritis	Liver qi invading the stomach	286
9	Gastritis	Spleen-stomach dampness-heat	250
10	Gastritis	Spleen-stomach deficiency cold	148
11	Gastritis	Stomach deficiency cold	148
12	Gastritis	Dampness-heat	132
13	Gastritis	Liver qi depression	106
14	Heart failure	Qi deficiency with blood stasis	133
15	Heart failure	Kidney yang deficiency	132
16	Hepatocirrhosis	Liver-kidney yin deficiency	126
17	Hepatocirrhosis	Kidney yin deficiency	126
18	Primary hypertension	Ascendant hyperactivity of liver yang	115
19	Primary hypertension	Yin deficiency with yang hyperactivity	115
20	Hepatocirrhosis	Liver depression and spleen deficiency	110

4. Discussion

Compared to a previous literature review [10, 11], we report a new quantitative route for the synthesis of related literature and provide new quantitative evidence on TCM Zheng studies.

A central problem is how to capture information from literature in a form that is suitable for analysis [12]. We address the information on Zheng and show that the frequencies of words in abstracts can be used to determine whether or not a given article discusses Zheng. For those articles that have been determined to discuss this topic, relevant information can be obtained. Furthermore, suitable annotations can be obtained. These evaluations are based on limited but increasing evidence from animal studies and clinical studies. Among other limitations, the lack of quantitative assessment has consistently been cited as a fundamental problem in existing studies, and mining exploration has been used in a recent review [1]. The purpose of this study was to provide a comprehensive overview of quantitative levels.

Over the past 30 years, an increasing number of Chinese researchers have focused their attention on developing evidence for Zheng and identifying the mechanism of Zheng. Recently, more studies were published in SCI indexed journals to introduce and evaluate the effectiveness of Zheng.

For Zheng, the highest numbers of Chinese-language articles were reported for experts' experiences, reviews, commentaries, animal studies, observational studies, and

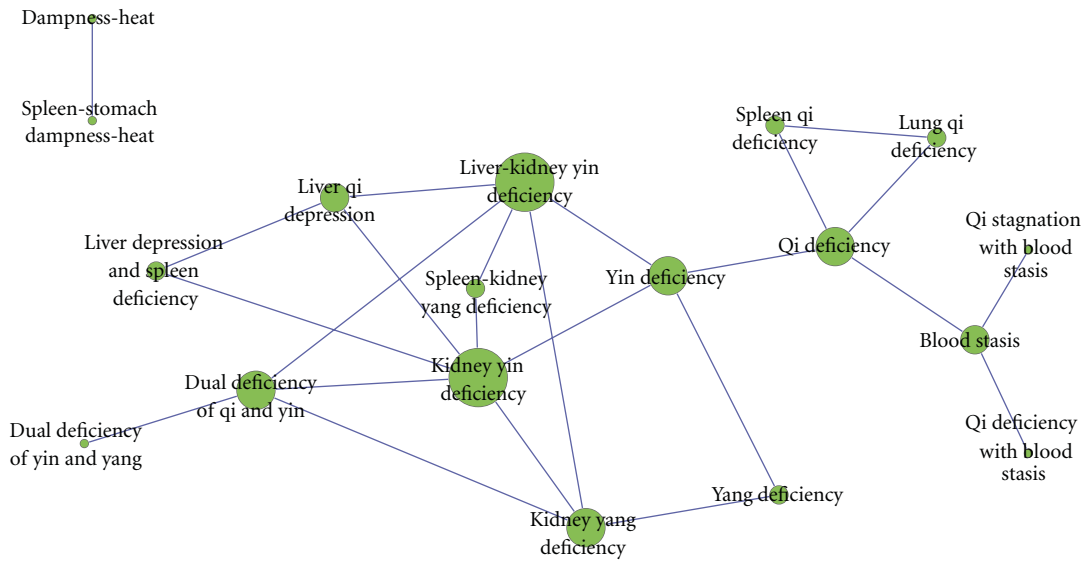


Figure 5: Overview of the Zheng-Zheng network. This network is generated from mining the SinoMed literature on TCM Zheng. The method of calculation is based on a data slicing algorithm that calculates the frequencies of the co-occurrence TCM Zhengs. Each node represents one type of Zheng. The size of the node indicates the frequency of Zheng publications; a larger node indicates more reports about the Zheng. The line width represents the frequency of co-occurrence of the connected Zhengs.

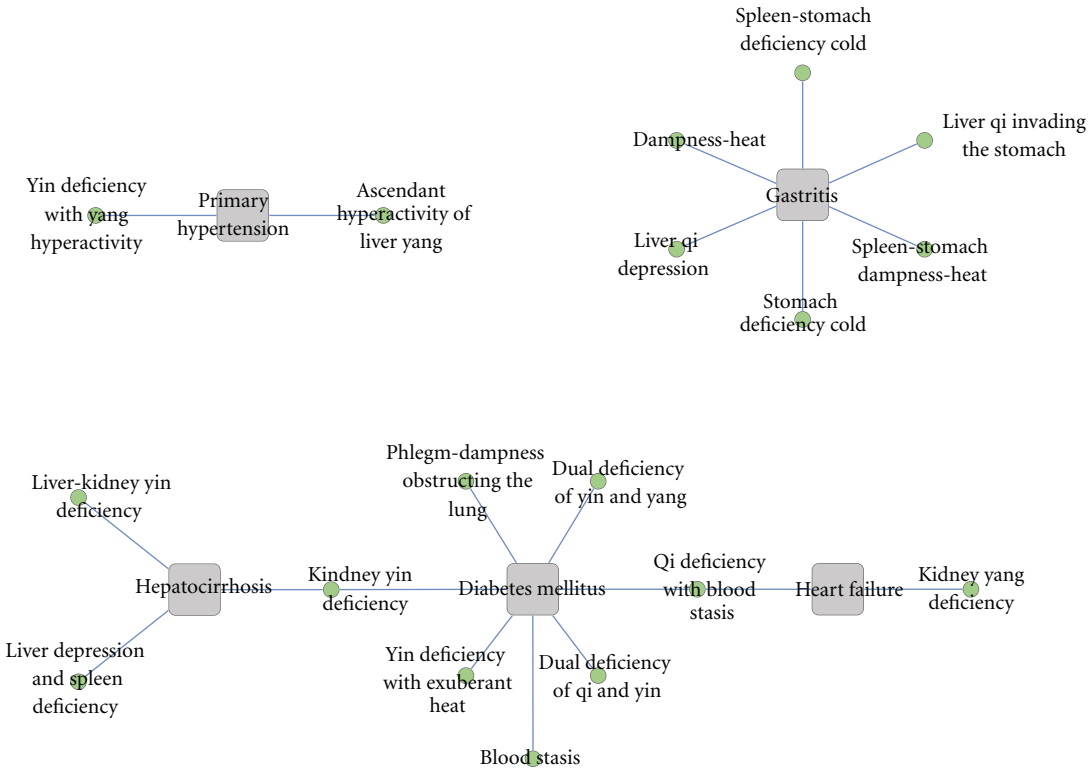


Figure 6: Overview of the disease-Zheng network. The disease-Zheng network is generated from a SinoMed literature analysis with the cooccurrence frequencies of disease. The method of calculating the frequency of co-occurrence is also based on a data slicing algorithm. In this figure, the square grey shape is a certain disease, and the round green shape is a TCM Zheng. If two diseases have a common Zheng, there is an edge connecting them. The upper left part identifies the relevant Zheng research on primary hypertension, with 2 TCM Zhengs for this disease. The upper right part represents the 6 most influential Zhengs in gastritis research. The section below each part represents the total number of shared Zheng among diabetes mellitus, hepatocirrhosis, and heart failure. The kidney yin deficiency Zheng can be found in both DM and Hepatocirrhosis, and Qi deficiency with blood stasis Zheng can be found in both DM and HF.

randomized controlled trials (RCTs). However, relatively low numbers were reported for animal studies and RCTs. It is difficult to develop an animal model that perfectly reproduces the symptoms of Zheng in patients [13]. Researchers attempt to overcome this limitation by combining the disease and Zheng [14]. The result shows the unambiguous identification of the authors' characteristics. Chinese authors are becoming more aggressive about submitting animal experimental studies for Zheng. However, it is important to note that many Chinese scientists in international institutes bring innovation to worldwide TCM Zheng research. We believe that there is a growing trend of collaboration in combining a disease and Zheng between TCM researchers and biomedical scientists in animal experimental Zheng studies. RCTs were not developed until the 1990s. Recently, more advanced trial designs are being developed and will provide explicit Zheng theories based on long-term experience [15, 16]. Nonetheless, there is a relatively small amount of evidence regarding RCTs with disease and Zheng designs for data mining.

The yin deficiency pattern is currently the preferred pattern for Zheng research compared to any other pattern because it is relatively major component of modern life. A yin deficiency may be due to excessive fluid loss or to the consumption of yin due to aging. As stated in the Nei Jing (Inner Classic), "At 40 years of age, yin is half consumed" [17]. If, due to overthinking, anxiety and worry, underexercise, faulty diet or erroneous medical treatments in modern life, the qi is damaged and becomes vacuous and weak, then the spleen will not be able to perform its various functions. As mentioned above, if yin does not nourish and enrich the liver and kidney, then the liver and kidney will not be able to governing coursing and discharging. Hence, the liver and kidney will become depressed. Thus, it is clear that liver and kidney deficiencies are mutually engendering in the mining results. For the yin deficiency pattern, more research is needed to investigate its contribution to preventing and reversing chronic diseases that are consequences of a modern lifestyle.

Similarly, damp heat typically complicates the diseases of many patients. In addition, dampness can be engendered internally, often due to spicy foods, alcohol, sugars, and sweets. Blood stasis is also a mechanism that is involved in most chronic disorders, especially when there is chronic severe pain at fixed locations. A study on blood stasis and activating blood circulation and removing stasis won the top prize of the National Science & Technology Progress Award in China [18]. In addition, there is less information available on the yang deficiency pattern compared to the yin deficiency pattern.

For disease and Zheng correlation research, the results of all selected studies showed that the number of DM studies was the highest, followed by the number of studies on gastritis and HF. The 5 most common diseases in the mining results are chronic diseases. These chronic diseases are a likely explanation for the report that the yin deficiency pattern is substantially higher than the yang deficiency pattern in Zheng studies, and CM is able to provide a worldwide contribution for patients who suffer from chronic diseases [19].

Similar to DM, more detailed patterns of gastritis were generally consistent with patterns found in clinical practice. However, relatively few mean concentrations for some of patterns were reported for primary hypertension, cirrhosis, and HF.

The results of this study suggest that DM and two diseases, cirrhosis and HF, share one common Zheng. One important concept in TCM is "Treating Different Diseases with the Same Therapy" (TDDST), which can be explained as the similar treatment of different diseases that have similar TCM patterns [9]. For disease and Zheng correlation research such as TDDST, explorations of the existing biomedical networks between diseases are challenging.

Despite the notable accomplishments of Zheng in TCM, it is impossible to exaggerate the importance of Zheng classification. We have been able to identify many of the classical formulas with one-to-one relationships to some diseases in the text-mining process. The formulas are often called effective formulas. These disease-TCM formulas are possible future trends in TCM basic and applied research.

In addition, Zheng studies can vary widely depending on actual academic environments. Thus, the availability of a comprehensive database that include Zheng determinants is likely to result in a more accurate and consistent assessment than when the assessment is based only on expert judgments.

There are some limitations to this approach. Because Zheng is a complex concept, many studies were selected, which complicated comparisons across studies that focus on different research methods. In addition, the classification of Zheng varies with conditions and the "standard" application of Zheng, which have changed over time [20, 21]. Additionally, experimental study records, clinical study records and other relevant records of Zheng were selected to investigate time trends. The proportion of nonexperimental or clinical studies has decreased, but the proportion of clinical studies has increased annually. However, there were insufficient data available to assess the effect of these changes. Consequently, the incorporation of time trends in review assessments is required to improve the mining method. A further limitation of using published literature is the extraction and interpretation of Zheng from reports that were written by different authors for different purposes. The description of the detailed experimental conditions was often unclear or absent, especially in Chinese-language articles. In addition, published reports may have been biased toward worst-case scenarios. Finally, Zheng in other research fields, such as epidemiological studies, has rarely been reported [22, 23].

5. Conclusions and Perspectives

5.1. Conclusions. Using this novel text-mining approach, contrast in Zheng was found when comparing different studies, and several determinants of Zheng were identified. The data described in this study can be used to assess Zheng research classifications based on titles and certain characteristics of abstracts. Furthermore, these data can guide efforts for future Zheng studies.

5.2. Perspectives. Based on our analysis of the literature, it seems that TCM Zheng-related studies will attract increasing interest worldwide, and more TCM Zheng studies will occur in the near future. In the era of evidence-based medicine, scientists will concentrate on studies that can provide solid evidence for compelling Zheng research, including RCTs, animal experimental studies, and bioinformatics research based on data from human samples instead of pure theoretical debates. Integrative studies on TCM Zheng and biomedical diseases will be a focus because TCM Zheng is considered a powerful tool for patient stratification that can supplement the present classification system based on biomedical disease. Optimal and innovative study designs, especially in Zheng-related clinical research and animal experimental studies, are urgently needed. High-quality, evidence-based studies in TCM Zheng-related research is expected to lead to innovation and breakthrough discoveries to establish a more accurate diagnostic system that will contribute to healthcare systems worldwide.

Authors' Contribution

M. Jiang, C. Zhang, and G. Zheng contributed equally to this paper.

Acknowledgments

This research is supported in part by the projects from the National Science Foundation of China (nos. 30825047, 30902003, and 81072982), from the MOST (Ministry of Science and Technology of the People's Republic of China, no. 2008IM020400), and from the China Academy of Chinese Medical Sciences (nos. Z0172 and Z0134).

References

[1] A. Lu, M. Jiang, C. Zhang, and K. Chan, "An integrative approach of linking traditional Chinese medicine pattern classification and biomedicine diagnosis," *Journal of Ethnopharmacology*, vol. 141, no. 2, pp. 549–556, 2012.

[2] S. K. Chen, "Discussion on definition of "traditional Chinese medicine and western medicine integration" and "integrated traditional Chinese and western medicine"," *Zhong Xi Yi Jie He Xue Bao*, vol. 1, no. 4, pp. 241–243, 2003.

[3] T. Ma, C. Tan, H. Zhang, M. Wang, W. Ding, and S. Li, "Bridging the gap between traditional Chinese medicine and systems biology: the connection of Cold Syndrome and NEI network," *Molecular BioSystems*, vol. 6, no. 4, pp. 613–619, 2010.

[4] C. Lu, Q. Zha, A. Chang, Y. He, and A. Lu, "Pattern differentiation in traditional chinese medicine can help define specific indications for biomedical therapy in the treatment of rheumatoid arthritis," *Journal of Alternative and Complementary Medicine*, vol. 15, no. 9, pp. 1021–1025, 2009.

[5] L. Cheng, N. Xuyan, X. Cheng et al., "Network-based gene expression biomarkers for cold and heat patterns of rheumatoid arthritis in traditional chinese medicine," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 203043, 17 pages, 2012.

[6] C. Gao, L. Cheng, Z. Qinglin et al., "A network-based analysis of traditional Chinese medicine cold and hot patterns in rheumatoid arthritis," *Complementary Therapies in Medicine*, vol. 20, no. 1-2, pp. 23–30, 2012.

[7] A. P. Lu and K. J. Chen, "Integrative medicine in clinical practice: from pattern differentiation in traditional Chinese medicine to disease treatment," *Chinese Journal of Integrative Medicine*, vol. 15, no. 2, p. 152, 2009.

[8] Y. Feng, Z. Wu, X. Zhou, Z. Zhou, and W. Fan, "Knowledge discovery in traditional Chinese medicine: state of the art and perspectives," *Artificial Intelligence in Medicine*, vol. 38, no. 3, pp. 219–236, 2006.

[9] G. Zheng, M. Jiang, X. He et al., "Discrete derivative: a data slicing algorithm for exploration of sharing biological networks between rheumatoid arthritis and coronary heart disease," *BioData Mining*, vol. 4, no. 1, article 18, 2011.

[10] A. P. Lu and K. J. Chen, "Chinese medicine pattern diagnosis could lead to innovation in medical sciences," *Chinese Journal of Integrative Medicine*, vol. 17, pp. 811–817, 2011.

[11] A. S. Ferreira and A. J. Lopes, "Chinese medicine pattern differentiation and its implications for clinical practice," *Chinese Journal of Integrative Medicine*, vol. 17, pp. 818–823, 2011.

[12] H. Shatkay and R. Feldman, "Mining the biomedical literature in the genomic era: an overview," *Journal of Computational Biology*, vol. 10, no. 6, pp. 821–855, 2003.

[13] C. Chai, J. Kou, D. Zhu, Y. Yan, and B. Yu, "Thinking of current situation of animal model of tradition Chinese medicine combining disease and syndrome," *Zhongguo Zhongyao Zazhi*, vol. 34, no. 20, pp. 2673–2675, 2009.

[14] Y. Wang, H. Y. Zhao, M. J. Liu et al., "Establishment of a rat model of rheumatoid arthritis with kidney deficiency syndrome," *Zhong Xi Yi Jie He Xue Bao*, vol. 9, pp. 973–982, 2011.

[15] J. L. Tang, S. Y. Zhan, and E. Ernst, "Review of randomised controlled trials of traditional Chinese medicine," *British Medical Journal*, vol. 318, no. 7203, pp. 160–161, 1999.

[16] M. Jiang, J. Yang, C. Zhang et al., "Clinical studies with traditional Chinese medicine in the past decade and future research and development," *Planta Medica*, vol. 76, no. 17, pp. 2048–2064, 2010.

[17] U. U. Paul, *Huang Di Nei Jing Su Wen: Nature, Knowledge, Imagery in an Ancient Chinese Medical Text*, University of California Press, Los Angeles, Calif, USA, 2003.

[18] "Study of blood stasis syndrome and promoting blood circulation to remove stasis win top prize of National Science & Technology Progress Award," *China Medical News*, vol. 19, p. 8, 2004.

[19] M. Jiang, C. Zhang, H. Cao, K. Chan, and A. Lu, "The role of Chinese medicine in the treatment of chronic diseases in China," *Planta Medica*, vol. 77, no. 9, pp. 873–881, 2011.

[20] I. K. Tsang, S. H. Huang, and B. E. Koehler, "Traditional Chinese medicine: an opportunity for discovery," *Journal of Rheumatology*, vol. 37, no. 10, pp. 1989–1990, 2010.

[21] E. Manheimer, S. Wieland, E. Kimbrough, K. Cheng, and B. M. Berman, "Evidence from the cochrane collaboration for traditional chinese medicine therapies," *Journal of Alternative and Complementary Medicine*, vol. 15, no. 9, pp. 1001–1014, 2009.

[22] Z. J. Zhang, Q. R. Tan, Y. Tong et al., "An epidemiological study of concomitant use of Chinese medicine and antipsychotics

in schizophrenic patients: implication for herb-drug interaction," *PLoS ONE*, vol. 6, no. 2, Article ID e17239, 2011.

[23] M. M. Lee and J. M. Shen, "Dietary patterns using Traditional Chinese Medicine principles in epidemiological studies," *Asia Pacific Journal of Clinical Nutrition*, vol. 17, supplement 1, pp. 79–81, 2008.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 135387, 9 pages
doi:10.1155/2012/135387

Research Article

Application of Multilabel Learning Using the Relevant Feature for Each Label in Chronic Gastritis Syndrome Diagnosis

Guo-Ping Liu,[1] Jian-Jun Yan,[2] Yi-Qin Wang,[1] Jing-Jing Fu,[1] Zhao-Xia Xu,[1] Rui Guo,[1] and Peng Qian[1]

[1] *Laboratory of Information Access and Synthesis of TCM Four Diagnosis, Basic Medical College, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*
[2] *Center for Mechatronics Engineering, East China University of Science and Technology, Shanghai 200237, China*

Correspondence should be addressed to Jian-Jun Yan, jjyan@ecust.edu.cn and Yi-Qin Wang, wangyiqin2380@sina.com

Received 13 January 2012; Accepted 22 March 2012

Academic Editor: Shi-Bing Su

Background. In Traditional Chinese Medicine (TCM), most of the algorithms are used to solve problems of syndrome diagnosis that only focus on one syndrome, that is, single label learning. However, in clinical practice, patients may simultaneously have more than one syndrome, which has its own symptoms (signs). *Methods*. We employed a multilabel learning using the relevant feature for each label (REAL) algorithm to construct a syndrome diagnostic model for chronic gastritis (CG) in TCM. REAL combines feature selection methods to select the significant symptoms (signs) of CG. The method was tested on 919 patients using the standard scale. *Results*. The highest prediction accuracy was achieved when 20 features were selected. The features selected with the information gain were more consistent with the TCM theory. The lowest average accuracy was 54% using multi-label neural networks (BP-MLL), whereas the highest was 82% using REAL for constructing the diagnostic model. For coverage, hamming loss, and ranking loss, the values obtained using the REAL algorithm were the lowest at 0.160, 0.142, and 0.177, respectively. *Conclusion*. REAL extracts the relevant symptoms (signs) for each syndrome and improves its recognition accuracy. Moreover, the studies will provide a reference for constructing syndrome diagnostic models and guide clinical practice.

1. Introduction

Although Traditional Chinese Medicine (TCM) and Western Medicine diagnose cases in clinical applications, their theoretical systems are totally different. The hard targets such as laboratory and imaging tests are very important for diagnosing diseases in Western Medicine whereas soft targets are much more important in the clinical diagnosis of TCM.

The so-called soft targets [1] mainly refer to targets that cannot be accurately measured and with poor repeatability. These targets are subjective and are collected through clinical observation of a doctor or the patient's self-report, which cannot be accurately measured using instruments or directly through other means.

Therefore, collecting information in the diagnosis of TCM is difficult because it cannot be measured accurately, with poor measurement repeatability, and are easily influenced by the study sample and environmental factors.

Given that the soft targets of TCM are subjective, fuzzy, and multidimensional, TCM has been considered as a mystical experience in the scientific world and has not been identified in a wide range.

In recent years, the standardization and objectification of TCM diagnosis have gradually become a research hotspot with the development of mathematical statistics, data mining, and pattern recognition technology.

The studies are revealing the rules between syndromes and the information of four diagnosis: inspection, auscultation and olfaction, inquiring, and palpation, and seeking information of four diagnosis for differential diagnosis or extracting classification rules for syndrome differentiation. The research can provide a reference for clinical syndrome

differentiation and reduce the subjectivity and ambiguity of diagnosis.

Some researchers have applied the structural equation model in studying chronic atrophic gastritis. The results show that chronic atrophic gastritis resulting from the most common syndromes correspond with diagnostic targets, which is agreement with the clinical practice of TCM [2]. An improved conjugate gradient learning algorithm is used to create the BP model with three layers for diabetes and nephropathy. The results show its advantages in predicting diabetes and nephropathy [3]. An entropy-based partition method for complex systems is applied to establish endothelial dysfunction diagnostic criteria for Yin deficiency syndrome. Moreover, the experimental results are highly consistent with the findings of clinical diagnosis [4]. Multilabel learning [5] combined with the frequency method is presented to select the symptoms that greatly contribute to coronary heart disease. The results show the improvement in the diagnosis of coronary heart disease. Su et al. [6] employed the correlation coefficient, similarity D, the angle cosine, and spectral similarity to study the correlation between the symptoms (signs) and the five syndromes of liver cirrhosis. The research can provide a basis for differentiating patients with nonspecific clinical manifestations.

Our research group focuses on the standardization and objectification of syndrome in TCM. We applied latent structure models [7] to study the association between symptoms of the spleen system. According to a social network theory, we used the associated density method [8, 9] to analyze the correlation between syndrome-syndrome of coronary heart disease and symptom-syndrome of chronic gastritis.

In the studies on syndrome standardization and objectification mentioned above, most of the algorithms were used to solve problems in diagnosing patients with disjoint syndromes, which belong to single-label learning. However, in clinical practice, strong relevance may be observed among different syndromes. Traditional single-label data-mining techniques, which could only build one model at a time, ignore the fact that one patient may be associated with more than one syndrome. In this study, a novel multilabel learning (MLL) technique is explored to solve this problem. Our group [5] applied a multilabel learning algorithm (ML-kNN) to construct a syndrome model for diagnosing CHD in TCM. The ML-kNN produces better results than the ranking Support Vector Machine (Rank-SVM), BPMLL, and kNN based on three criteria, namely, average precision, coverage, and ranking loss.

Compared with traditional learning methods, multilabel learning more effectively identifies syndrome information in TCM and solves problems such as single samples with several syndromes. However, the relationship between features and class labels is not concerned in multilabel learning.

Chronic gastritis (CG) is a common disease and is classified under spleen and stomach diseases in TCM. According to preliminary studies, we applied the feature selection methods to select significant symptoms (signs) associated with each syndrome in CG. In addition, we further applied multilabel learning algorithm to construct the syndrome models of inquiry diagnosis for CG in TCM to provide a reference for the syndrome standardization and objectification of CG.

In this paper, the first section includes the introduction of the research progress in the field of TCM diagnosis, the purpose of the study, and its significance. In Section 2, we introduce the data-collecting methods, which include a variety of feature selection methods and a multilabel learning method designated as REAL. Standardizing a scale of inquiry information is discussed in the results section. The optimal symptom set is obtained for each syndrome using feature selection. The results of diagnostic models constructed are discussed based on the REAL method. Then the results of REAL are compared with other multilabel learning algorithms. Section 3, the results of feature selection and diagnostic models are analyzed based on TCM theory. The last section concludes and indicates several issues for future studies.

2. Material and Methods

2.1. Research Subjects. Chronic gastritis (CG) samples were collected from a clinic, in-patient department, and gastroscopy room of the digestive system department of the Longhua Hospital and the Shuguang Hospital of Shanghai University of Traditional Chinese Medicine, the Xinhua Hospital, the Putuo District Central Hospital, and the Shanghai Hospital of Traditional Chinese Medicine. This work was approved by the Shanghai Society of Medical Ethics. All patients signed an informed consent form. A total of 919 valid subjects were enrolled after excluding cases with TCM inquiry diagnosis scales that lacked information or cannot be diagnosed with CG. Among the 919 patients, 354 were male (38.5%, with an average age of 44.61 yr $\pm$ 14.54 yr) and 565 were female (61.5%, with an average age of 48.70 yr $\pm$ 12.74 yr).

2.1.1. Inclusion Criteria. Including criteria were

(1) patients who meet the diagnostic standards for CG and TCM syndromes, and

(2) patients who were informed and have agreed to join this investigation.

2.1.2. Diagnostic Standards.

Western Diagnostic Standards. The Consensus of National Seminar on CG held by the Chinese Medical Association Digestive Diseases Branch in 2006 [10] was refered to diagnose whether a patient has CG based on gastroscopy results, pathologic results, and clinical performance.

Chinese Diagnosis Standard. Diagnosis Standard includes the following eight syndromes (patterns) referring to "Guideline for Clinical Research of New Traditional Chinese Medicine" [11] issued by the Ministry of Health and "National Standard of People's Republic of China: Syndrome Part of TCM Clinical diagnosis and Treatment Terminology" [12] issued by the China State Bureau of Technical Supervision,

(1) damp heat accumulating in the spleen-stomach,

(2) dampness obstructing the spleen-stomach,

(3) spleen-stomach qi deficiency,

(4) spleen-stomach cold deficiency,

(5) liver stagnation,

(6) stagnated heat in the liver-stomach,

(7) stomach yin deficiency,

(8) blood stasis in the stomach collateral.

2.1.3. Exclusion Criteria. Excluding criteria were

(1) mentally ill patients and patients with other severe systemic diseases,

(2) patients who have difficulty in describing their conditions, and

(3) patients who are not informed or refuse to cooperate.

2.2. Method for Establishing TCM Inquiry Diagnosis Scales. The research group was composed of Shanghai senior clinical experts on the digestive system, clinical doctors, and researchers. The final TCM inquiry diagnosis scales were drafted based on past experience in the production of scales [13], a wide range of literature about TCM spleen and stomach diseases, related documents in core magazines, and journals for over 15 years and reports about the frequency of symptoms associated with syndromes in CG diseases in TCM. The scales were also amended and fixed by two rounds of expert consultation and statistical tests. The scales include eight dimensions such as cold or heat, sweat, head, chest and abdomen, urine and stool, diet and taste, sleep, mood, woman aspects and contents of disease history, inspection, and palpation. More than 113 variables were ultimately included in these scales.

2.3. Investigation Methods. The clear definitions of symptoms, the specific methods, and the order of inquiry diagnosis are given in the scales. All samplers must have undergone unified training. The group members assemble regularly and discuss the information of typical patients to ensure the consistency of the collected data.

2.4. Diagnosis Methods. Three senior chief doctors with plenty of experience in clinical practices were invited for inquiry diagnosis of the cases in terms of the CG diagnostic standards made by our research group. If two of them have the same diagnosis results, the case was included. Otherwise, the case was not adopted until at least two of them came to the same conclusion.

2.5. Data Input and Process Methods. We have the following methods

(1) Build a database with Epidata software.

(2) Input data two times independently.

(3) The Epidata software compares the two data sets and checks out mistakes.

(4) Check the investigation form logically in case of filling errors.

2.6. Feature Extraction Methods. To obtain the proper set of symptoms for each syndrome, we employed four feature selection methods, namely, mutual information (MI) [14], information gain (IG) [15, 16], conditional mutual information method (CMIM) [17], and minimum redundancy maximum relevance (MRMR) [18], to investigate the relationship between the symptoms and the six common syndromes (patterns), such as the accumulation of damp heat in the spleen-stomach, dampness obstructing the spleen-stomach, spleen-stomach qi deficiency, spleen-stomach cold deficiency, liver stagnation, and stagnated heat in the liver-stomach.

2.7. Multilabel Learning Methods. Many real-world problems involving ambiguous objects lose useful information when analyzed using the traditional single-label algorithm. Thus, it will be harmful to the learning performance. To minimize this information loss, multilabel learning was proposed.

Most traditional multilabel classification approaches to learning methods in vector spaces are used based on the assumption that the instances should have the same set of features in the input space for each label. However, for specific labels, not all the features have strong correlations. ML-kNN is the lazy multilabel learning algorithm based on k-nearest neighbor techniques (kNN) [19]. Similar to the kNN algorithm, it finds the k nearest neighbors for each test instance; however, in ML-kNN, the label of each test instance is estimated directly using the k nearest neighbors in instance. We applied a new algorithm called REAL to fit the characteristics for inquiry diagnosis in TCM based on ML-kNN. The REAL algorithm extracts the best feature subset correlated with a certain label as its input space and then calculates the posterior probability combined with the ML-kNN algorithm. The REAL algorithm is shown in Algorithm 1.

2.8. Experimental Design and Evaluation. Different characteristics were selected using the REAL algorithm. We selected 112, 100, 70, 60, 50, 40, 30, 20, 10, and 5 symptoms (signs), which correlated with each syndrome to build a syndrome model to study the influence of the different symptoms (signs) on the diagnostic model.

Considering each example could simultaneously be associated with multiple labels, performance evaluation in multilabel learning is different from single-label learning. The following five multilabel evaluation parameters presented in [20] are used in this paper.

Average Precision. It evaluates the average fraction of labels ranked above a particular label $y \in Y$, which actually are in Y. The performance is perfect when $\text{avgprec}_S(f) = 1$; the

Step 1: The best feature subset is extracted for each label based on feature selection.
Step 2: Search for the k nearest neighbors based on the distance between two training instances for each labels, which is calculated in the corresponding feature subspace instead of the whole feature space.
Step 3: Calculate the posterior probability with the k nearest neighbors and further the confidential threshold value.
Step 4: Estimate the posterior probabilities of test instances.

Algorithm 1: REAL algorithm.

bigger the value of $\text{avgprec}_S(f)$, the better the performance one has.

$$\text{avgprec}_S(f) = \frac{1}{p}\sum_{i=1}^{p}\frac{1}{|Y_i|} \times \sum_{y\in Y_i}\frac{\left|\left\{y' \mid \text{rank}_f(x_i,y') \le \text{rank}_f(x_i,y), y' \in Y_i\right\}\right|}{\text{rank}_f(x_i,y)}. \quad (1)$$

Coverage. It evaluates how far on the average we need to go down the list of labels to cover all the proper labels of the instance. It is loosely related to precision at the level of perfect recall. The smaller the value of $\text{coverage}_S(f)$, the better the performance one has.

$$\text{coverage}_S(f) = \frac{1}{p}\sum_{i=1}^{p}\max_{y\in Y_i}\text{rank}_f(x_i,y) - 1, \quad \text{rank}_f(x_i,y) = 1 - f(x_i,y). \quad (2)$$

Ranking Loss. It evaluates the average fraction of label pairs that are reversely ordered for the instance. The performance is perfect when $\text{rloss}_S(f) = 0$; the smaller the value of $\text{rloss}_S(f)$, the better the performance,

$$\text{rloss}_S(f) = \frac{1}{p}\sum_{i=1}^{p}\frac{1}{|Y_i|\left|\overline{Y_i}\right|} \times \left|\left\{(y_1,y_2) \mid f(x_i,y_1) \le f(x_i,y_2), (y_1,y_2) \in Y_i \times \overline{Y_i}\right\}\right|, \quad (3)$$

where $\overline{Y}$ denotes the complementary set of Y in $y \cdot y = \{1, 2, \ldots, Q\}$ be the finite set of labels.

Hamming Loss. It evaluates how many times instance-label pairs are misclassified; that is, a label not belonging to the instance is predicted, or a label belonging to the instance is not predicted one has,

$$\text{hloss}_\Gamma(f) = \frac{1}{m}\sum_{i=1}^{m}\frac{1}{n}\left|f(x_i)\Delta Y_i\right|, \quad (4)$$

where Δ denotes the symmetric difference between two sets.

One-Error. It evaluates how many times the top-ranked label is not in the set of proper labels of the instance. The performance is perfect when $\text{one-error}_\Gamma(f) = 0$ we have.

$$\text{one-error}_\Gamma(f) = \frac{1}{m}\sum_{i=1}^{m}\left[\!\left[\arg\max_{y\in Y} f(x_i,y)\right]\!\right] \notin Y_i. \quad (5)$$

For any predicted π, $[\![\pi]\!]$ equals 1 if π holds and 0 if otherwise. Note that, for single-label classification problems, a one-error is identical to an ordinary classification error.

3. Results and Discussion

3.1. The Results of the Finest Symptoms (Signs) Subsets

3.1.1. The Results of Finest Subsets of Specific Symptoms (Signs). In the research of multilabel classification of syndrome diagnosis for CG, feature selection methods such as mutual information, IG, CMIM, and MRMR were combined with multilabel learning. The prediction accuracy was highest when 20 features were selected for classification. Based on the results, the features selected by IG are more suitable for TCM theory than those using other algorithms.

(i) 12 specific symptoms (signs), including yellow tongue coating and greasy tongue coating, were extracted for the pattern of damp heat accumulating in the spleen-stomach.

(ii) 12 specific symptoms (signs), including white and greasy tongue coating, were extracted for the pattern of dampness obstructing the spleen-stomach.

(iii) 11 specific symptoms (signs), including fatigue and tongue with teeth marks, were extracted for the pattern of spleen-stomach qi deficiency.

(iv) 8 specific symptoms (signs), including cold limbs and preference for warm temperature, were extracted for the pattern of spleen-stomach cold deficiency.

(v) 9 specific symptoms (signs), including white aggravating after anxiety or anger, distending pain in the chest, and hypochondriac area, were extracted for the pattern of liver stagnation.

(vi) 12 specific symptoms (signs), including burning pain and red tongue, were extracted for the pattern of stagnated heat in liver-stomach.

The detailed information about symptoms (signs) is displayed in Table 1.

Table 1: The finest subsets of specific symptoms (signs).

Symptoms (signs)	Syndromes (patterns)					
	Damp-heat accumulating in the spleen-stomach	Dampness obstructing the spleen-stomach	Spleen-stomach qi deficiency	Spleen-stomach deficiency cold	Liver stagnation	Stagnated heat in liver-stomach
1	Yellow tongue coating	Greasy tongue coating	Fatigue	Cold limbs	Aggravating after anxiety or anger	Red tongue
2	Greasy tongue coating	Thick tongue coating	White tongue coating	Preference for warm	Distending pain in the chest and hypochondriac area	Burning pain
3	Red tongue	White tongue coating	Tongue with teethmarks	White tongue coating	Belching	Distending pain in the chest and hypochondriac area
4	Thick tongue coating	Whitish tongue	Pale-white tongue	Cold pain	Pain of unfixed location	Preference for cold
5	Retrosternal burning sensation	Tongue with teethmarks	Fat tongue	Whitish tongue	Gastric distension	Yellow tongue coating
6	Dry tongue coating	Fat tongue	Whitish lips	Loose stool	Aggravating after diet	An empty sensation in the stomach
7	Greasy taste	Dark-red tongue	Loose stool	Heaviness of the body	Preference for pressure	Dry stool
8	Dark-red tongue	Slippery tongue coating	Dizziness	Thin tongue coating	Preference for warm	Thin tongue
9	Mixed yellow and white tongue coating	Slippery pulse	Thin tongue coating		Fixed pain	Thirsty
10	Bitter taste in the mouth	Cold limbs	Heaviness of the body			Red lips
11	Preference for cold	Bluish or purple tongue	Whitish complexion			Soure taste
12	Slippery pulse	Hesitant pulse				Insomnia

3.1.2. The Results of Finest Subsets of Negative Symptoms (Signs). They are as follows

(i) 8 negative symptoms (signs) including white and thin tongue coating were extracted for the pattern of damp-heat accumulating in the spleen-stomach.

(ii) 12 negative symptoms (signs) including red and dark red tongue were extracted for the pattern of dampness obstructing the spleen-stomach.

(iii) 11 negative symptoms (signs) including thick tongue coating, mixed yellow, and white tongue coating were extracted for the pattern of spleen-stomach qi deficiency.

(iv) 8 negative symptoms (signs) including red lips and good appetite, but easily gets hungry, were extracted for the pattern of spleen-stomach cold deficiency.

(v) 9 negative symptoms (signs), including fatigue and pain when exposed to cold, were extracted for the pattern of liver stagnation.

(vi) 12 negative symptoms (signs) including tongue with teeth marks and fat tongue were extracted for the pattern of stagnated heat in liver-stomach.

The detailed information about negative symptoms (signs) is displayed in Table 2.

3.2. The Results of Syndrome Classification Using Multilabel Learning Methods

3.2.1. Comparison of Average Accuracy with Different Number of Features. Using the REAL algorithm, we selected 112, 100, 70, 60, 50, 40, 30, 20, 10, and 5 symptoms (signs), which correlated with each syndrome to build a syndrome classification model to study the influence of the different symptoms (signs) on the diagnostic model.

The abscissa represents the number of the selected features, and the vertical axis represents their prediction accuracy in Figure 1.

As shown in Figure 1, the average accuracy changes with the number of symptoms (signs). When the number

Table 2: The finest subsets of negative symptoms (signs).

Symptoms (Signs)	Syndromes (Patterns)					
	Damp-heat accumulating in the spleen-stomach	Dampness obstructing the spleen-stomach	Spleen-stomach qi deficiency	Spleen-stomach deficiency cold	Liver stagnation	Stagnated heat in liver-stomach
1	White tongue coating	Red tongue	Red lips	Red lips	Fatigue	Tongue with teethmarks
2	Thin tongue coating	Dark-red tongue	Thick tongue coating	Stabbing pain	Thick tongue coating	Thick tongue coating
3	Fat tongue	Thin tongue coating	Mixed yellow and white tongue coating	Good appetite but fast hunger	Bitter taste	Greasy tongue coating
4	Tongue with teethmarks	Yellow tongue coating	Greasy tongue coating	Thick tongue coating	Cold pain	Fat tongue
5	Whitish Lips	Distending pain in the chest and hypochondriac area	Red tongue	Fetid mouth odor	Greasy tongue coating	Whitish tongue
6	Whitish complexion	Wiry pulse	Dark-red tongue	Red tongue	Rapid pulse	White tongue coating
7	Whitish tongue	Whitish lips	Yellow tongue coating	Heat sensation in both palms and soles	Thin tongue coating	Slippery pulse
8	Dark -purple lips	Yellow urine	Retrosternal burning sensation	Thin tongue	Loose stool	Cold limbs
9			Large pulse	Yellow tongue coating	Deep pulse	
10				Preference for eating cold food	Heaviness of the body	
11				Dry tongue coating	Rotten tongue coating	
12				Hesitant pulse		

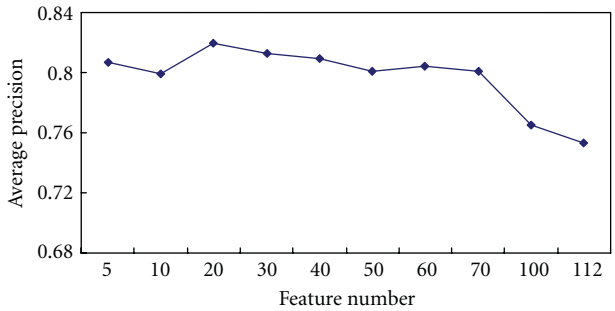


Figure 1: The average accuracy rate with different number of symptoms (signs) by using REAL methods.

of selected symptoms (signs) was 20, the average accuracy peaked at 82%. Then, it decreased gradually with increasing number of symptoms (signs).

3.2.2. Comparison of Performance of Different Multilabel Learning Algorithms. We selected 20 symptoms (signs) to build the models and compared the five evaluation parameters obtained using ML-kNN, Ensembles of Classifier Chains (ECCs), BSVM, BP-MLL, Rank-SVM, and REAL algorithms. The result is shown in Table 3.

As indicated in Table 3, the highest was 82%, obtained by REAL, whereas the lowest average precision was 54%, obtained using BP-MLL. For the indicators coverage, hamming loss, and ranking loss, the values obtained using the REAL algorithm were lowest at 0.160, 0.142, and 0.177, respectively. In summary, the results obtained using the REAL algorithm were the most accurate.

3.2.3. The Comparison of Accuracy Rates of Various Syndromes Using Different Multilabel Methods (the 20 Features Are Selected in REAL Method). The results of the REAL method were compared with the other multilabel learning methods, namely, BP-MLL, Rank-SVM, ECC, BSVM, and ML-kNN. The recognition accuracies of the six common syndromes of CG are shown in Table 4.

As shown in Table 4, for the pattern of damp heat accumulation in the spleen-stomach, the REAL algorithm achieved the highest accuracy rate, followed by ECC, BSVM, Rank-SVM, ML-kNN, and BP-MLL. For the pattern of dampness obstructing the spleen-stomach, the REAL

Table 3: Performance of different multilabel learning algorithms.

Group (mean ± std)	ML-kNN	ECC	BSVM	BP-MLL	RANK-SVM	REAL
Average precision	0.759 ± 0.029	0.802 ± 0.016	0.802 ± 0.016	0.540 ± 0.023	0.707 ± 0.022	0.820 ± 0.029
Coverage	0.200 ± 0.023	0.186 ± 0.019	0.174 ± 0.023	0.345 ± 0.039	0.237 ± 0.016	0.160 ± 0.020
Hamming loss	0.167 ± 0.014	0.148 ± 0.016	0.156 ± 0.014	0.304 ± 0.014	0.214 ± 0.014	0.142 ± 0.019
One error	0.375 ± 0.050	0.261 ± 0.024	0.307 ± 0.022	0.755 ± 0.029	0.449 ± 0.034	0.283 ± 0.055
Ranking loss	0.167 ± 0.025	0.190 ± 0.025	0.130 ± 0.017	0.334 ± 0.040	0.206 ± 0.014	0.117 ± 0.018

Table 4: Comparison of recognition accuracy for six common syndromes.

Syndromes (Patterns)	ML-kNN	ECC	BSVM	BP-MLL	Rank-SVM	REAL
Damp-heat accumulating in the spleen-stomach	0.869 ± 0.036	0.899 ± 0.025	0.884 ± 0.025	0.247 ± 0.035	0.880 ± 0.028	0.901 ± 0.030
Dampness obstructing the spleen-stomach	0.737 ± 0.044	0.789 ± 0.052	0.800 ± 0.035	0.683 ± 0.052	0.762 ± 0.044	0.830 ± 0.038
Spleen-stomach qi deficiency	0.689 ± 0.065	0.741 ± 0.037	0.712 ± 0.023	0.538 ± 0.039	0.679 ± 0.068	0.699 ± 0.041
Spleen-stomach deficiency cold	0.966 ± 0.017	0.958 ± 0.019	0.943 ± 0.027	0.966 ± 0.017	0.793 ± 0.036	0.966 ± 0.023
Liver stagnation	0.827 ± 0.056	0.820 ± 0.043	0.826 ± 0.049	0.831 ± 0.054	0.801 ± 0.047	0.840 ± 0.063
Stagnated heat in liver-stomach	0.908 ± 0.023	0.906 ± 0.034	0.901 ± 0.030	0.910 ± 0.022	0.799 ± 0.048	0.910 ± 0.019

algorithm also had the highest accuracy rate, followed by BSVM, ECC, Rank-SVM, ML-kNN, and BP-MLL.

For the pattern of spleen-stomach qi deficiency, the accuracy rate obtained from ECC was the highest, followed by BSVM, REAL, ML-kNN, Rank-SVM, and BP-MLL. For the pattern of spleen-stomach cold deficiency, REAL, ML-kNN and BP-MLL had the highest accuracy rate at 96.6%, followed by ECC, BSVM, and Rank-SVM. For the pattern of liver stagnation, the REAL algorithm achieved the highest accuracy rate, followed by BP-MLL, ML-kNN, BSVM, ECC, and Rank-SVM. For the pattern of stagnated heat in the liver-stomach, BP-MLL and REAL algorithm achieved the highest accuracy rate, followed by ML-kNN, ECC, BSVM, and Rank-SVM.

From the results, the comprehensive performance of REAL method was the best, with the accuracy rates in the six syndromes, except for the pattern of spleen-stomach qi deficiency.

3.3. Discussion. A syndrome is a unique TCM concept. It is an abstractive conception of a variety of symptoms and signs. It is a pathological summarization of a certain stage of a disease, and it covers disease location, etiology, and the struggle between the body's resistance and pathogenic factors. Different syndromes have different clinical manifestations.

Symptoms, which are the external manifestations of a disease and a syndrome, refer to subjective abnormalities and the abnormal signs of patients elicited by doctors using the four diagnostic methods.

The etiology, location, nature, the struggle between the body's resistance and pathogenic factors, and the condition at a certain stage of the disease process are highly summarized using syndrome differentiation. Syndrome differentiation involves three steps: (a) determining symptoms and signs through inspection, auscultation, inquiry, and palpation; (b) making an overall analysis of the information; (c) making a diagnostic conclusion. All these steps are based on TCM theory.

Figure 2 shows the TCM diagnosis of the network structure diagram. Network structure can be compared to a tree, where the root node is composed of a number of leaf nodes. $X1$, $X2$,..., $X7$ leaf nodes are directly observed, and we call them manifest variables, which denote the symptoms and signs in TCM. $Z1$, $Z2$, $Z3$, and $Z4$ are the root nodes that are indirectly measured through their manifestations, and we call them latent variables, which represent the syndromes of chronic gastritis in TCM. The syndrome can be observed alone or with others, such as $Z1$ and $Z2$, or $Z2$ and $Z3$, which may appear together.

D denotes the disease. In this study, it represents chronic gastritis, which is a disease defined in Western Medicine. Chinese medical diagnosis of chronic gastritis may contain syndromes like latent variables $Z1$, $Z2$, $Z3$ ... and so on.

3.3.1. The Finest Symptoms (Signs) Feature Subsets for Each Syndrome. Feature selection is a hot topic in the field of machine learning. It studies how to select the most effective feature subset from a set of original feature sets to reduce

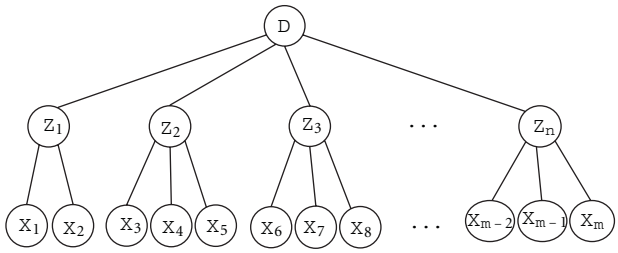


Figure 2: Syndrome diagnostic schemes.

the feature space dimension and enhance the generalization ability of the model.

Feature selection not only removes the redundant and irrelevant features of the data, but also significantly reduces the cost of data mining.

Information gain is a widely used feature selection method [21]. It was first proposed for text classification and was then used in other areas such as image processing and bioinformatics.

Currently, those feature selection methods [22] have been used in TCM diagnosis for selecting symptoms (signs) and building diagnostic models. Many studies have shown that these feature selection methods select key features effectively and also remove irrelevant features. Some symptoms and signs in TCM have certain specific meanings that can be used for determining the syndrome.

When making a diagnosis is difficult using positive aspects, doctors can diagnose by eliminating symptoms and signs of similar syndromes.

Negative information [23] denotes some symptoms that have a negative meaning in the diagnosis of certain diseases, or some information that are impossible to be observed in some diseases.

The purpose of this study is to recognize the common syndromes of CG using IG combined with multilabel learning. The six finest symptoms (signs) subsets were selected by correlating the six common syndromes of CG, which include the specific and negative symptoms (signs). The experimental results show that the six finest symptoms (signs) subsets are basically in accordance with the TCM theory, clinical practice, and the previous Chinese diagnostic standard.

However, individual symptoms (signs) such as the preference for pressure and warm temperature, fixed pain with the syndrome of liver depression and qi stagnation do not agree with the TCM theory, which may be due to the fact that several syndromes appear together.

3.3.2. Comparison between REAL and Other Multilabel Learning Methods. Compared with conventional learning methods, multilabel learning identifies syndromes in TCM more effectively and solves problems of one sample being associated with several syndromes.

In clinical practice, relevance among different syndromes may exist. The syndrome complex of one patient is mainly composed of several syndromes. For example, spleen-stomach qi deficiency syndrome usually exists with dampness obstructing the middle energizer syndrome, qi stagnation syndrome, turbid phlegm syndrome, or blood stasis syndrome.

In multilabel data, there is a relationship among labels. However, this relationship may be bound to be ignored inevitably by using the single-label learning. For this reason, multilabel learning algorithms are developed to facilitate the correlation of the labels.

Compared with other traditional multilabel learning methods, the REAL algorithm found the relevant symptom subset of each syndrome with feature selection. Moreover, the REAL algorithm identified the syndrome information of CG in TCM more effectively and accurately.

In addition, the REAL algorithm assisted in extracting the corresponding specificity and negative symptoms (signs) through feature selection. Extracted features are not only used for identifying the syndrome of chronic gastritis, but it also improves the syndrome diagnostic accuracy of chronic gastritis.

4. Conclusions

To fully understand the characteristics of multilabel data of TCM in syndrome diagnosis, feature selection was combined with a multilabel learning algorithm.

Applying the REAL method extracts the relevant symptoms (signs) for each syndrome and improves the accuracy of syndrome diagnosis in CG.

The study showed that the six finest symptoms (signs) subsets agree with the theory and clinical practice of TCM. In addition, the study will serve as references for establishing diagnostic criteria and a diagnostic model for CG and a better guide for clinical practice. Further studies will focus on building an intelligent diagnostic system for CG with application of the method on biomedical data sets.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant no. 30901897 and 81173199), the Shanghai 3th Leading Academic Discipline Project (Grant no. S30302), and the National Natural Science Foundation of China (Grant no. 30701072.)

References

[1] S. M. Zheng, L. S. Hu, Y. L. Li, and R. Biao, "Screening methods of traditional Chinese medicine," *Chinese Journal of Basic Medicine in Traditional Chinese Medicine*, vol. 14, pp. 304–305, 2008.

[2] G. C. Li, C. T. Li, L. P. Huang et al., "An investigation into regularity of syndrome classification for chronic atrophic gastritis based on structural equation model," *Journal of Nanjing University of Traditional Chinese Medicine*, vol. 22, pp. 217–220, 2006.

[3] Y. J. Bai, Q. G. Meng, H. B. Shen et al., "Study on nonlinear modelling of TCM syndrome of DN based on improved BP neural network model," *Journal of Beijing University of Traditional Chinese Medicine*, vol. 31, pp. 308–311, 2008.

[4] H. L. Gao, Y. L. Wu, J. Chen et al., "The complex system based on entropy method to partition the syndrome diagnosis standard research of application," *China Journal of Traditional Chinese Medicine and Pharmacy*, vol. 23, pp. 307–309, 2008.

[5] G. P. Liu, G. Z. Li, Y. L. Wang, and Y. Q. Wang, "Modelling of inquiry diagnosis for coronary heart disease in traditional Chinese medicine by using multi-label learning," *BMC Complementary and Alternative Medicine*, vol. 10, article 37, 2010.

[6] Y. Su, L. Wang, and H. Zhang, "Posthepatitic cirrhosis of TCM syndrome and disease information similarity analysis," *Chinese Journal of Integrated Traditional and Western Medicine*, vol. 29, pp. 398–402, 2009.

[7] G. P. Liu, Y. Q. Wang, Z. X. Xu et al., "Based on the structure of traditional Chinese medicine of spleen hidden information objectified by analysis," *Journal of Traditional Chinese Medicine*, vol. 52, pp. 1372–1375, 2011.

[8] G. P. Liu, Y. L. Wang, Y. Q. Wang et al., "Coronary Heart Disease of TCM syndrome association," *Journal of Liaoning University of Traditional Chinese Medicine*, vol. 38, pp. 821–824, 2011.

[9] G. P. Liu, Y. Q. Wang, F. F. Li et al., "Association analysis and distribution of chronic gastritis syndromes based on associated density," in *Proceedings of the IEEE International Conference on Bioinformatics and Biomedicine Workshops (BIBMW '10)*, pp. 790–794, December 2010.

[10] Chinese Medical Association Digestive Diseases Branch, "Consensus of national seminar on chronic gastritis," *Chinese Journal of Digestive Endoscopy*, vol. 24, pp. 58–63, 2007.

[11] X. Y. Zheng, *Chinese Herbal Medicine Clinical Research Guiding Principles (for Trial Implementation)*, China Medical Science and Technology Press, Beijing, China, 2004.

[12] State Bureau of Technical Supervision, *National Standards of Peoples Republic of China: Syndrome Part of TCM Clinical Diagnosis and Treatment*, Standards Press of China, Beijing, China, 1997.

[13] G. P. Liu, Y. Q. Wang, Y. Dong et al., "Development and evaluation of Scale for heart system Inquiry of TCM," *Journal of Chinese Integrative Medicine*, vol. 7, pp. 1222–1225, 2009.

[14] W. C. Kenneth and H. Patrick, "Word association norms, mutual information, and lexicography," in *Proceedings of the 27th Annual Meeting on Association for Computational Linguistics (ACL '89)*, vol. 27, pp. 76–83, Vancouver, Canada, 1989.

[15] J. R. Quinlan, "Induction of decision trees," *Machine Learning*, vol. 1, no. 1, pp. 81–106, 1986.

[16] T. Mitchell, *Machine Learning*, McCraw Hill, 1996.

[17] F. Fleuret, "Fast binary feature selection with conditional mutual information," *Journal of Machine Learning Research*, vol. 5, pp. 1531–1555, 2004.

[18] H. Peng, F. Long, and C. Ding, "Feature selection based on mutual information: criteria of Max-Dependency, Max-Relevance, and Min-Redundancy," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, vol. 27, no. 8, pp. 1226–1238, 2005.

[19] R. E. Schapire and Y. Singer, "BoosTexter: a boosting-based system for text categorization," *Machine Learning*, vol. 39, no. 2, pp. 135–168, 2000.

[20] M. L. Zhang and Z. H. Zhou, "ML-KNN: a lazy learning approach to multi-label learning," *Pattern Recognition*, vol. 40, no. 7, pp. 2038–2048, 2007.

[21] M. Y. You, Y. Chen, and G. Z. Li, "Disequilibrium problem of the new feature selection algorithm: Im-IG," *Shandong University Journal*, vol. 40, pp. 123–128, 2010.

[22] H. Shao, G. Z. Li, and G. P. Liu, "Symptom selection for multi-label data of inquiry diagnosis in traditional Chinese medicine," *Science China Information Sciences*, vol. 41, pp. 1372–1387, 2011.

[23] Y. J. Xu, *TCM tongue pulse diagnosis system and tongue diagnosis digital pulse*, Ph.D. thesis, Beijing University of Chinese Medicine.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 837245, 5 pages
doi:10.1155/2012/837245

Research Article

Intelligent ZHENG Classification of Hypertension Depending on ML-kNN and Information Fusion

Guo-Zheng Li,[1,2] Shi-Xing Yan,[1] Mingyu You,[1] Sheng Sun,[1] and Aihua Ou[2]

[1] *Department of Control Science and Engineering, Tongji University, Shanghai 201804, China*
[2] *The Department of Clinical Epidemiology and The Cardiovascular Medicine of Chinese Medical, Guang Dong Provincial Hospital of Traditional Chinese Medicine, Guangzhou 510120, China*

Correspondence should be addressed to Mingyu You, myyou@tongji.edu.cn and Aihua Ou, ouaihua2@163.com

Received 14 February 2012; Accepted 3 April 2012

Academic Editor: Shao Li

Hypertension is one of the major causes of heart cerebrovascular diseases. With a good accumulation of hypertension clinical data on hand, research on hypertension's ZHENG differentiation is an important and attractive topic, as Traditional Chinese Medicine (TCM) lies primarily in "treatment based on ZHENG differentiation." From the view of data mining, ZHENG differentiation is modeled as a classification problem. In this paper, ML-kNN—a multilabel learning model—is used as the classification model for hypertension. Feature-level information fusion is also used for further utilization of all information. Experiment results show that ML-kNN can model the hypertension's ZHENG differentiation well. Information fusion helps improve models' performance.

1. Introduction

Hypertension is one of the major causes of heart cerebrovascular diseases. 25%–35% adults over the world have hypertension. There are over 972 million hypertension patients, of which 60%–70% are over 70 years old [1, 2]. With the fast development of electronic medical record (EMR) system, there exists a good accumulation of clinical cases about hypertension. As diagnostic knowledge and herb formula of Traditional Chinese Medicine (TCM) are mostly distilled from clinical practice, researches on these clinical cases may help promote the understanding toward TCM theory, make progress on the development of diagnosis technology, and also contribute to the objection and modernization of TCM.

ZHENG, also translated as syndrome, in TCM means a characteristic profile of all clinical manifestations that can be identified by a TCM practitioner. TCM lies primarily in "treatment based on ZHENG differentiation" [3]. Only after successful differentiation of ZHENG, can effective treatment of TCM be possible [4]. Traditionally, techniques of ZHENG differentiation are learned by successors of a particular TCM practitioner only and learning effect is always confined to the successors' personal talents. With the unprecedented growth of clinical data, this way is no longer proper, which makes it difficult to discover new knowledge from the data mountain. Data mining is a distinguished technology to track the underlying information. Many research works have been dedicated to TCM data mining [5–7], all of which indicate a promising future for auto differentiation of ZHENG in TCM.

In the field of data mining, differentiation of ZHENG is modeled as a classification problem. For traditional classification methods, every instance should have one and only one label. However, TCM diagnostic result usually consists of several ZHENG. In other words, one patient could have more than one ZHENG. Professionally, it is called multilabel data, the learning of which is a rather hot topic recently in the fields of data mining and machine learning. International workshops about multilabel learning are held in the recent three years, respectively, to promote the development of this topic [8, 9]. Multilabel learning has been applied to TCM by Liu et al. [7], who compared the performance of ML-kNN and kNN on a coronary heart disease dataset. Li et al. and Shao et al. proposed embedded multilabel feature selection method MEFS [10] and wrapper multilabel feature selection

Table 1: Information from inspection diagnosis.

Pale whit complexion	Lusterless complexion	Sallow complexion	Reddened complexion	Bleak complexion	Facial hot flashes	Flushed complexion
Hot eyes	Blue lips	Dark purple lips	Lusterless lips	Red ear	Reddish urine	Yellow urine
Clear abundant urine	Lassitude of spirit	No desire to speak	Listlessness	Palpitate with fear	Impatient	Irritability

Table 2: Information from tongue diagnosis.

Pale tongue	Red tongue	Dark red tongue	Pale red tongue	Crimson tongue	Teeth-marked tongue	Tender tongue
Tender and red tongue	Bluish purple tongue	Enlarged and pale tongue	Red margins and tip of the tongue	Petechial on tongue	Enlarged tongue	Dark tongue body
Sublingual collateral vessels tongue	Thin fur	Yellow fur	White slimy fur	Few fur	White fur	Thin yellow fur
Yellow slimy fur	No fur	Thin white fur	Slimy fur	Thick slimy fur	White slippery fur	

method HOML [11], respectively, to improve multilabel classification's performance on a coronary heart disease dataset.

One characteristic of TCM ZHENG differentiation is "fusion use of four classical diagnostic methods." Inspection, auscultation and olfaction, inquiry and palpation are the four classical diagnostic methods in TCM. How to use information from these four diagnostic methods to make better ZHENG differentiation is an important research area in TCM field. Some theories of Traditional Chinese Medicine diagnosis even claim that only by using information from all the four classical diagnostic methods can we differentiate correctly the ZHENG [4]. And "fusion use of the four classical diagnostic methods" is treated as an important direction in computerization of TCM diagnosis [12]. In fact, it is called information fusion in the field of data mining. Therefore, fusion of information from different sources should be considered seriously in building ZHENG classification with multilabel learning techniques. Nowadays, no researchers have tried to bring techniques of information fusion into the field of multilabel learning. Wang et al. have done some work in TCM information fusion using traditional single-label methods, which mainly focus on the data acquisition and medical analysis on experiment results [12, 13]. But as described above, multilabel learning should be more appropriate for ZHENG classification. So more attention should be paid on the research of information fusion for multilabel learning.

In this paper, we try to build TCM ZHENG classification models on hypertension data using multilabel learning and information fusion. The rest of the paper is arranged as follows. Section 2 describes materials and methods, including the data source, data preprocessing, feature-level information fusion, and ML-kNN. Experimental results and discussions are shown in Section 3. Finally Section 4 draws conclusions on this paper.

2. Materials and Methods

2.1. Data Source. The hypertension datasets used in this paper are from LEVIS Hypertension TCM Database. The data are from the in-patient, out-patient cases of Cardio Center, Cardiovascular Internal Department, Nerve Internal Department, and Medical Examination Center, and so forth in Guangdong Provincial Hospital of TCM in China during November 2006 to December 2008, as well as some cases from on-the-spot investigation in Li Wan District Community in Guangzhou of China during March 2007 to April 2007. With strict control measures, 775 reliable TCM hypertension clinical cases are recorded in this database. 148 features, including 143 TCM symptoms from inspection, auscultation and olfaction, inquiry and palpation, and 5 common indexes including gender, age, hypertension duration, SBPmax, and DBPmax, are investigated and collected in this database. It also stores the 13 labels (TCM ZHENG) of each case. Academic and noncommercial users may access it at http://levis.tongji.edu.cn/datasets/index_en.jsp.

2.2. Data Preprocessing. According to the theory of TCM, the characteristics of the LEVIS Hypertension TCM Database, and our research target that evaluation of the performance of multilabel classification model on datasets with information from particular diagnostic methods only (we call them single-diagnosis datasets later) and on dataset with fusional information of all diagnostic methods (called fusional-diagnosis dataset), five single-diagnosis datasets are retrieved from the LEVIS Hypertension TCM Database. The information contained in each datasets is shown in Tables 1, 2, 3, 4, and 5, which comes, respectively, from inspection diagnosis, tongue diagnosis, inquiry diagnosis, palpation diagnosis, and other diagnoses. Analyzing the 775 cases, 4 cases are found to have empty value in one of the features mentioned above in the five tables. Thus, these 4 cases are removed from all

Table 3: Information from inquiry diagnosis.

Headache	Dizzy	Swelling pain of head-eye	Vertigo	Wrapped head	Heavy-headedness	Stretching
Empty pain	Dizzy vision	Visual deterioration	Blurred vision	Dry	Eyes bulge	Deaf
Tinnitus	Chest pain	Distending pain in hypochondrium	Soreness of waist	Weakness of knees	Oppression in chest	Stuffiness in chest
Weakness of limb	Abdominal distention	Numbness	Anorexia	Dry mouth	Insomnia	Dreamy
Bitter taste in mouth	Bland taste in the mouth	Somnolence	Constipation	Short urine	Frequent nocturia	Sloppy stool
Heat in the palms and soles	Torrid	Cold body	Cold limbs	Fear of cold	Exing heat in the chest palms and soles	

Table 4: Information from palpation diagnosis.

Fine	Rough	Fine rapid	Slippery wiry	Fine rapid wiry	Slippery	Weak
Fine wiry	Rough wiry	Slippery rapid	Rapid	Intermittent bound	Soggy slippery	Rapid wiry
Wiry	Fine weak	Rough sunken	Fine wiry	Soggy	Fine rough	Fine sunken

the five single-diagnosis datasets to ensure smooth progress of the following tasks: information fusion and classification model building.

In the above data sets, we find some labels appear rarely, which will severely hurt severely performance of classification methods. We randomly choose part of the data set in this work. Firstly, labels are selected to decrease the degree of imbalance. In this case, we chose labels 6, 10, and 12, as they have the largest number of positive cases and multilabel method should predict at least 3 labels simultaneously. Secondly, cases are selected that are marked negative on all the selected labels to be the pending removable set, so that the entire positive cases in any label are preserved. Finally, randomly remove some cases from the pending removable set to decrease imbalance. Here, 500 cases are put into the pending removable set and 100 cases are selected from the set to form one dataset with remaining cases each time. So finally, we get five datasets and the performance of our model is evaluated according to the average performance on all datasets. The final used data set may be downloaded from: http://levis.tongji.edu.cn/datasets/htn-ecam.zip.

2.3. Feature-Level Information Fusion. In this work, we only discuss information fusion on the level of feature [14, 15]. Let $A = \{a_1, a_2, \ldots, a_n\}$, $B = \{b_1, b_2, \ldots, b_m\}$, C, D, E denote, respectively, the 5 feature vectors with different dimensions illustrated in Tables 1–5. The target is to combine these five feature sets in order to yield a new feature vector, Z, which would better represent the individual or help build better classification model [14]. Specifically, information fusion is accomplished by simply augmenting the information (feature) obtained from multiple diagnostic methods. The vector Z is generated by augmenting vectors A to B, C, D, and E one after the other. The concrete stages are described below:

(1) *Feature Normalization*. The individual feature values of particular vectors, such as a_{11} and b_{m2}, may exhibit significant variations both in their range and distribution. The goal of feature normalization is to modify the location (mean) and scale (variance) of the values to ensure that the contribution of each vector to the final vector Z is comparable. Min-max normalization techniques were used in this work. It computes the value x' after normalization using the formula, $x' = (\mathrm{x} - \min(\mathrm{Fx}))/(\max(\mathrm{Fx}) - \min(\mathrm{Fx}))$, where x and x' denote, respectively, a feature value before and after normalization and Fx is the feature value set that contains all values of a specific feature. Normalizing all feature values via this method, we get the modified feature vectors A', B, C', D', and E'.

(2) *Feature Concatenation*. Augment the 5 feature vectors, which results in a new feature vector, $Z' = \{a_1', \ldots, a_n', b'_1, \ldots, b_m', \ldots, e_1', \ldots, e_l'\}$.

2.4. Multilabel Learning: ML-kNN. As illustrated in Section 1, multilabel learning model is believed to be more suitable classification model for TCM clinical data. Specifically, we constructed models of the relationship between symptoms and ZHENG by means of the multilabel k-nearest neighbor (ML-kNN) algorithm [16] in this study. ML-kNN is a lazy multilabel learning algorithm developed on the basis of kNN algorithm, which regards an instance as a point in synthesis space. kNN's idea is to search for k training instances nearest to the testing instance, and then predict the label of the test instance according to the

Table 5: Information from other diagnosis.

Night sweating	Palpitate	Muscular twitching and cramp	Sputum	Facial paralysis	Spermatorrhoea	Palpitation
Nausea vomiting	Dry in the throat	Stiffness of the neck	Forgettery	Short breath	Lusterless of hair	Luxated tooth
Heavy body	Impotence	Shortness of breath	Retch nausea sputum	Fat		

Table 6: Experimental results of ML-kNN on Six datasets.

Dataset type	Inspection	Tongue	Inquiry	Palpation	Others	Fusional
Average precision	0.80	0.77	0.79	0.78	0.77	0.81
Coverage	0.42	0.40	0.41	0.42	0.39	0.44
Hamming loss	−0.13	−0.13	−0.13	−0.13	−0.13	−0.14
macroF1 measure	0.01	0.01	0.00	0.00	0.00	0.01
microF1 measure	0.01	0.01	0.00	0.00	0.01	0.01
One error	−0.34	−0.38	−0.35	−0.38	−0.38	−0.32
Ranking loss	−0.28	−0.31	−0.29	−0.29	−0.32	−0.25

nearest instances' labels. Compared with other algorithms, advantage of kNN lies in its simpler training process, better efficiency, and competitive performance. Based on the theory of kNN, ML-kNN also aims to find k nearest instances for each test instance. But rather than judging labels directly by nearest instances, ML-kNN utilizes the "maximum a posteriori estimation" principle to determine the label set based on statistical information derived from the label sets of neighboring instances. The concrete steps are demonstrated below [7]:

(1) calculate the conditional probability distribution of each instance associated to each label;

(2) calculate the distance between the x_i test instance and the training instances; then find k nearest instances for x_i. Repeat for each test instance;

(3) according to the labels of k training instances and the conditional probability associated to each label, forecast the probability of the x_i instance and then acquire the forecast results (≥ 0.5 is taken here); Repeat for each test instance;

(4) evaluate the forecast results according to multilabel evaluation criteria.

3. Results and Discussions

3.1. Experiment Setting and Procedure. Firstly, five single-diagnosis datasets are retrieved from LEVIS Hypertension TCM Database as illustrated in Section 2.1. Secondly, data preprocessing is conducted on all the five datasets as described in Section 2.2. Thirdly, feature-level information fusion mentioned in Section 2.3 is applied to the single-diagnosis datasets and yields fusional-diagnosis dataset. There are five single-diagnosis datasets and one fusional-diagnosis dataset. Fourthly, ML-kNN is used to train models and test models on all the 6 datasets with parameter k set to be 10; to better reveal performance of models, 10-fold cross-validation is conducted, and the average results of each fold are taken as the final results.

3.2. Evaluation Criterion. In order to measure and compare effectively and comprehensively the performance of ML-kNN, multiple evaluation criterions are computed, including Precision, Macroaverage F1-Measure, Microaverage F1-Measure, Coverage, Hamming Loss, One Error, and Ranking Loss. Each criterion has its own characteristic which display one aspect of a model's performance. More information about these criterions can be found in [9].

3.3. Experimental Results and Discussions. Table 6 summarizes the experimental results on the five single-diagnosis datasets and the one fusional-diagnosis dataset. All the seven evaluation criterions are configured to be the bigger the better, even for negative number (the closer to zero, the better).

From the Table 6, we can find the following.

(1) The model built on inspection-diagnosis dataset performs the best in all the evaluation criterions, among the 5 models built on single-diagnosis datasets, which demonstrates that inspection may be the best way to differentiate ZHENG about hypertension.

(2) For all evaluation criterions, performance of fusional-diagnosis model is the best, which may prove strongly the TCM theory that "fusion use of the four classical diagnostic methods" is essential and help improve the accuracy of ZHENG differentiation.

4. Conclusions

In this paper, we attempted to use feature-level information fusion technique and ML-kNN algorithm to improve

performance of intelligent ZHENG classification, which is a tough but essential task in TCM. Instead of using traditional learning methods, according to the characteristics of TCM clinical cases, a popular multilabel learning method, ML-kNN, is used as the classification model. Information fusion to properly combine information from different diagnostic methods is used to improve classification performance, which confirms the TCM theory of "comprehensive analysis of data gained by four diagnostic methods."

In future, we will continue this study to solve the imbalance in the data set and try model level information fusion.

Acknowledgments

This work was supported by the Natural Science Foundation of China under grant nos. 61005006 and 61105053, as well as the Fundamental Research Funds for the Central Universities.

References

[1] J. Guo and A. H. Ou, "The prevention status of primary hypertension in communities of our country," *Chinese General Medicine*, pp. 1354–1356, 2009.

[2] J. F. Vilela-Martin, R. O. Vaz-de-Melo, C. H. Kuniyoshi, A. N. R. Abdo, and J. C. Yugar-Toledo, "Hypertensive crisis: clinical-epidemiological profile," *Hypertension Research*, vol. 34, no. 3, pp. 367–371, 2011.

[3] *The Inner Canon of Emperor Huang*, Chinese Medical Ancient Books Publishing House, 2003.

[4] T. T. Deng, *Practical TCM Diagnostics*, People's Medical Publishing House, Beijing, China, 2004.

[5] X. Zhou, Y. Peng, and B. Liu, "Text mining for traditional Chinese medical knowledge discovery: a survey," *Journal of Biomedical Informatics*, vol. 43, no. 4, pp. 650–660, 2010.

[6] S. K. Poon, J. Poon, M. McGrane et al., "A novel approach in discovering significant interactions from TCM patient prescription data," *International Journal of Data Mining and Bioinformatics*, vol. 5, no. 4, pp. 353–368, 2011.

[7] G. P. Liu, G. Z. Li, Y. L. Wang, and Y. Q. Wang, "Modelling of inquiry diagnosis for coronary heart disease in traditional Chinese medicine by using multi-label learning," *BMC Complementary and Alternative Medicine*, vol. 10, page 37, 2010.

[8] Y.-H. Liu, G.-Z. Li, H.-Y. Zhang, J. Y. Yang, and M. Q. Yang, "Feature selection for gene function prediction by using multi-label lazy learning," *International Journal of Functional Informatics and Personalised Medicine*, vol. 1, no. 3, pp. 223–233, 2008.

[9] G. Tsoumakas, I. Katakis, and I. Vlahavas, "Mining multi-label data," in *Data Mining and Knowledge Discovery Handbook*, O. Maimon and L. Rokach, Eds., pp. 667–685, Springer, Boston, Mass, USA, 2009.

[10] G. Z. Li, M. You, L. Ge, J. Y. Yang, and M. Q. Yang, "Feature selection for semi-supervised multi-label learning with application to gene function analysis," in *Proceedings of the 1st ACM International Conference on Bioinformatics and Computational Biology (ACM-BCB '10)*, pp. 354–357, Niagara Falls, NY, USA, August 2010.

[11] H. Shao, G. Z. Li, G. P. Liu, and Y. Wang, "Symptom Selection for Multi-label Data of Inquiry Diagnosis in Traditional Chinese Medicine," *SCIENCE CHINA Information Sciences*, vol. 54, no. 1, pp. 1–13, 2011.

[12] Y. Q. Wang, "Progress and prospect of objectivity study on four diagnostic methods in Traditional Chinese Medicine," in *Proceedings of the IEEE International Conference On Bioinformatics and Biomedicine Workshops (BIBMW '10)*, p. 3, 2010.

[13] Y. Q. Wang, Z. X. Xu, F. F. Li, and H. X. Yan, "Research ideas and methods about objectification of the four diagnostic methods of Traditional Chinese Medicine," *Acta Universitatis Traditionis Medicalis Sinensis Pharmacologiaeque Shanghai*, vol. 23, pp. 4–8, 2009.

[14] A. Ross and R. Govindarajan, "Feature level fusion using hand and face biometrics," in *Biometric Technology for Human Identification II*, A. K. Jain and N. K. Ratha, Eds., vol. 5779 of *Proceedings of SPIE*, pp. 196–204, Orlando, Fla, USA, March 2005.

[15] A. Ross and A. Jain, "Information fusion in biometrics," *Pattern Recognition Letters*, vol. 24, no. 13, pp. 2115–2125, 2003.

[16] M. L. Zhang and Z. H. Zhou, "ML-KNN: a lazy learning approach to multi-label learning," *Pattern Recognition*, vol. 40, no. 7, pp. 2038–2048, 2007.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 912852, 14 pages
doi:10.1155/2012/912852

Research Article

Automated Tongue Feature Extraction for ZHENG Classification in Traditional Chinese Medicine

Ratchadaporn Kanawong,[1] Tayo Obafemi-Ajayi,[1] Tao Ma,[2] Dong Xu,[1] Shao Li,[2] and Ye Duan[1]

[1] *Department of Computer Science and Informatics Institute, University of Missouri, Columbia, MO 65211, USA*
[2] *MOE Key Laboratory of Bioinformatics and Bioinformatics Division, TNLIST/Department of Automation, Tsinghua University, Beijing 100084, China*

Correspondence should be addressed to Shao Li, shaoli@mail.tsinghua.edu.cn and Ye Duan, duanye@missouri.edu

Received 13 February 2012; Accepted 30 March 2012

Academic Editor: Shi-Bing Su

ZHENG, Traditional Chinese Medicine syndrome, is an integral and essential part of Traditional Chinese Medicine theory. It defines the theoretical abstraction of the symptom profiles of individual patients and thus, used as a guideline in disease classification in Chinese medicine. For example, patients suffering from gastritis may be classified as Cold or Hot ZHENG, whereas patients with different diseases may be classified under the same ZHENG. Tongue appearance is a valuable diagnostic tool for determining ZHENG in patients. In this paper, we explore new modalities for the clinical characterization of ZHENG using various supervised machine learning algorithms. We propose a novel-color-space-based feature set, which can be extracted from tongue images of clinical patients to build an automated ZHENG classification system. Given that Chinese medical practitioners usually observe the tongue color and coating to determine a ZHENG type and to diagnose different stomach disorders including gastritis, we propose using machine-learning techniques to establish the relationship between the tongue image features and ZHENG by learning through examples. The experimental results obtained over a set of 263 gastritis patients, most of whom suffering Cold Zheng or Hot ZHENG, and a control group of 48 healthy volunteers demonstrate an excellent performance of our proposed system.

1. Introduction

Traditional Chinese Medicine (TCM) has a long history in the treatment of various diseases in East Asian countries and is also a complementary and alternative medical system in Western countries. TCM takes a holistic approach to medicine with emphasis on the integrity of the human body and the close relationship between a human and its social and natural environment [1]. TCM applies different therapeutic methods to enhance the body's resistance to diseases and prevention. TCM diagnosis is based on the information obtained from four diagnostic processes, that is, *looking, listening, and smelling, asking, and touching.* The most common tasks are taking the pulse and inspecting the tongue [2]. For thousands of years, Chinese medical practitioners have diagnosed the health status of a patients' internal organs by inspecting the tongue, especially the patterns on the tongue's surface. The tongue mirrors the viscera. The changes of tongue can objectively manifest the states of a disease, which can help differentiate syndromes, establish treatment methods, prescribe herbs, and determine prognosis of disease.

ZHENG (TCM syndrome) is an integral and essential part of TCM theory. It is a characteristic profile of all clinical manifestations that can be identified by a TCM practitioner. ZHENG is an outcome after analyzing all symptoms and signs (tongue appearance and pulse feeling included). All diagnostic and therapeutic methods in TCM are based on the differentiation of ZHENG, and this concept is as ancient as TCM in China [3]. ZHENG is not simply an assemblage of disease symptoms but rather can be viewed as the TCM theoretical abstraction of the symptom profiles of individual patients. *As noted in the abstract, ZHENG is also used as a guideline in TCM disease classification. For example, patients*

suffering from the same disease may be grouped into different ZHENGs, whereas different diseases may be grouped as the same ZHENG. The Cold ZHENG (Cold syndrome) and the Hot ZHENG (Cot syndrome) are the two key statuses of ZHENG [3]. *Other ZHENGs include Shen-Yang-Xu ZHENG (Kidney-Yang deficiency syndrome), Shen-Xu ZHENG (Kidney deficiency syndrome), and Xue-Yu ZHENG (Blood Stasis syndrome)* [4].

In this paper, we explore new modalities for the clinical characterization of ZHENG using various supervised machine-learning algorithms. Using an automated tongue-image diagnosis system, we extract objective features from tongue images of clinical patients and analyze the relationship with their corresponding ZHENG data and disease prognosis (specifically stomach disorders, i.e., gastritis) obtained from clinical practitioners. We propose a system that learns from the clinical practitioner's subjective data on how to classify a patient's health status by extracting meaningful features from tongue images using a rich set of features based on color-space models. Our premise is that Chinese medical practitioners usually observe the tongue color and coating to determine ZHENG such as Hot or Cold ZHENG, and to diagnose different stomach disorders including gastritis. Hence, we propose using machine-learning techniques to establish the relationship between the tongue image features and the ZHENG by learning through examples. We are also interested in the correlation between the Hot and Cold patterns observed in ZHENG gastritis patients and their corresponding symptom profiles.

Various types of features have been explored for tongue feature extraction and tongue analysis, including texture [5], color [6–8], shape [9], spectrum [8], among others. A systematic tongue feature *set, comprising of a combination of geometric features (size, shape, etc.), cracks, and textures, was later proposed by Zhang et al.* [10]. Computer-aided tongue analysis systems based on these types of features have also been developed [11, 12]. Our goal is to provide a set of objective features that can be extracted from patients' tongue images, based on the knowledge of ZHENG, which improves accuracy of an objective clinical diagnosis. Our proposed tongue feature set is based on an extensive color model.

This paper is organized as follows: in Section 2, we provide a TCM descriptive view of the physiology of the tongue. An overview of the proposed feature extraction and learning framework along with a complete description of the color space model feature set is presented in Section 3. Our experimental results and analysis in a tongue image dataset from gastritis patients with Cold ZHENG and Hot ZHENG are discussed in Section 4 before drawing our conclusions and proposing plans for future work in Section 5.

2. Tongue Diagnosis in TCM

TCM believes that the tongue has many relationships and connections in the human body, both to the meridians and the internal organs. It is, therefore, very useful and important during inspection for confirming TCM diagnosis as it can present strong visual indicators of a person's overall physical and mental harmony or disharmony. In TCM, the tongue is divided into tongue tip, tongue margins, tongue center, and tongue root. *Figure 1(a) shows each part of the tongue and its correspondence to different internal organs according to TCM while Figure 1(b) illustrates how we geometrically obtain an approximation of these regions from the tongue image.* The tongue tip reflects the pathological changes in the heart and lungs, while the bilateral sides of the tongue reflect that of the liver and gallbladders. The pathological changes in the spleen and stomach are mirrored by the center of tongue, while changes in the kidneys, intestines, and bladder section correspond to the tongue root.

In this paper, we focus on the patients with stomach disorders, gastritis. Hence, we are interested in extracting features not only from entire tongue image but also specifically from the middle region, as this corresponds to the stomach organ, according to TCM. We extract the middle rectangular region, illustrated in Figure 1(b), as our approximation for the tongue middle region.

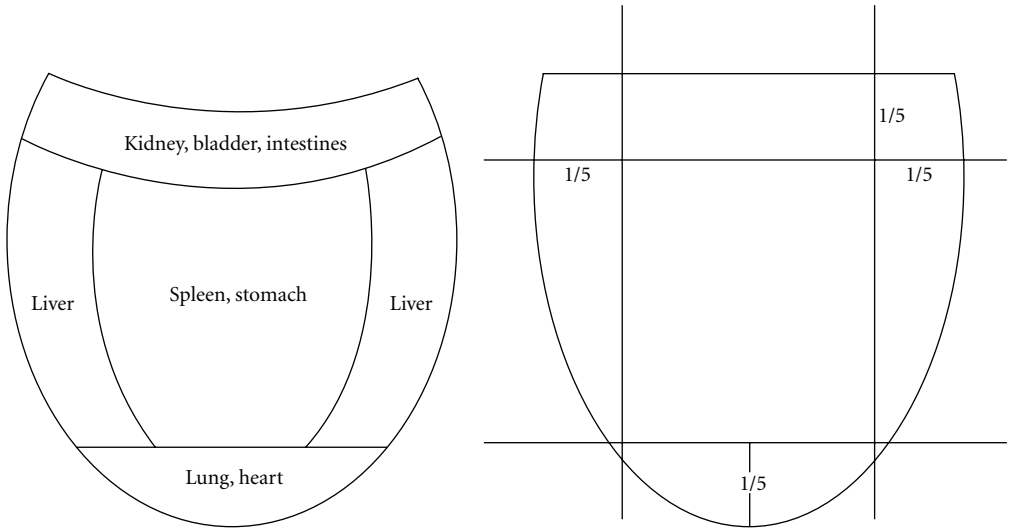
The practitioner examines the general and local shape as well as the color of the tongue and its coating. According to TCM, the normal tongue is pale red with thin white coating. Some signs of imbalance or pathology are red body, yellow coating, or thick coating like mozzarella cheese, and so forth. Some characteristic changes occur in the tongue in some particular diseases. Most tongue attributes are on the tongue surface. A TCM doctor looks at several attributes of tongue body: color, moisture, size, shape, and coating. These signs not only reveal overall states of health but they also correlate to specific organ functions and disharmonies, especially in the digestive system.

The two main characteristics of the tongue in TCM ZHENG diagnosis are the color and the coating. The color of the patient's tongue color provides information about his/her health status. For example [13], dark red color can indicate inflammation or ulceration, while a white tongue indicates cold attack, mucus deposits, or a weakness in the blood leading to such conditions as anemia [12]. Moreover, a yellow tongue points out a disorder of the liver and gallbladder, and blue or purple implies stagnation of blood circulation and a serious weakening of the part of the digestive system that corresponds to the area of the tongue where the color appears.

The coating on the tongue is discriminated by not only its presence but also its color. The color could be yellow, white, and other colors. However, the color in image is not the exact true color of the tongue. To properly identify the color of the tongue coating, we applied the specular component technique presented in our prior work on tongue detection and analysis [2]. Figure 2 illustrates different tongue images of patients and their corresponding ZHENG class.

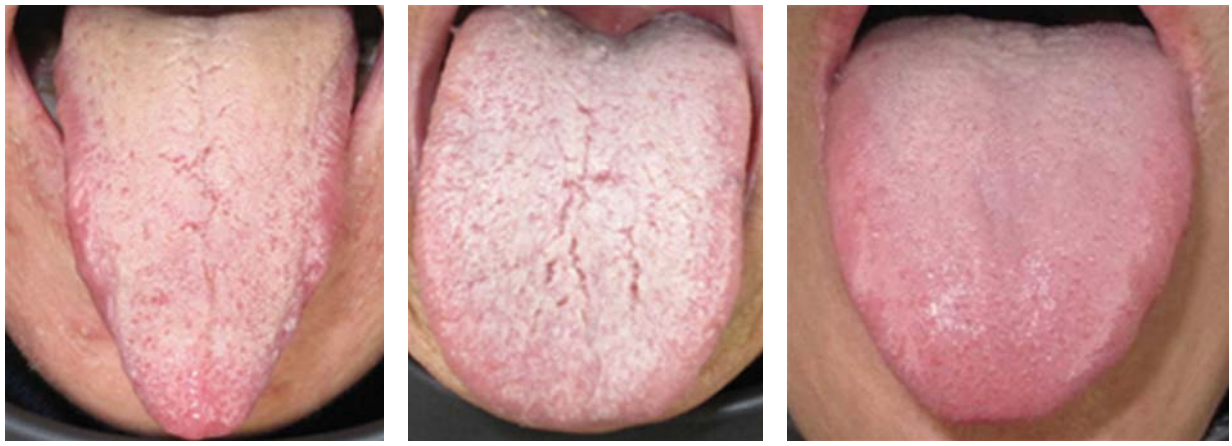
3. Tongue Feature Extraction and Classification Framework

3.1. Feature Extraction for Tongue Image Analysis. Our goal is to compute a set of objective features $\vec{F_j} = \{F_n\}$ from each tongue image j that can be fed into our learning



(a) organ layout of tongue regions (b) geometrical layout of tongue regions

Figure 1: Tongue areas and their correspondence to internal organs in TCM.



(a) Hot ZHENG (b) Cold ZHENG (c) Normal

Figure 2: Tongue images of patients with different ZHENG classification. "Normal" represents a healthy person.

system so that we can predict not only the color and coating on the tongue, but also different ZHENGs of the gastritis patients. These features are designed to capture different color characteristics of the tongue. While a single feature may not be very discriminative, our premise is that the aggregation of these features will be discriminative. We leave it to the learning algorithm to determine the weight/contribution of each feature in the final classification.

Most color spaces are represented in tuples of number, normally three or four color components. Color components determine the position of the color in the color space used. There are many color spaces defined for different purposes. We designed a set of 25 features that span the entire color-space model. They can be grouped under eight categories: RGB, HSV, YIQ, Y'CbCr, XYZ, L*a*b*, CIE Luv, and CMYK.

In this section, we first describe in detail how we compute each feature $\vec{f}^i$ per ith pixel in the image. Then, we explain how each feature per pixel is aggregated to obtain $\vec{F}_j = \{F_n\}$ per tongue image j.

3.1.1. RGB. RGB is an additive color system, based on trichromatic theory in which red, green, and blue light components are added together to produce a specific pigment. The RGB model encodes the intensity of red, green, and blue, respectively. (R_i, G_i, B_i) for each pixel is an unsigned integer between 0 and 255. Each RGB feature $\{f_n^i \mid n = 1, \ldots, 3\}$ represents the normalized intensity value of the red, green, and blue component, respectively, of the ith pixel in the image. We denote the normalized value of each component

as $r_i = R_i/255$, $g_i = G_i/255$, and $b_i = B_i/255$. Thus, $f_1^i = r_i$; $f_2^i = g_i$; $f_3^i = b_i$.

All the remaining color-space model features described in our feature set derive their value from the RGB feature set.

3.1.2. HSV. HSV color space represents color using a 3-tuple set of hue, saturation, and value. It separates the luminance component of the color from chrominance information. The HSV model (H_i, S_i, V_i) is obtained by a linear transformation of thenormalized RGB color space $\{r_i, g_i, b_i\}$.

For each pixel p_i, let $\widetilde{M}_i = \max\{r_i, g_i, b_i\}$ represent the maximum value of the pixel's RGB triple set while $\widetilde{m}_i = \min(r_i, g_i, b_i)$, the minimum value of the set. We also denote the difference between maximum and minimum values of each RGB tuple by $\Delta_i = \widetilde{M}_i - \widetilde{m}_i$. The HSV components $\{H_i, S_i, V_i\}$ are computed from RGB color space $\{r_i, g_i, b_i\}$ as follows:

$$V_i = \widetilde{M}_i,$$

$$S_i = \begin{cases} 0, & \widetilde{M}_i = 0, \\ \dfrac{\Delta_i}{\widetilde{M}_i}, & \text{otherwise}, \end{cases}$$

$$H_i = \begin{cases} 0, & \Delta_i = 0, \\ \dfrac{g_i - b_i}{6 \cdot \Delta_i}, & \widetilde{M}_i = r_i, \\ \left(\dfrac{b_i - r_i}{\Delta_i} + 2\right) \cdot \dfrac{1}{6}, & \widetilde{M}_i = g_i, \\ \left(\dfrac{r_i - g_i}{\Delta_i} + 4\right) \cdot \dfrac{1}{6}, & \widetilde{M}_i = b_i. \end{cases} \tag{1}$$

Thus, the HSV features are $f_4^i = H_i$; $f_5^i = S_i$; $f_6^i = V_i$.

3.1.3. YIQ. The YIQ color model is the television transmission color space for a digital standard. The Y component represents the perceived luminance, while I and Q components are the color information. I character is referred to "in-phase" term and Q letter stands for "quadrature." I and Q can place color in a graph representing I as X axis and Q as Y axis. The YIQ system takes advantage of human color perceiver characteristics [14, 15].

The YIQ model (Y_i, I_i, Q_i) is obtained by a linear transformation of the normalized RGB color space $\{r_i, g_i, b_i\}$ as follows:

$$\begin{bmatrix} Y_i \\ I_i \\ Q_i \end{bmatrix} = \begin{bmatrix} 0.299 & +0.587 & +0.114 \\ 0.596 & -0.274 & -0.322 \\ 0.211 & -0.523 & +0.312 \end{bmatrix} \begin{bmatrix} r_i \\ g_i \\ b_i \end{bmatrix}. \tag{2}$$

The $\{Y_i, I_i, Q_i\}$ values are each normalized to obtain $\{y_i, i_i, q_i\} \in [0, 1]$. Thus, the YIQ features are $f_7^i = y_i$; $f_8^i = i_i$; $f_9^i = q_i$.

3.1.4. Y'CbCr. Like YIQ, Y'CbCr is the television transmission color spaces but it is in analogue spaces for the NTSC system. YCbCr color space detaches RGB into the luma component, the blue-difference and red-difference chroma components. The transformation equation from RGB (unnormalized) model to YCbCr is defined as

$$\begin{bmatrix} Y_i' \\ \mathrm{Cb}_i \\ \mathrm{Cr}_i \end{bmatrix} = \begin{bmatrix} 0.299 & +0.587 & +0.114 \\ -0.169 & -0.331 & +0.500 \\ 0.500 & -0.419 & -0.081 \end{bmatrix} \begin{bmatrix} R_i \\ G_i \\ B_i \end{bmatrix}. \tag{3}$$

Similar to the YIQ features, the $\{Y_i', Cb_i, Cr_i\}$ values are each normalized to obtain$\{y_i', cb_i, cr_i\} \in [0, 1]$. Thus the YIQ features are $f_{10}^i = y_i'$; $f_{11}^i = cb_i$; $f_{12}^i = cr_i$.

3.1.5. XYZ. Brightness and chromaticity are two principal components of color that interact with human vision. XYZ are developed under CIE XYZ color space [16]. The XYZ values can be obtained by a linear transformation of the gamma corrected value of the RGB normalized color space $\{r_i, g_i, b_i\}$.

The gamma-corrected function is defined as

$$\gamma(t) = \begin{cases} \dfrac{t}{12.92}, & \text{if } t \le 0.04045, \\ \left(\dfrac{t + a}{1 + a}\right)^{2.4}, & \text{otherwise}, \end{cases} \tag{4}$$

where $a = 0.055$. Thus, XYZ model consisting of $\{X_i, Y_i'', Z_i\}$ components is given by

$$\begin{bmatrix} X_i \\ Y_i'' \\ Z_i \end{bmatrix} = \begin{bmatrix} 0.4124 & 0.3576 & 0.1805 \\ 0.2126 & 0.7152 & 0.0722 \\ 0.0193 & 0.1192 & 0.9505 \end{bmatrix} \begin{bmatrix} \gamma(r_i) \\ \gamma(g_i) \\ \gamma(b_i) \end{bmatrix}. \tag{5}$$

The $\{X_i, Y_i'', Z_i\}$ values are each normalized to obtain $\{x_i, y_i'', z_i\} \in [0, 1]$. Thus, the XYZ features are defined as $f_{13}^i = x_i$; $f_{14}^i = y_i''$; $f_{15}^i = z_i$.

*3.1.6. L*a*b*.* CIE L*a*b* color space is a nonlinear transformation of the CIE XYZ color space [17]. CIE L*a*b* try to imitate the logarithmic response of the human eye. The L^* component is designed to match closely with human perception of lightness. The other two components describe the chroma.

The forward transformation of CIE XYZ color space to CIE L*a*b* is computed as follows:

$$\begin{aligned} L_i^* &= 116\varphi\left(\frac{Y_i''}{\delta_2}\right) - 16, \\ A_i &= 500\left[\varphi\left(\frac{X_i}{\delta_1}\right) - \varphi\left(\frac{Y_i''}{\delta_2}\right)\right], \\ B_i &= 200\left[\varphi\left(\frac{Y_i''}{\delta_2}\right) - \varphi\left(\frac{Z_i}{\delta_3}\right)\right], \end{aligned} \tag{6}$$

where

$$\varphi(t) = \begin{cases} t^{1/3}, & \text{if } t > \left(\dfrac{6}{29}\right)^3, \\ \dfrac{1}{3}\left(\dfrac{29}{6}\right)^2 t + \dfrac{4}{29}, & \text{otherwise}, \end{cases} \tag{7}$$

and $\{\delta\}$ denotes the D65 white point given by $\{0.950456, 1.0, 1.088754\}$.

The L*a*b* values $\{L^*{}_i, A_i, B_i\}$ are normalized as $\{l_i^*, a_i, b_i\} \in [0, 1]$. Hence, the CIE L*a*b* color features are given by $f_{16}^i = l_i^*$; $f_{17}^i = a_i$; $f_{18}^i = b_i$.

3.1.7. CIE Luv. CIE Luv, or L*u*v*, is color-space-computed from the transformation of the CIE XYZ color space by International Commission on Illumination (CIE) in order to perceptual uniformity [17]. Similar to CIE L*a*b*, the D65 white point is referred by $\{\delta\}$:

$$L_i'' = \begin{cases} \left(\frac{29}{3}\right)^3 \left(\frac{Y_i''}{\delta_2}\right), & \text{if } \frac{Y_i''}{\delta_2} \le \left(\frac{6}{29}\right)^3, \\ 116\left(\frac{Y_i''}{\delta_2}\right)^{1/3} - 16, & \text{otherwise,} \end{cases}$$
$$U_i = 13L_i''\left(\frac{4X_i}{X_i + 15Y_i'' + 3Z_i} - k_1\right), \quad (8)$$
$$V_i = 13L_i''\left(\frac{9Y_i''}{X_i + 15Y_i'' + 3Z_i} - k_2\right),$$

where $k_1 = 0.2009$, $k_2 = 0.4610$, under the standard luminance C. The normalized $\{L_i'', U_i, V_i\}$ values are denoted by $\{l_i'', u_i, v_i\} \in [0,1]$. Therefore, $f_{19}^i = l_i''$; $f_{20}^i = u_i$; $f_{21}^i = v_i$.

3.1.8. CMYK. The CMYK color space is a subtractive color system mainly used in the printing industry [16]. The components consist of cyan, magenta, yellow, and neutral black. It is a common way to translate RGB display on monitors to CMYK values for printing.

Let $\widetilde{M}_i = \max\{r_i, g_i, b_i\}$ represent the maximum value of the pixel's RGB triple set. The CMYK color space, denoted by $\{C_i, M_i, Y_i^*, K_i\}$, can be computed from the RGB model as follows:

$$K_i = 1 - \widetilde{M}_i,$$
$$C_i = \frac{\widetilde{M}_i - r_i}{\widetilde{M}_i},$$
$$M_i = \frac{\widetilde{M}_i - g_i}{\widetilde{M}_i}, \quad (9)$$
$$Y_i^* = \frac{\widetilde{M}_i - b_i}{\widetilde{M}_i},$$

Thus, the CMYK features are computed as $f_{22}^i = C_i$; $f_{23}^i = M_i$; $f_{24}^i = Y_i^*$; $f_{25}^i = K_i$.

3.1.9. Aggregate Operators for the Feature Vectors. To train our classification model using this set of features, we need to combine the features per pixel into one composite feature vector $\vec{F}_j = \{F_n\}$ per tongue image (or region) j. We aggregate the pixel features using two different statistical averages (mean and median) and the standard deviation values. We derive five variations of feature vectors for our automated tongue ZHENG classification system using the following operators: mean, median ($\operatorname{med}\vec{\mathbf{F}}$), standard deviation ($\sigma\vec{\mathbf{F}}$), "mean plus standard deviation" ($\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$), and "median plus standard deviation" ($\{\operatorname{med}\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$).

Let N denote the number of pixels in a given tongue image (or region) j. The mean feature vector is denoted by $\mu\vec{\mathbf{F}}_j = \{\mu F_n\}$, where μF_n is given by

$$\mu F_n = \frac{\sum_{i=1}^N f_n^i}{N}, \quad n = 1, \ldots, 25. \quad (10)$$

The median feature vector, denoted by $\operatorname{med}\vec{\mathbf{F}}_j = \{\operatorname{med} F_n\}$, is computed as $\operatorname{med} F_n = \operatorname{mid}\{\operatorname{sort}(F_{\text{set}})\}$, $n = 1, \ldots, 25$. Standard deviation depicts the margin of difference between a given feature value and its average value among all the pixels in the given region. Thus, the standard deviation feature vector is denoted by $\sigma\vec{\mathbf{F}}_j = \{\sigma F_n\}$, where σF_n is given by

$$\sigma F_n = \sqrt[2]{\frac{\sum_{i=1}^N (f_n^i - \mu F_n)}{N}}, \quad n = 1, \ldots, 25. \quad (11)$$

The "mean plus standard deviation," denoted by $\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$, is a concatenation of the mean feature vector and the standard deviation feature vector. Similarly, the "median plus standard deviation" feature vector, denoted by $\{\operatorname{med}\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$, is a concatenation of the median feature vector and the standard deviation feature vector. Thus, the total number of features in both concatenated feature vectors is 50 each.

3.2. Supervised Learning Algorithms for ZHENG Classification. We apply three different supervised learning algorithms (AdaBoost, support vector machine, multilayer perceptron network) to build classification models for training and evaluating the proposed automated tongue based diagnosis system. Each model has its strength and weakness, which we describe briefly below. We empirically evaluate their performance over our dataset.

3.2.1. AdaBoost. An ensemble of classifiers is a set of classifiers whose individual predictions are combined in some way (typically by voting) to classify new examples. Boosting is a type of ensemble classifier which generates a set of weak classifiers using instances drawn from an iteratively updated distribution of the data, where in each iteration the probability of incorrectly classified examples is increased and the probability of the correctly classified examples is decreased. The ensemble classifier is a weighted majority vote of the sequence of classifiers produced.

The AdaBoost algorithm [18] trains a weak or base-learning algorithm repeatedly in a series of round $t = 1, \ldots, T$. Given a training set $\{x_i, y_i\}_{i=1,\ldots,n}$, where x_i belongs to some domain X and $y_i \in Y = \{-1, +1\}$ (the corresponding binary class labels), we denote the weight of ith example in round t by $D_t(i)$. Initially, all weights are set equally and so $D_1(i) = 1/n$, for all i. For each round t, a weak learner is trained using the current distribution D_t. When we obtain a weak hypothesis h_t with error $\epsilon_t =$

$\Pr_{i\sim D_t}[h_t(x_i) \neq y_i.]$, if $\epsilon_t > 1/2$, we end training; otherwise, we set $\alpha_t = (1/2)\ln((1-\epsilon_t)/\epsilon_t)$ and update D_{t+1} as

$$D_{t+1}(i) = \frac{D_t(i)}{Z_t} \times \begin{cases} e^{-\alpha_t} & \text{if } h_t(x_i) = y_i, \\ e^{\alpha_t} & \text{if } h_t(x_i) \neq y_i, \end{cases} \tag{12}$$

where Z_t is a normalization factor.

The final hypothesis is given by $H(x) = \text{sign}\left(\sum_{t=1}^{T} a_t h_t(x)\right)$.

3.2.2. Support Vector Machine. The support vector machine (SVM) [19] is one of the best-known general purpose learning algorithms. The goal of the SVM is to produce a model which predicts target values of data instances in the testing set given a vector of feature attributes. It attempts to maximize the margin of separation between the support vectors of each class and minimize the error in case the data is nonlinearly separable. The SVM classifiers usually perform well in high-dimensional spaces, avoid overfitting, and have good generalization capabilities.

For a given a training set $\{x_i, y_i\}_{i=1,\ldots,n}$, the SVM model for an instance x can be written as [20]

$$f(x) = \sum_{i=1}^{n} y_i \alpha_i k(x_i, x) + b, \tag{13}$$

where k is the kernel function used (polynomial kernel in this work), α_i is the Lagrange multiplier, and b is a constant.

In our work, we utilize the sequential minimal optimization (SMO) algorithm [21], which gives an efficient way of solving the dual problem of the support vector machine optimization problem.

3.2.3. Multilayer Perceptron Networks. The multilayer perceptron network (MLP) [22] is a feed-forward neural network with one or more layers that are hidden from the input and output nodes. Neural networks have the ability to learn complex data structures and approximate any continuous mapping [23]. The model of each neuron in the network includes a nonlinear activation function that is differentiable such as the sigmoid. The units each perform a biased weighted sum of their inputs and pass this activation level through the transfer function to produce their output given by

$$\varphi(x) = f\left(w^T x + \theta\right), \tag{14}$$

where w is the synaptic vector, x is the input vector, θ is the bias constant, and T is the transpose operator. For K-class classification, the MLP uses back propagation to implement nonlinear discriminants. There are K outputs with softmax as the output nonlinearity.

3.3. Dataset Labeling and Preprocessing. Our proposed system relies on a labeled dataset, to effectively build an automated tongue-based ZHENG classification system. Our dataset is comprised of tongue images from 263 gastritis patients and a control group of 48 healthy volunteers. Most of the gastritis patients have been classified as Hot or Cold ZHENG and are identified with a color label (yellow or white) based on the color of the coating of their tongue, as determined by their Chinese doctors. The doctors also carry out a detailed profile of the ZHENG symptoms for each patient based on clinical evaluations. The list of the main symptom profile terms is summarized in Table 1.

We are also interested in the relationship between TCM diagnosis and Western medicine diagnosis; hence, for a subset of the patients, we are provided with their corresponding Western medical gastritis pathology. They are grouped into two categories: superficial versus atrophic. In Western medicine, the doctors are also interested in knowing whether the *Helicobacter Pylori* (HP) bacterium found in the stomach is present (positive) or absent (negative) in the patients with chronic gastritis. Thus, we are provided with that information for a subset of the patients. It was not feasibleto obtain all the different information collected per patient. Table 2 summaries the population of each subset for four different labels (ZHENG, Coating, Pathology, and HP).

4. Results and Analysis

4.1. Experimental Setup. In this section, we evaluated the performance of our proposed ZHENG classification system using the three classification models (AdaBoost, SVM, and MLP) described in Section 3.2. We compared the performance of training the classifier models using the set of features extracted from the entire tongue image versus the middle tongue region only. As mentioned in Section 2, in TCM, it is believed that the middle tongue region provides discriminant information for diagnosing stomach disorders. *Hence, we extract features from the middle tongue region, as described in Figure 1(b), to evaluate the performance compared to extracting features from the entire tongue region.* In training and testing our classification models, we employ a 3-fold cross-validation strategy. This implies that the data is split into three sets; one set is used for testing and the remaining two sets are used for training. The experiment is repeated with each of the three sets used for testing. The average accuracy of the tests over the three sets is taken as the performance measure. For each classification model, we varied the parameters to optimize its performance. We also compare the results obtained using the five different variations of the feature vector (mean = $\mu\vec{\mathbf{F}}$, median = $\text{med}\,\vec{\mathbf{F}}$, standard deviation = $\sigma\vec{\mathbf{F}}$, mean + standard deviation = $\{\mu\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$, and median + standard deviation = $\{\text{med}\,\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$), as described in Section 3.1. We also apply Information Gain attribute evaluation on the feature vectors to quantify and rank the significance of individual features. *Lastly, we apply the Best First feature selection algorithm to select the "significant" features before training the classifiers to compare the performance of training the classifiers with the whole feature set against selected features.*

The performance metrics used are the classification accuracy (CA) and the average F-measure. CA is defined as the percentage of correctly classified instances over the entire set of instances classified. In our dataset, as described

Table 1: Symptom profile terms of Cold ZHENG and Hot ZHENG.

Subjects	Terms (keywords)
Cold-ZHENG related symptoms	Cold (chill, coldness), hot diet/drink preferred, desires warm environment, pale flushing of face, not thirsty, no bad mouth breath, no acidic saliva, clear urine, loose stool, high and short pitch voice, and feeling cold at limbs.
Hot-ZHENGrelated symptoms	Fever (heat, hot), cold diet/drink preferred, desires cold environment, red flushing of face, thirsty, obvious bad mouth breath, acidic saliva, yellow urine, hard stool, constipation, and feeling hot at limbs.

Table 2: Data label summary for the gastritis patients.

Data labels	Population
ZHENG: Hot/Cold	132/68
Coating: yellow/white	147/67
Pathology: superficial/atrophic	84/144
HP bacterium: positive/negative	72/167

in Table 2, for each data label, the population of both classes (which we denote by $\{C_1,\ C_2\}$) is not uniformly distributed. Hence, evaluating the performance of our classifiers using simply the classification accuracy does not paint an accurate picture of the discriminative power of the classifier. Since the dataset distribution is skewed, we can achieve a high accuracy but very poor performance in discriminating between both classes. Thus, we judge our classifiers using the average F-measure obtained for both binary classes. The F-measure combines precision and recall. It measures how well an algorithm can predict an instance belonging to a particular class. Let TP represent true positive, which we define as the number of instances that are correctly classified as C_1 for a given test set, while TN denotes true negative, the equivalent for C_2 instances. Let FP represent false positive, which we define as the number of instances that are incorrectly classified as C_1 for a given test set, while FN denotes false negative, the equivalent for C_2 instances. Precision = TP/(TP + FP) and Recall = TP/(TP + FN). Thus, the F-measure is defined as

$$F\text{-measure} = \frac{2 \cdot \text{Recall} \cdot \text{Precision}}{\text{Recall} + \text{Precision}}. \tag{15}$$

For both binary classes $\{C_1,\ C_2\}$, let $(|C_1|,\ |C_2|)$ denote the total number of instances belonging to class C_1 and C_2, respectively, then the average F-measure is defined as

$$\overline{F\text{-measure}} = \frac{|C_1| \cdot F\text{-measure}(C_1) + |C_2| \cdot F\text{-measure}(C_2)}{|C_1| + |C_2|}. \tag{16}$$

In all the tables illustrating the different experimental results, we highlight the best $\overline{F\text{-measure}}$ obtained along with the corresponding classification accuracy of the classifier.

4.2. Classification Results Based on Tongue Coating and ZHENG for Gastritis Patients. The experimental results presented in this section analyze the discrimination among the gastritis patients based on their tongue coating color and ZHENG category. Table 3 summarizes the results obtained using our proposed color-space feature vector to train the classifiers to automatically classify the color of the coating of a gastritis patient's tongue as yellow or white. We can observe from Table 3 that the combination of the median and standard deviation feature values ($\{\text{med}\,\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$) yields the best result for both the entire tongue region and the middle tongue region only. The results for both regions are also very comparable.

When using the entire tongue region, the top three significant features for the color coating classification, ranked by the information gain attribute, were $\{\sigma F_9,\ \text{med}\,F_{12},\ \sigma F_2\}$, which denote the standard deviation of Q chroma (YIQ model), the median of Cr component (YCbCr), and the standard deviation of Green Channel (RGB), respectively. For the middle tongue region only, the top three were $\{\sigma F_9,\ \sigma F_{20},\ \text{med}\,F_4\}$ which denote the standard deviation of Q chroma (YIQ model), the standard deviation of u component (L*u*v*), and the median of the Hue (HSV). It is also interesting to observe that out of the top ten significant features using the entire region versus the middle tongue region, they both have six of those features in common.

The result obtained on ZHENG classification between the Hot and Cold groups is shown in Table 4. For the ZHENG classification, using the standard deviation feature values ($\sigma\vec{\mathbf{F}}$) performs best when dealing with the entire tongue region while the $\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$ feature vector is the top performer for the middle tongue region only.

For ZHENG classification between Hot and Cold *syndromes* for gastritis patients, when using the entire tongue region, only one feature was considered significant by the information gain attribute: σF_9, that is, which is the standard deviation of Q chroma (YIQ model). For the middle tongue region, the most important feature is σF_{20}, the standard deviation of u component (L*u*v*). Even though the noteworthy feature in the entire tongue area and the middle tongue area is not the same, both Q components in YIQ color space and u component in L*u*v* color space show the difference from green to red in chromaticity diagram.

Table 5 summarizes the results obtained when we train different classifiers to detect the presence of the HP bacteria in a gastritis patient using the color feature vector. The classification result obtained in learning the pathology groups of the patients (superficial versus atrophic) is shown in Table 6. Both cases are not very strong, which illustrates a weak correlation between the western medicine diagnosis and the tongue information utilized by Chinese medical practitioners. No feature was identified as significant in either case.

Table 3: Tongue coating color classification: yellow versus white for gastritis patients.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.681	69.16	0.757	76.64	0.752	76.17	0.761	77.57	0.796	80.84	0.773	78.04
$\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.743	74.77	0.792	79.44	0.774	77.57	0.764	76.64	0.799	80.37	0.767	77.10
$\text{med}\,\vec{\mathbf{F}}$	0.758	76.64	0.728	74.30	0.724	72.90	0.735	74.77	0.789	79.44	0.766	77.10
$\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.763	76.64	**0.801**	**80.37**	0.767	77.10	0.781	78.50	0.775	77.10	**0.811**	**81.31**
$\sigma\vec{\mathbf{F}}$	0.747	75.70	0.797	79.91	0.783	78.50	0.747	74.77	0.777	77.57	0.783	78.97

Table 4: ZHENG classification between Hot and Cold syndromes for gastritis patients.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.618	63.50	0.716	71.50	0.710	71.00	0.622	63.50	0.710	70.50	0.663	67.00
$\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.750	75.00	0.680	67.50	0.723	72.00	0.664	68.00	0.735	73.50	0.740	74.00
$\text{med}\,\vec{\mathbf{F}}$	0.647	65.50	0.649	64.50	0.676	68.00	0.684	71.00	0.661	67.00	0.690	69.00
$\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.738	74.50	0.665	66.00	0.726	72.50	0.685	70.00	0.708	72.00	**0.761**	**76.00**
$\sigma\vec{\mathbf{F}}$	**0.763**	**76.50**	0.709	71.00	0.709	71.00	0.676	69.00	0.704	70.00	0.719	72.00

Table 5: Detection of presence of HP bacteria (positive versus negative) in gastritis patients.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.679	71.97	0.681	68.20	0.673	68.20	0.696	71.97	0.686	70.29	0.682	70.29
$\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.644	66.11	0.680	67.78	**0.713**	**71.97**	0.632	64.85	0.681	68.20	0.681	67.78
$\text{med}\,\vec{\mathbf{F}}$	0.655	67.78	0.666	67.36	0.666	67.78	**0.699**	**71.55**	0.644	69.04	0.676	68.20
$\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.655	67.78	0.686	68.20	0.695	69.87	0.633	65.27	0.631	64.44	0.684	68.20
$\sigma\vec{\mathbf{F}}$	0.661	68.20	0.695	71.13	0.702	70.29	0.594	61.09	0.669	66.95	0.649	65.27

Table 6: Classification between superficial and atrophic pathology of the gastritis patients.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.604	63.16	0.642	64.47	0.627	63.16	**0.658**	**66.67**	0.631	63.16	0.622	62.72
$\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.633	65.35	0.662	65.79	**0.702**	**71.05**	0.604	61.40	0.630	63.60	0.621	62.28
$\text{med}\,\vec{\mathbf{F}}$	0.633	64.47	0.601	62.72	0.640	64.04	0.623	65.79	0.632	63.16	0.623	62.28
$\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.657	66.23	0.660	65.79	0.697	69.74	0.613	62.72	0.645	64.47	0.663	66.23
$\sigma\vec{\mathbf{F}}$	0.637	64.91	0.697	70.18	0.659	66.23	0.631	64.04	0.629	63.16	0.639	64.47

Table 7: Tongue Classification between superficial and atrophic in Cold syndrome patients.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.579	58.33	0.658	66.67	0.633	63.33	0.651	65.00	0.639	65.00	0.633	63.33
$\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.716	71.67	0.647	65.00	0.680	68.33	0.643	65.00	0.649	65.00	0.662	66.67
$\text{med}\,\vec{\mathbf{F}}$	0.600	60.00	0.714	71.67	0.733	73.33	0.633	63.33	0.613	66.67	0.633	63.33
$\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.717	71.67	0.698	70.00	0.700	70.00	**0.684**	**68.33**	0.598	60.00	0.667	66.67
$\sigma\vec{\mathbf{F}}$	0.701	70.00	**0.761**	**76.67**	0.745	75.00	0.579	58.33	0.598	60.00	0.601	60.00

Table 8: Tongue classification between superficial and atrophic in Hot syndrome patients.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.768	77.06	0.755	75.23	0.735	73.39	0.710	71.56	0.735	76.15	0.680	67.89
$\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.741	74.31	**0.845**	**84.40**	0.764	76.15	0.680	68.81	0.777	77.06	0.780	77.98
$\text{med}\,\vec{\mathbf{F}}$	0.718	72.48	0.708	72.48	0.718	71.56	0.686	68.81	0.706	70.64	0.736	73.39
$\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.715	71.56	0.817	81.65	0.815	81.65	0.672	67.89	0.774	77.06	**0.808**	**80.73**
$\sigma\vec{\mathbf{F}}$	0.770	77.06	0.818	81.65	0.817	81.65	0.675	67.89	0.792	78.90	0.781	77.98

Table 9: Tongue classification between Hot syndrome and Cold syndrome in superficial patients.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.583	59.68	0.773	77.42	0.705	70.97	0.705	70.97	0.773	77.42	0.726	72.58
$\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.740	74.19	**0.839**	**83.87**	0.765	77.42	0.690	69.35	**0.839**	**83.87**	0.757	75.81
$\text{med}\,\vec{\mathbf{F}}$	0.628	62.90	0.740	74.19	0.743	74.19	0.675	67.74	0.710	70.97	0.658	66.13
$\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.774	77.42	**0.839**	**83.87**	0.755	75.81	0.774	77.42	**0.839**	**83.87**	0.774	77.42
$\sigma\vec{\mathbf{F}}$	0.834	83.87	0.757	75.81	0.838	83.87	0.819	82.26	0.791	79.03	0.750	75.81

Table 10: Tongue Classification between Hot syndrome and Cold syndrome in atrophic patients.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.539	55.14	0.642	63.55	0.645	64.49	0.572	58.88	**0.762**	**75.70**	0.615	61.68
$\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.662	67.29	0.681	69.16	0.698	70.09	0.638	64.49	0.702	69.16	0.685	68.22
$\text{med}\,\vec{\mathbf{F}}$	0.612	61.68	0.646	63.55	0.666	66.36	0.611	62.62	0.606	62.62	0.638	64.49
$\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.704	71.03	0.657	64.49	0.677	68.22	0.604	60.75	0.701	69.16	0.703	70.09
$\sigma\vec{\mathbf{F}}$	0.696	70.09	0.691	68.22	**0.734**	**73.83**	0.650	64.49	0.675	66.36	0.645	63.55

Table 11: Classification between normal tongue and tongue with coating.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.803	82.82	0.831	82.44	0.795	80.53	0.771	78.63	0.774	77.48	0.764	75.95
$\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.829	83.59	0.851	85.11	0.848	85.50	0.812	81.68	0.814	81.68	0.816	82.44
$\text{med}\,\vec{\mathbf{F}}$	0.785	80.53	0.803	83.21	0.814	83.21	0.776	80.53	0.791	78.63	0.784	79.39
$\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.814	83.21	0.835	83.59	**0.861**	**86.26**	0.817	83.59	0.823	82.06	0.824	82.44
$\sigma\vec{\mathbf{F}}$	0.818	83.21	0.839	83.59	0.851	85.11	**0.837**	**84.73**	0.786	79.39	0.818	82.44

Table 12: Tongue classification between normal group and ZHENG gastritis group.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.765	78.63	0.809	80.24	0.784	78.63	0.781	79.44	0.770	76.61	0.762	76.61
$\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.836	84.68	0.852	84.68	**0.857**	**85.89**	0.820	82.66	0.798	80.65	0.826	82.26
$\text{med}\,\vec{\mathbf{F}}$	0.756	77.82	0.795	81.45	0.784	78.63	0.772	78.23	0.817	81.45	0.785	78.63
$\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.802	81.45	0.845	84.27	0.844	84.68	0.779	79.44	0.837	83.47	**0.869**	**87.10**
$\sigma\vec{\mathbf{F}}$	0.826	83.47	0.849	84.68	0.843	84.27	0.799	81.05	0.780	77.02	0.833	83.87

Table 13: Tongue classification between normal group and Hot ZHENG.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.671	70.00	0.781	77.78	0.708	72.22	0.741	75.00	0.773	77.22	0.755	76.11
$\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.804	80.56	0.792	79.44	0.816	81.67	0.780	78.89	0.764	77.22	0.799	79.44
$\text{med}\,\vec{\mathbf{F}}$	0.721	72.78	0.711	72.22	0.739	75.00	0.727	73.89	0.739	73.33	0.744	74.44
$\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.796	80.00	0.814	82.78	0.797	80.00	0.781	79.44	0.752	75.00	0.798	79.44
$\sigma\vec{\mathbf{F}}$	0.768	77.22	**0.828**	**82.22**	0.826	82.78	0.736	75.00	0.766	77.22	**0.805**	**80.56**

Table 14: Tongue classification between normal group and Cold ZHENG.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.690	68.97	0.759	75.86	0.676	68.10	0.714	71.55	0.741	74.14	0.731	73.28
$\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.742	74.14	**0.785**	**78.45**	0.748	75.00	**0.826**	**82.76**	0.759	75.86	0.750	75.00
$\text{med}\,\vec{\mathbf{F}}$	0.686	68.97	0.745	75.00	0.757	75.86	0.672	67.24	0.750	75.00	0.742	74.14
$\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$	0.759	75.86	0.774	77.59	0.734	73.28	0.768	76.72	0.733	73.28	0.811	81.03
$\sigma\vec{\mathbf{F}}$	0.741	74.14	0.733	73.28	0.734	73.28	0.679	68.10	0.723	72.41	0.708	70.69

Tables 7–10 illustrate how experimental results reflect the analysis of the classification *between* two pathology types of gastritis patients according to ZHENG category. Table 7 summarizes the results obtained using our proposed color-space feature vector to train the classifiers to automatically classify between Superficial group and Atrophic group for patients labeled as Cold ZHENG. The results obtained on classification between superficial group and atrophic group for Hot ZHENG patients is shown in Table 8. We can observe from Table 7 that the $\sigma\vec{\mathbf{F}}$ feature vector performed best for the entire tongue region while the $\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$ feature vector yielded the best result for the middle tongue region.

Similarly, from Table 8 we can observe that for the Hot ZHENG patients, for the middle tongue region, the $\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$ feature vector also performed best. However, $\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$ feature vector performs best when dealing with the entire tongue region.

When using the entire tongue region, the top three significant features for the pathology classification between superficial and atrophic in Cold ZHENG, ranked by the information gain attribute, were $\{\sigma F_9, \sigma F_6, \sigma F_1\}$ which denote the standard deviation of Q chroma (YIQ model), the standard deviation of value component (HSV), and the standard deviation of Red Channel (RGB), respectively.

In Table 8, when using the entire tongue region, the top three significant features for the pathology classification between superficial and atrophic in Hot syndrome, ranked by the information gain attribute, were $\{\mu F_{22}, \mu F_{25}, \mu F_3\}$ which denote the mean of Cyan Ink (CMYK model), the mean of Black Ink (CMYK model), and the mean of Blue Channel (RGB), respectively. For the middle tongue region only, the top three were $\{\sigma F_{22}, \sigma F_{25}, \text{med}\,F_{25}\}$, which denote the standard deviation of Cyan Ink (CMYK model), the standard deviation of Black Ink (CMYK model), and the median of Black Ink (CMYK model).

The next set of experimental results focus on training our classifier using our proposed color-space feature vector to discriminate Hot ZHENG from Cold ZHENG in each pathology group. Table 9 summarizes the results obtained to train the classifiers to automatically classify between Hot and Cold ZHENG for superficial gastritis patients. Table 10 reflects the results for gastritis patients. We can observe from Table 9 that both $\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$ and $\{\text{med}\,\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$ feature vectors perform the best for both the entire tongue region and the middle tongue region. From results in Table 10, using the standard deviation feature values ($\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$) performs best when dealing with the entire tongue region while the ($\{\mu\vec{\mathbf{F}}, \sigma\vec{\mathbf{F}}\}$) feature vector is the top performer for the middle tongue region.

When using the entire tongue region, the top three significant features for the ZHENG classification between Hot syndrome and Cold syndrome in the patients who are superficial, ranked by the information gain attribute, were $\{\sigma F_9, \text{med}\,F_3, \text{med}\,F_{18}\}$, which denote the standard deviation of Q chroma (YIQ model), the median of Blue Channel (RGB), and the median of the blue sensitivity Z component, respectively. For the middle tongue region only, the top three were med F_{24}, σF_{19}, and med F_5 which denote

Table 15: Tongue classification between normal group and superficial patients.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.655	65.91	0.737	74.24	0.754	75.76	0.694	69.70	0.687	68.18	0.704	70.45
$\{\mu\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$	0.679	68.18	0.751	75.00	0.774	77.27	0.749	75.00	0.744	74.24	0.719	71.97
$\text{med}\,\vec{\mathbf{F}}$	0.675	67.42	0.737	74.24	0.737	73.48	0.733	73.48	0.677	67.42	0.739	73.48
$\{\text{med}\,\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$	0.695	70.45	0.759	75.76	**0.811**	**81.06**	0.749	75.00	**0.762**	**75.76**	0.726	72.73
$\sigma\vec{\mathbf{F}}$	0.687	68.94	0.735	74.24	0.706	70.45	0.726	72.73	0.742	74.24	0.749	75.00

Table 16: Tongue classification between normal group and atrophic patients.

Feature vector	Entire tongue						Middle tongue					
	AdaBoost		SVM		MLP		AdaBoost		SVM		MLP	
	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA	F-meas	CA
$\mu\vec{\mathbf{F}}$	0.733	75.52	0.803	80.21	0.781	79.17	0.754	77.08	0.770	78.13	0.699	70.83
$\{\mu\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$	0.736	73.96	0.772	78.13	**0.837**	**83.85**	0.798	80.73	0.782	78.65	0.802	80.21
$\text{med}\,\vec{\mathbf{F}}$	0.726	73.96	0.754	77.08	0.751	75.52	0.726	75.52	0.749	74.48	0.753	75.52
$\{\text{med}\,\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$	0.738	74.48	0.816	82.29	0.818	81.77	0.751	75.52	0.792	78.65	**0.848**	**84.90**
$\sigma\vec{\mathbf{F}}$	0.761	77.08	0.787	79.69	0.799	80.21	0.772	78.13	0.798	80.21	0.791	79.69

the median of Yellow Ink (CMYK), the standard deviation of lightness component (Luv model), and the median of saturation (HSV). It is also interesting to observe that by comparing the set of the top five significant features using the entire region versus the set from the middle tongue region, they both have the Yellow Ink (CMYK) in common.

When using the entire tongue region, there is only one significant feature difference for the ZHENG classification between *Hot syndrome and Cold syndrome* in patients who are atrophic, ranked by the information gain attribute, σF_9 which denotes the standard deviation of Q chroma (YIQ model). For the middle tongue region only, there were two significant features: $\{\mu F_{19}, \mu F_3\}$ which denote the mean of the blue sensitivity Z component (XYZ) and the mean of the Blue Channel (RGB).

4.3. Classification Results for Gastritis Patients versus Control Group. The experimental results presented in this section analyze the discrimination between the gastritis patients and control group. Table 11 summarizes the results obtained using our proposed color-space feature vector to train the classifiers to automatically classify patients with coating on tongue versus healthy patients with normal tongue (without coating). We can observe from Table 11 that the $\{\text{med}\,\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$ feature vector yields the best result for the entire tongue region while for the middle tongue region, it was the $\sigma\vec{\mathbf{F}}$ feature vector.

When using the entire tongue region, the top three significant features for distinguishing between normal tongue and tongue with coating, ranked by the information gain attribute, were $\{\sigma F_1, \sigma F_6, \sigma F_{25}\}$ which denote the standard deviation of Red Channel (RGB), the standard deviation of value component (HSV), and the standard deviation of Black Ink (CMYK) respectively. For the middle tongue region only, there were only two significant features: $\{\sigma F_{13}, \sigma F_{14}\}$ which denote the standard deviation of lightness component (L*a*b) and the standard deviation of a^* component (L*a*b*). It is also interesting to observe that by comparing the set of the top 10 significant features using the entire region versus the set from the middle tongue region, they both have the lightness and a^* component (L*a*b*) in common.

The results obtained from the classification between the normal group and the entire set of patients with ZHENG syndrome is shown in Table 12. The $\{\mu\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$ feature vector performs best when dealing with the entire tongue region while the $\{\text{med}\,\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$ feature vector is the top performer for the middle tongue region.

When using the entire tongue region, the top three significant features for the classification between the normal group and the gastritis group, ranked by the information gain attribute, were $\{\sigma F_1,\ \sigma F_6,\ \sigma F_{25}\}$ which denote the standard deviation of Red Channel (RGB), the standard deviation of value component (HSV), and the standard deviation of Black Ink (CMYK) respectively. For the middle tongue region only, the top three were: $\{\text{med}\,F_1,\ \text{med}\,F_6,\ \sigma F_{13}\}$ which denote the median of Red Channel (RGB), the median of Value component (HSV), and the standard deviation of lightness component (L*a*b*).

Tables 13 and 14 show the results of training our classifiers to discriminate between the normal group and the Hot ZHENG patients only, and then normal group versus Cold ZHENG patients only. Table 13 illustrates the results for normal versus hot ZHENG. We can observe that the $\sigma\vec{\mathbf{F}}$ feature vector performs best both for the entire tongue region and the middle tongue region. From Table 14, when only the normal versus Cold ZHENG patients is considered,

Table 17: Comparison between using selected features versus Whole feature set for classification.

Classification experiment type	Feature selection		Whole feature	
	F-measure	Accuracy	F-measure	Accuracy
Coating (yellow versus white)	0.764	77.10%	0.801	80.37%
ZHENG (Hot versus Cold)	0.642	65.00%	0.763	76.50%
HP Bacteria (positive versus negative)	0.636	72.38%	0.713	71.97%
Gastritis patients (superficial versus atrophic)	0.656	68.42%	0.702	71.05%
Cold ZHENG patients (superficial versus atrophic)	0.750	75.00%	0.761	76.67%
Hot ZHENG patients (superficial versus atrophic)	0.776	77.98%	0.845	84.40%
Superficial Patients (Hot versus Cold ZHENG)	0.807	80.65%	0.839	83.87%
Atrophic patients (Hot versus Cold ZHENG)	**0.782**	**78.50**%	**0.734**	**73.83**%
Normal tongue versus tongue with coating	0.833	85.88%	0.861	86.26%
Normal group versus ZHENG patients	0.834	84.68%	0.857	85.89%
Normal group versus Hot ZHENG	0.808	81.11%	0.828	82.22%
Normal group versus Cold ZHENG	0.750	75.00%	0.785	78.45%
Normal group versus superficial patients	0.765	76.52%	0.811	81.06%
Normal group versus atrophic patients	0.762	78.13%	0.837	83.85%

the same feature vector, $\{\mu\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$, performs best for both cases, however, considering only the middle tongue region outperforms using the entire tongue region.

When using the entire tongue region, the top three significant features for the classification between the normal group and the gastritis *patients with Hot syndrome*, ranked by the information gain attribute, were $\{\sigma F_1,\ \sigma F_6,\ \sigma F_{25}\}$ which denote the standard deviation of Red Channel (RGB), the standard deviation of value component (HSV), and the standard deviation of Black Ink (CMYK), respectively. For the middle tongue region only, there were only two significant features: $\{\sigma F_{13}, \sigma F_{14}\}$ which denote the standard deviation of lightness component (L*a*b) and the standard deviation of a^* component (L*a*b*). When the set of the top ten significant features using the entire region versus the set from the middle tongue region are compared, they both have the lightness and a^* component (L*a*b*) in common.

When using the entire tongue region, the top three significant features for the classification between the normal group and the gastritis *patients with Cold syndrome*, ranked by the information gain attribute, were $\{\sigma F_{25},\ \sigma F_{22},\ \sigma F_1\}$ which denotethe standard deviation of Black Ink (CMYK), the standard deviation of Cyan Ink (CMYK), and the standard deviation of Red Channel (RGB), respectively. For the middle tongue region only, the top three were $\{\sigma F_{13}, \mu F_{22}, \sigma F_{14}\}$ which denote the standard deviation of lightness component (L*a*b), the mean of Cyan Ink (CMYK), and the standard deviation of a^* component (L*a*b*).

Table 15 show the results of training our classifiers to discriminate between the normal group and the superficial patients while Table 16 shows the result for normal group versus the atrophic patients. When using the entire tongue region, the top three significant features for the classification between the normal group and the superficial group, ranked by the information gain attribute, were $\{\sigma F_1,\ \sigma F_6,\ \sigma F_{25}\}$ which denote the standard deviation of Red Channel (RGB), the standard deviation of value component (HSV), and the standard deviation of Black Ink (CMYK), respectively. For the middle tongue region, the top three were $\{\text{med}F_9, \text{med}F_1,\ \text{med}F_6\}$ which denote the median of Q chromatic component (YIQ), the median of Red Channel (RGB), and the median of Value component (HSV).

When using the entire tongue region, the top three significant features for the classification between the normal group and the atrophic group, ranked by the information gain attribute, were $\{\mu F_{25},\ \mu F_{22},\ \mu F_1\}$ which denote the mean of Black Ink (CMYK model), the mean of Cyan Ink (CMYK model), and the mean of Red Channel (RGB), respectively. For the middle tongue region, the top three were $\{\text{med}\,F_{16}, \sigma F_{13},\ \sigma F_{23}\}$ which denote the median of red sensitivity X component (XYZ), the standard deviation of lightness (L*a*b*), and the standard deviation of Cyan Ink (CMYK).

4.4. Analysis of Classification Results. From the experimental results presented in Sections 4.2 and 4.3, we can draw the following conclusions. Firstly, concerning the performance of the different classification models, we observe that the MLP and SVM models usually outperformed the AdaBoost model. The multilayer perceptron neural network seems most adequate for learning the complex relationships between the color features of the tongue images and the ZHENG/coating classes. However, both the MLP and SVM models have many parameters to consider and optimize while the AdaBoost is a much simpler model. In the AdaBoost model, we use a decision tree as our base weak learner and vary the number of classifiers to optimize its performance.

Secondly, we observe that when making discriminations within the gastritis patients group (hot versus cold ZHENG, yellow versus white coating, etc.), it was more profitable to apply the feature vectors on the entire tongue image. When classifying the normal groups versus the ZHENG groupings, usually, it improved classifier performance to apply the feature vectors to the middle tongue regions only.

Thirdly, we also observe that from the evaluation of the variations of the feature vectors used, taking into account both the average and the standard deviation usually resulted in an excellent performance. It seemed like the mean outperformed the median slightly, overall, that is, $\{\mu\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$. In a few cases, simply considering variation of the spread of the values over the region ($\{\sigma\vec{\mathbf{F}}\}$) yielded the best performance. Thus, we can conclude that when deriving a feature vector for the tongue image, the mean (or median) as well as the standard deviation (which takes into account the variation of the spread on the region) is very important.

Lastly, we observe that though we were not able to effectively discriminate between the pathology groups (superficial versus atrophic and also the presence of the HP bacterium using our color-space feature vectors, we were able to classify them much better when we took into account the ZHENG classes. This further strengthens the notion that our proposed color-space feature vectors are able to discriminate between the hot and cold ZHENG patients in addition to discerning a ZHENG patient from a non-ZHENG (healthy) patient.

4.5. Applying Feature Selection Algorithm. The classification results presented in Sections 4.2 and 4.3 were obtained using the whole feature set. For each experiment carried out on the entire tongue region, we also applied information gain attribute evaluation to rank the significance of the features. In this section, we apply feature selection algorithm (Best First) to select only a subset of features, which are deemed significant, before training the classifiers. Our goal is to see if this would yield a better result than using the whole feature set. The Best First algorithm searches the space of attribute subsets by greedy hill climbing augmented with a backtracking facility.

The summary of the results obtained is shown in Table 17. The normal group refers to the healthy (non-ZHENG) control group. We present the best classification result obtained for each experiment based on using the five variations of the feature vectors ($\mu\vec{\mathbf{F}}$, $\mathrm{med}\,\vec{\mathbf{F}}$, $\sigma\vec{\mathbf{F}}$, $\{\mu\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$, $\{\mathrm{med}\,\vec{\mathbf{F}},\ \sigma\vec{\mathbf{F}}\}$) and the three different classification models (Adaboost, SVM, and MLP). As we can observe from Table 17, using the whole feature set to train the classifiers yielded a better result in all cases except for the Atrophic Patients (Hot versus Cold ZHENG) experiment. Thus, we can conclude the overall, using the aggregate of the proposed feature sets is more discriminative even though some features are more significant than others.

5. Conclusion and Future Work

In this paper, we propose a novel color space-based feature set for use in the clinical characterization of ZHENG using various supervised machine-learning algorithms. Using an automated tongue-image diagnosis system, we extract these objective features from tongue images of clinical patients and analyze the relationship with their corresponding ZHENG data and disease prognosis (specifically gastritis) obtained from clinical practitioners. Given that TCM practitioners usually observe the tongue color and coating to determine ZHENG (such as Cold or Hot ZHENG) and to diagnose different stomach disorders including gastritis. We propose using machine-learning techniques to establish the relationship between the tongue image features and ZHENG by learning through examples.

The experimental results obtained demonstrate an excellent performance of our proposed system. *Our future work will focus on improving the performance of our system by exploring additional tongue image features that can be extracted to further strengthen our classification models. We plan to explore ways to improve our methodology to more accurately classify the ZHENGs such as including a preprocessing step of coating separation prior to the feature extraction phase. Lastly, we plan to evaluate the classification of the other ZHENG types mentioned in Section 1.*

Acknowledgment

S. Li and T. Ma are supported in part by the NSFC (no. 90709013).

References

[1] T. Ma, C. Tan, H. Zhang, M. Wang, W. Ding, and S. Li, "Bridging the gap between traditional Chinese medicine and systems biology: the connection of Cold Syndrome and NEI network," *Molecular BioSystems*, vol. 6, no. 4, pp. 613–619, 2010.

[2] R. Kanawong, W. Xu, D. Xu, S. Li, T. Ma, and Y. Duan, "An automatic tongue detection and segmentation framework for computer-aided tongue image analysis," *International Journal of Functional Informatics and Personalized Medicine*. In press.

[3] S. Li, Z. Q. Zhang, L. J. Wu, X. G. Zhang, Y. D. Li, and Y. Y. Wang, "Understanding ZHENG in traditional Chinese medicine in the context of neuro-endocrine-immune network," *IET Systems Biology*, vol. 1, no. 1, pp. 51–60, 2007.

[4] S. Li, "Network systems underlying traditional Chinese medicine syndrome and herb formula," *Current Bioinformatics*, vol. 4, no. 3, pp. 188–196, 2009.

[5] C. C. Chiu, H. S. Lin, and S. L. Lin, "A structural texture recognition approach for medical diagnosis through tongue," *Biomedical Engineering Application Basis & Communications*, vol. 7, no. 2, pp. 143–148, 1995.

[6] Y. G. Wang, J. Yang, Y. Zhou, and Y. Z. Wang, "Region partition and feature matching based color recognition of tongue image," *Pattern Recognition Letters*, vol. 28, no. 1, pp. 11–19, 2007.

[7] C. H. Li and P. C. Yuen, "Tongue image matching using color content," *Pattern Recognition*, vol. 35, no. 2, pp. 407–419, 2002.

[8] Z. Liu, J. Q. Yan, D. Zhang, and Q. L. Li, "Automated tongue segmentation in hyperspectral images for medicine," *Applied Optics*, vol. 46, no. 34, pp. 8328–8334, 2007.

[9] B. Pang, D. Zhang, and K. Wang, "The bi-elliptical deformable contour and its application to automated tongue segmentation in chinese medicine," *IEEE Transactions on Medical Imaging*, vol. 24, no. 8, pp. 946–956, 2005.

[10] D. Zhang, Z. Liu, and J. Q. Yan, "Dynamic tongueprint: a novel biometric identifier," *Pattern Recognition*, vol. 43, no. 3, pp. 1071–1082, 2010.

[11] C. C. Chiu, "A novel approach based on computerized image analysis for traditional Chinese medical diagnosis of the tongue," *Computer Methods and Programs in Biomedicine*, vol. 61, no. 2, pp. 77–89, 2000.
[12] C. C. Chiu, "The development of a computerized tongue diagnosis system," *Biomedical Engineering*, vol. 8, no. 4, pp. 342–350, 1996.
[13] C. H. Horng, "The principles and methods of tongue diagnosis," in *Tongue Diagnosis*, Lead Press, Taipei, Taiwan, 1993.
[14] Z. K. Huang and Z. F. Wang, "Bark classification using RBPNN in different color space," *Neural Information Processing*, vol. 11, no. 1, 2007.
[15] C. M. Tsai and Z. M. Yeh, "Contrast compensation by fuzzy classification and image illumination analysis for back-lit and front-lit color face images," *IEEE Transactions on Consumer Electronics*, vol. 56, no. 3, pp. 1570–1578, 2010.
[16] A. Ford and A. Roberts, *Colour Space Conversions*, 1998.
[17] M. Tkalcic and J. F. Tasic, "Colour spaces: perceptual, historical and applicational background," in *Proceedings of the IEEE Region 8 EUROCON 2003. Computer as a Tool*, 2003.
[18] Y. Freund and R. E. Schapire, "A decision-theoretic generalization of on-line learning and an application to boosting," *Journal of Computer and System Sciences*, vol. 55, no. 1, pp. 119–139, 1997.
[19] C. J. C. Burges, "A tutorial on support vector machines for pattern recognition," *Data Mining and Knowledge Discovery*, vol. 2, no. 2, pp. 121–167, 1998.
[20] B. Scholkopf and A. Smole, *Learning with Kernals: Support Vector Machines, Regularization, Optimization, and Beyond*, The MIT Press, Cambridge, Mass, USA, 2002.
[21] J. Platt, "Sequential minimal optimization: a fast algorithm for training support vector machines," in *Advances in Kernel Methods—Support Vector Learning*, B. Scholkopf, C. Burges, and A. Smola, Eds., MIT Press, Cambridge, Mass, USA, 1998.
[22] E. Alpaydin, *Introduction to Machine Learning*, MIT Press, Cambridge, Mass, USA, 2004.
[23] A. Bouzerdoum, A. Havstad, and A. Beghdadi, "Image quality assessment using a neural network approach," in *Proceedings of the 4th IEEE International Symposium on Signal Processing and Information Technology (ISSPIT '04)*, 2004.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 696983, 7 pages
doi:10.1155/2012/696983

Research Article

Relationship between Blood Stasis Syndrome Score and Cardioankle Vascular Index in Stroke Patients

Ki-Ho Cho, Kyoo-Pil Kim, Byung-Cheol Woo, Young-Jee Kim, Joo-Young Park, Seung-Yeon Cho, Seong-Uk Park, Woo-Sang Jung, Jung-Mi Park, and Sang-Kwan Moon

Department of Cardiovascular and Neurologic Disease, College of Oriental Medicine, Kyung Hee University, Seoul 130-702, Republic of Korea

Correspondence should be addressed to Sang-Kwan Moon, skmoon@khu.ac.kr

Received 13 February 2012; Revised 30 March 2012; Accepted 5 April 2012

Academic Editor: Wei Jia

Blood stasis syndrome (BSS) in traditional Asian medicine has been considered to correlate with the extent of atherosclerosis, which can be estimated using the cardioankle vascular index (CAVI). Here, the diagnostic utility of CAVI in predicting BSS was examined. The BSS scores and CAVI were measured in 140 stroke patients and evaluated with respect to stroke risk factors. Receiver operating characteristic (ROC) curve analysis was used to determine the diagnostic accuracy of CAVI for the diagnosis of BSS. The BSS scores correlated significantly with CAVI, age, and systolic blood pressure (SBP). Multiple logistic regression analysis showed that CAVI was a significant associate factor for BSS (OR 1.55, $P = 0.032$) after adjusting for the age and SBP. The ROC curve showed that CAVI and age provided moderate diagnostic accuracy for BSS (area under the ROC curve (AUC) for CAVI, 0.703, $P < 0.001$; AUC for age, 0.692, $P = 0.001$). The AUC of the "CAVI+Age," which was calculated by combining CAVI with age, showed better accuracy (0.759, $P < 0.0001$) than those of CAVI or age. The present study suggests that the CAVI combined with age can clinically serve as an objective tool to diagnose BSS in stroke patients.

1. Introduction

Blood stasis syndrome (BSS) is defined by retardation or cessation of the blood flow and is regarded as the cause or product of many chronic diseases in traditional Asian medicine. Traditionally, the diagnosis of BSS depended on subjective diagnostic methods such as inspection and palpation of the patient [1]. In 1983, Terasawa et al. developed a diagnostic criterion for "Oketsu" (BSS in Japanese), which comprises numerous symptom scores and is among the most widely accepted BSS scores [2–4]. Recent reports have suggested that BSS is correlated with haemorheologic changes such as the deterioration of erythrocyte deformability, elevation of blood viscosity, and acceleration of erythrocyte aggregation, as well as microcirculatory dysfunction [3, 4]. However, the diagnosis of BSS must still be complemented by scientific and objective methods.

BSS is considered to be closely related to senile diseases such as atherosclerosis, ischaemic heart disease, and stroke [5], as well as rheumatoid arthritis, Behçet's disease, hyperuricaemia, and various inflammatory conditions [3]. With regard to atherosclerosis, the carotid intima-media thickness (IMT) has been reported to be closely correlated with the BSS [6], and, besides, treatment of BSS has received recent attention as a therapeutic principle in traditional Chinese medicine (TCM) for atherosclerosis [5]. In addition, atherosclerosis is known to be correlated with arterial stiffness [7]. The cardio-ankle vascular index (CAVI) is thought to be a noninvasive and useful method to evaluate the arterial stiffness [8], and it has been used to estimate the extent of atherosclerosis [7, 9]. Therefore, the CAVI is likely to provide supplementary information for the diagnosis of BSS. However, to our knowledge, no study has addressed the correlation between the CAVI and the BSS score.

The purpose of this study was to assess the relationship between the BSS score and the CAVI in stroke patients and to estimate the role of the CAVI as a diagnostic tool for BSS using the ROC curve.

Table 1: Diagnostic criteria for blood stasis syndrome (BSS).

Symptom	Score	
	Male	Female
Dark-rimmed eyes	10	10
Areas of dark pigmentation of facial skin	2	2
Rough skin	2	5
Livid lips	2	2
Livid gingival	10	5
Livid tongue	10	10
Telangiectasis/vascular spiders	5	5
Subcutaneous hemorrhage	2	10
Palmar erythema	2	5
Resistance and tenderness on pressure of the left paraumbilical region	5	5
Resistance and tenderness on pressure of the right paraumbilical region	10	10
Resistance and tenderness on pressure of the umbilical region	5	5
Resistance and/or tenderness on pressure of the ileocecal region	5	2
Resistance and/or tenderness on pressureof the sigmoidal region	5	5
Resistance and/or tenderness on pressureof the subcostal region	5	5
Hemorrhoids	10	5
Dysmenorrhea	—	10

A total score larger than 20 is diagnosed as a BSS and that not exceeding 20 is diagnosed as a non-BSS. Mild symptoms are designated by half points.

2. Methods

2.1. Subjects. From April 2006 to May 2007, 810 patients who were hospitalized with ischaemic or haemorrhagic stroke diagnosed by brain CT or MRI were recruited in the Kyung Hee University Oriental Medicine Hospital. We excluded patients in the acute stage within 10 days after stroke onset and whose BSS scores could not be assessed because of impaired cognitive function. The remaining 140 patients were included in this study. Written informed consent was obtained from all patients after the Institutional Review Board of Kyung Hee Oriental Medicine Hospital approved the study protocol.

2.2. Estimation of Blood Stasis Syndrome Score. For the evaluation of the BSS score, we used the "Oketsu" scoring system, with the diagnostic criteria developed by Terasawa et al. (Table 1) [2]. This BSS scoring system consists of 17 inquiries with 3 scales of points that are determined after extensive multivariate analyses; the resultant score in this system has been reported to have a quantitative relationship with haemorheology data [3]. According to the BSS score, patients were classified into 2 categories: a "non-BSS" state (BSS score ≤ 20) and a "BSS" state (BSS score > 20).

2.3. Measurement of the CAVI. The arterial stiffness was assessed by determining the CAVI (VaSera VS-1000; Fukuda Denshi, Tokyo, Japan). In accordance with the device manufacturer's instructions, the subjects rested in the supine position for at least 10 min before measurements were obtained. The cuffs were attached to the 4 extremities, and electrocardiographic electrodes were attached to the upper extremities. A microphone was placed on the sternal angle for phonocardiography. The CAVI was automatically calculated using a waveform analyser in the VaSera VS-1000 [10, 11]. The complete measurement of all CAVIs was usually completed in <5 min.

2.4. Clinical Assessments. Information regarding the potential vascular risk factors for each subject, including tobacco smoking, history of MI, and the use of antihypertensive and hypoglycaemic agents, was recorded from patient interviews and medical records. For current smoking, subjects must have reported smoking at least 100 cigarettes over their lifespan and a current smoking frequency of occasional or every day, at the time of interview. History of myocardial infarction (MI) and left ventricular hypertrophy (LVH) were confirmed by reviewing the medical records or by electrocardiography. Hypertension was defined as the presence of a history of hypertension, a systolic blood pressure (SBP) of ≥ 140 mmHg, or a diastolic pressure of ≥ 90 mmHg. SBP as a variable for analysis was recorded from the higher brachial SBP, which was checked during the measurement of the CAVI. DM was diagnosed if the subject was currently undergoing treatment with insulin or oral hypoglycaemic agents, or if the fasting blood glucose level was ≥ 140 mg/dL. Blood was drawn for biochemical analyses, including estimation of serum level of total cholesterol and creatinine following an overnight fast in the initial study.

2.5. Statistical Analysis. The data were expressed as case numbers or means ± standard deviation (SD). To compare the means of continuous variables, Student's t-test was applied. Categorical variables were analysed using a chi-square analysis or Fisher's exact test. Correlations between continuous variables were determined using the Pearson's correlation coefficient. The variables found to be associated with BSS were further tested by a multiple logistic regression analysis to investigate the independent factors for BSS. To assess the ability of each variable to discriminate the BSS, the areas under the receiver operating characteristic (ROC) curves (AUC) were calculated. In addition, the asymptotic 95% confidence interval (CI) and P values under the null hypothesis (true area = 0.50) were calculated. An AUC of >0.9 was considered excellent; 0.8–0.9, very good; 0.7–0.8, good; 0.6–0.7, average; <0.6, poor [10, 12]. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed with SPSS version 12.0 (SPSS Inc., Chicago, IL, USA), whereas the ROC curves were calculated by MedCalc version 12.1.4.0.

3. Results

Characteristics of the participants are shown in Table 2. Of 140 stroke patients, 118 (84.3%) were diagnosed with BSS.

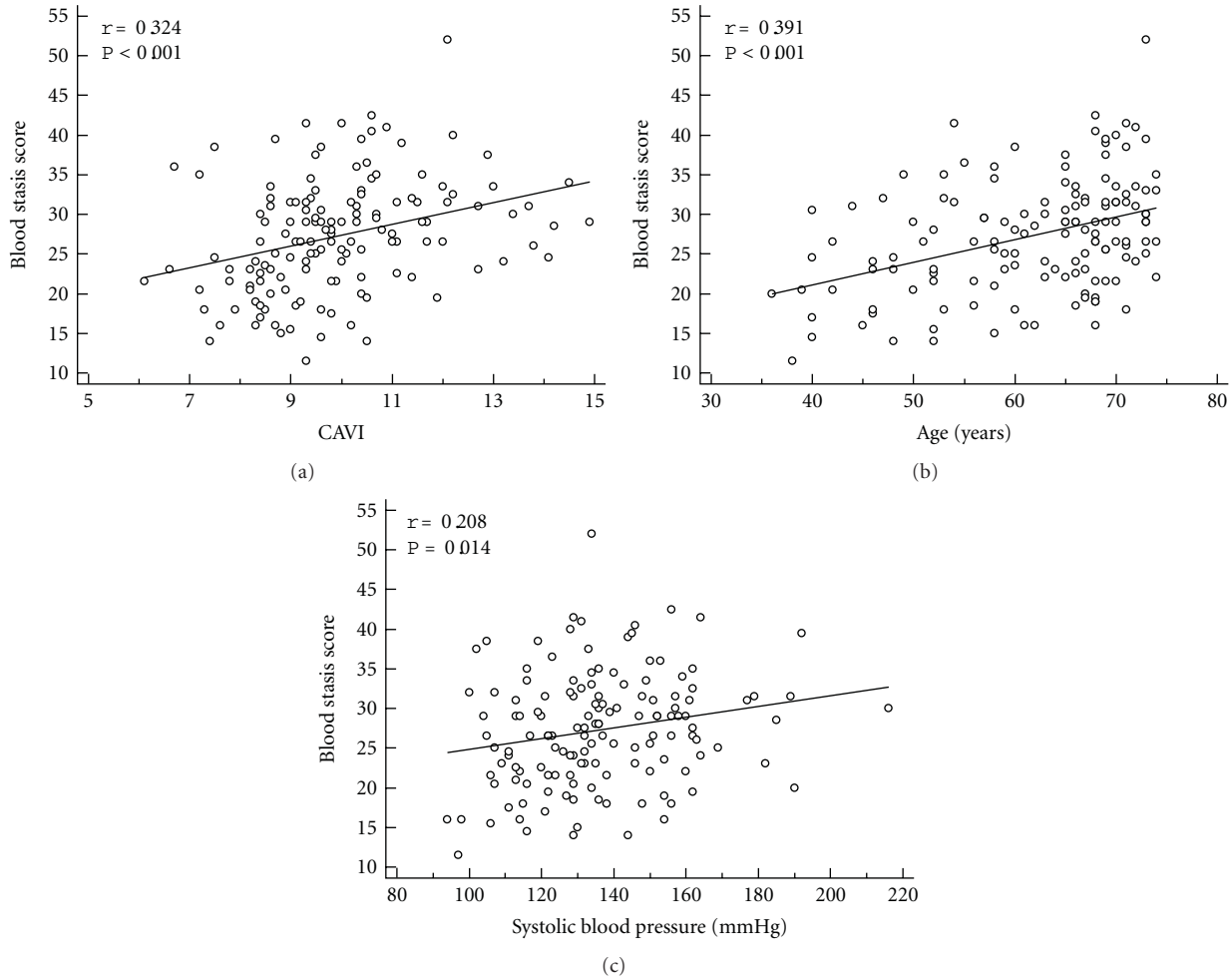


Figure 1: The correlation between the blood stasis score and the cardio-ankle vascular index (CAVI) was significant ($n = 140$, r [Pearson's correlation coefficient] = 0.324, $P < 0.001$) in stroke patients (a). In addition, the age (b) and systolic blood pressure (c) were significantly correlated with the blood stasis score ($r = 0.391$, $P < 0.001$; $r = 0.208$, $P = 0.014$, resp.).

The BSS group was found to have higher CAVI ($P < 0.01$), age ($P < 0.01$), and SBP ($P = 0.04$) than the non-BSS group. However, other variables, including stroke risk factors, did not statistically differ between individuals with and without BSS.

The correlation between the BSS scores and CAVI was significant ($r = 0.324$, $P < 0.001$) in stroke patients. In addition, the age and SBP were significantly correlated with the BSS scores ($r = 0.391$, $P < 0.001$; $r = 0.208$, $P = 0.014$, resp.) (Figure 1).

A multiple logistic regression analysis showed that the CAVI was a significant associate factor for BSS (OR 1.55, $P = 0.032$) after adjusting for the age and SBP, both of which were not significant in the model (Table 3).

ROC curves were generated for CAVI, age, and SBP to determine their possible diagnostic utility for distinguishing the BSS groups from the non-BSS groups (Figure 2). The CAVI and age showed modest utility with ROC curves that were higher and shifted more to the left than those of SBP, which showed poor utility. Based on the area under the ROC curve (AUC), by which the accuracy of the test is measured, the AUC of the CAVI and age showed average accuracy (0.703 and 0.692, resp.), with no significant difference between these values. However, the SBP indicated an AUC of 0.630, which did not reject the null hypothesis (true area = 0.50) (Table 4). To find a better discriminator of BSS, a new variable "CAVI+Age" was calculated by combining the CAVI with age as follows: the age was categorized into ages <40, 40–49, 50–59, 60–69, and ≥70; then converted into 1, 2, 3, 4, and 5, respectively; finally added to CAVI scores. The AUC of the "CAVI+Age" (0.759) showed better accuracy than those of the CAVI or age although there was no significant difference among those values (Table 4) (Figure 2).

To determine the optimal threshold for the diagnosis of BSS, the intersection point between the sensitivity and the 100-specificity curves of the CAVI, age, and "CAVI+Age" was used. The optimal cut-off points for the CAVI, age, and "CAVI+Age" were 9.2, 62 years, and 12.7, respectively. Using the threshold of 9.2 for the CAVI, 62 years for the age, and 12.7 for the "CAVI+Age", the sensitivities were 70.3%, 62.7%, and 72.9%, respectively, and the specificities were 63.6%, 68.2%, and 77.3%, respectively (Figure 2).

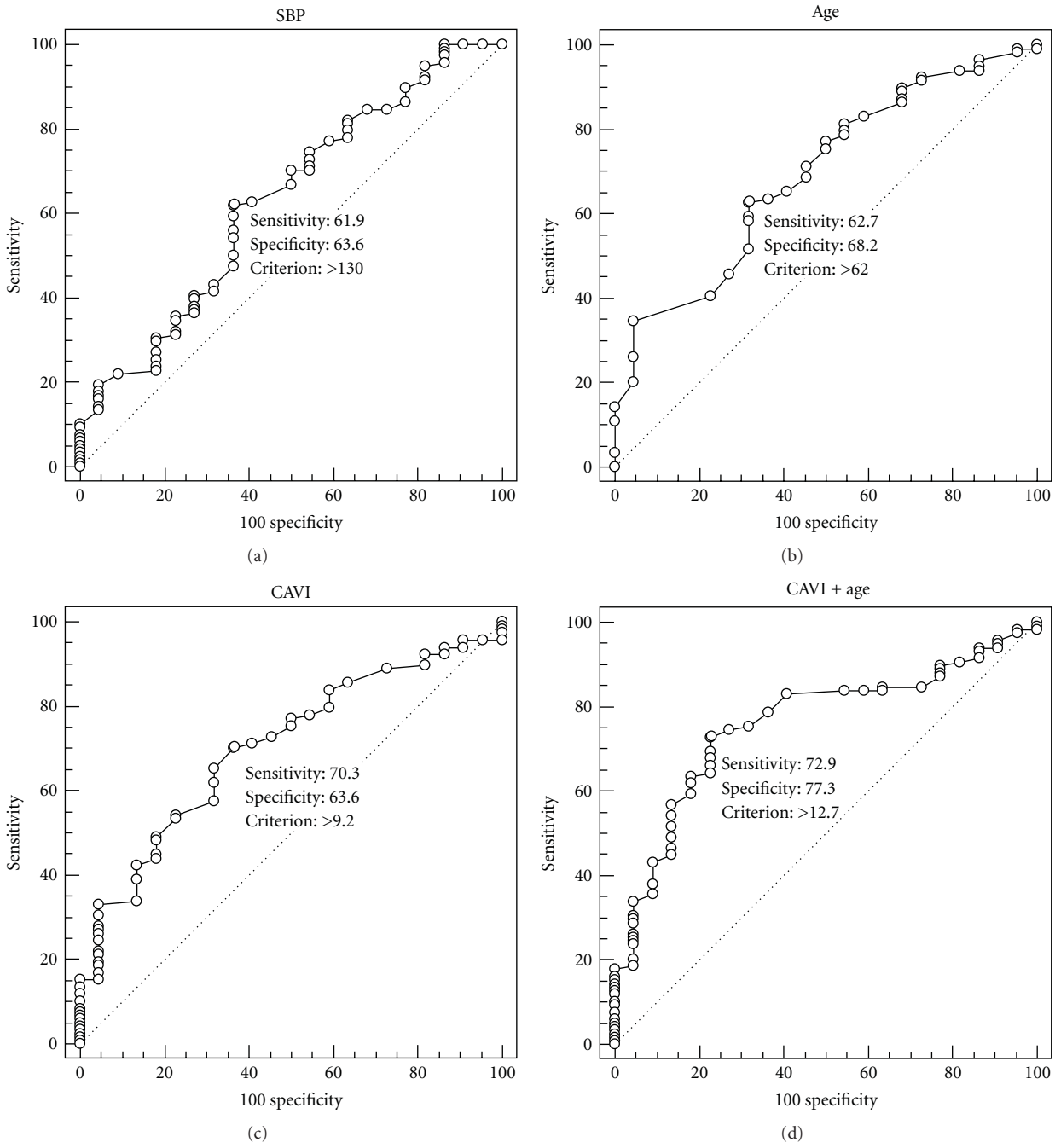


Figure 2: The diagnostic accuracy of the systolic blood pressure (SBP), age, CAVI, and CAVI+Age for predicting blood stasis syndrome (BSS) in stroke patients. The ROC curves depicted that the CAVI and age showed modest diagnostic utility for BSS with the CAVI+Age indicating good diagnostic accuracy, while SBP provided poor diagnostic utility. In each graph, the solid diagonal line was the line of no discrimination (area = 0.5), and the optimal cut-off points were indicated on the curves.

4. Discussion

Ancient Chinese medical texts describe a disorder of the blood circulation, which causes various symptoms such as BSS, reduced blood flow, and cessation of flow. This phenomenon is commonly observed in Asian countries but termed differently as "Yu Xue" in Chinese, "Eohyul" in Korean, or "Oketsu" in Japanese. For the diagnosis of BSS, Terasawa et al. developed a diagnostic criterion of "Oketsu," which has become one of the most widely accepted methods for BSS scoring [2, 3]. However, the diagnosis of BSS still needs to be complemented by scientific and objective methods [3]. In the present study, the ROC analysis indicated that the AUC of the CAVI and age showed fair diagnostic accuracy for BSS. Furthermore, The AUC of the "CAVI+Age" showed better accuracy than those of the CAVI or age.

BSS has been reported to be closely correlated with atherosclerosis [6], as well as disorders of the peripheral

Table 2: Characteristics of the study participants.

Variables	Blood stasis syndrome		P-value
	No ($n = 22$)	Yes ($n = 118$)	
Male sex, %	50.0 (11)	47.5 (56)	0.83
Mean age, y	56.0 (10.6)	62.8 (9.3)	0.003
Age, %			0.004
<40	4.5 (1)	1.7 (2)	
40–49	27.3 (6)	9.3 (11)	
50–59	22.7 (5)	20.3 (24)	
60–69	40.9 (9)	42.4 (50)	
≥70	4.5 (1)	26.3 (31)	
Total cholestrol, mg/dL	176.14 (36.73)	179.79 (39.69)	0.69
Systolic blood pressure, mmHg	127.32 (20.11)	137.97 (21.83)	0.04
Serum creatinine, mg/dL	0.69 (0.25)	0.73 (0.30)	0.53
Height, cm	161.60 (7.58)	161.34 (8.49)	0.90
CAVI	9.01 (1.12)	10.09 (1.72)	0.006
History MI, %	0 (0)	0 (0)	—
LVH, %	4.5 (1)	5.9 (7)	0.80
Diabetes, %	18.2 (4)	30.5 (36)	0.24
Hypertension, %	63.6 (14)	65.3 (77)	0.88
Current smoke, %	22.7 (5)	17.8 (21)	0.59
Stroke type			0.89
Infarction, %	81.8 (18)	80.5 (95)	
Hemorrhage, %	18.2 (4)	19.5 (23)	

Data are mean (SD) or % (n). P value represents significance of differences between groups using t-test, χ^2 test. CAVI: Cardio-ankle vascular index; MI: Myocardial infarction; LVH: left ventricular hypertrophy.

Table 3: Multiple logistic regression analysis of associated variables for blood stasis syndrome.

Variables	Odds ratio*	95% Confidence interval	P value
CAVI	1.55	1.04–2.32	0.032
Age	1.04	0.99–1.10	0.090
SBP	1.01	0.99–1.04	0.267

CAVI: Cardio-ankle vascular index; SBP: Systolic blood pressure.
*Adjusted for all the other variables shown in this table.

Table 4: Area under the receiver-operator characteristic (ROC) curve for the CAVI+Age, CAVI, age, and systolic blood pressure as discriminators of blood stasis syndrome among stroke patients.

Variables	Area under ROC curve	95% Confidence interval	P value
CAVI + Age	0.759	0.680–0.827	<0.0001
CAVI	0.703	0.620–0.777	0.0003
Age	0.692	0.609–0.767	0.0010
SBP	0.630	0.545–0.710	0.0522

CAVI: Cardio-ankle vascular index; SBP: Systolic blood pressure.

microcirculation, rheumatoid arthritis, systemic lupus erythaematosus (SLE), disseminated intravascular coagulation (DIC), and various allergic responses [3]. With regard to the relationship between BSS and atherosclerosis, Lei et al. reported that the carotid IMT was closely correlated with BSS in patients with dyslipidaemia. In addition, Ma and Chen indicated in a review paper that the treatment of BSS has received recent attention as a therapeutic principle in TCM for atherosclerosis [5]. In this regard, a traditional Chinese drug to relieve BSS, the Xuefuzhuyu pill, was reported to be beneficial to retard the progress of atherosclerosis [13, 14]. In Japan, Keishi-bukuryo-gan-ryo, which is one of the most important prescriptions for improving BSS, has been reported to prevent the progression of atheromatous plaque by strengthening the antioxidant defence system [15] and exerting a protective effect on the endothelium [11]. These studies support the relationship between BSS and atherosclerosis. Therefore, the diagnostic methods for atherosclerosis are likely to complement the diagnosis of BSS in an objective manner.

Atherosclerosis is known to be correlated with arterial stiffness [16] and the progression of coronary artery sclerosis [7, 16]. The aortic (carotid-femoral) pulse wave velocity (cfPWV) is a well-established index of central arterial stiffness. However, one drawback of this index is that the accuracy of cfPWV measurements by Doppler imaging or tonometry depends greatly on the skill and experience of the practitioner. The recent introduction of the volume plethysmographic method allows the measurement of brachial ankle pulse wave velocity (baPWV) and the cardio ankle vascular index (CAVI) with minimal technical skill [17]. Furthermore, the CAVI, which is independent of the blood pressure, can clinically serve as a predictive marker of

the extent of coronary artery disease (CAD) and has been reported to increase the diagnostic performance of CAD over baPWV [7, 18]. The CAVI was also reported to be a useful clinical marker for evaluating atherosclerosis and arteriolosclerosis in patients with essential hypertension [19]. Therefore, the CAVI is thought to be an easy, noninvasive and useful method to estimate the extent of atherosclerosis, and it is likely to provide supplementary information on the diagnosis of BSS.

In the present study, we used ROC analysis, which is a useful tool to evaluate the performance of diagnostic tests [20] to evaluate the diagnostic performance of the CAVI for BSS diagnosis. Generally, an ROC curve is a plot of sensitivity on the y axis against "1-specificity" on the x axis for varying values of the threshold t. The AUC provides an overall summary of the diagnostic accuracy. The AUC equals 0.5 when the ROC curve corresponds to random chance, and 1.0 under conditions of perfect accuracy. When the estimated AUC is <0.5, the test is less predictive than chance [20]. In this study, the AUC of the CAVI and age were 0.703 and 0.692, respectively, thereby demonstrating average diagnostic accuracy for predicting BSS in stroke patients. Although there was no significant difference between these outcome measures in the comparison of the AUC, the multiple logistic regression analysis showed that the CAVI was a significant factor for BSS after adjusting for the age and SBP, both of which were not significant in the model. Thus, we suggest that the CAVI might be more valuable for discerning the presence of BSS in stroke patients than the age.

The CAVI has been reported to correlate with age [7]. In humans, aging is a considered a strong contributing factor for atherosclerosis. With aging, the degenerated and decreased elastic fibres of the media in the large arterial wall can lead to an increase in collagen fibres and matrix. Such changes increase the aortic stiffness. It has been also reported that the cfPWV and baPWV increased with age, which was associated with an increase in aortic stiffness [21, 22]. Therefore, it is conceivable for age to have a fair diagnostic accuracy for BSS diagnosis, considering the relationship between CAVI and BSS score. Furthermore, we found that the AUC of the combined variable "CAVI+Age" demonstrated better accuracy than those of the CAVI or age, which suggests that the CAVI combined with age might be a better discriminator of BSS than the CAVI alone.

The present study has several limitations. First, the ROC curve was difficult to interpret because of the small sample size. The ROC analysis in the present study thus represents a preliminary trial that should be extended to a larger cohort. In addition, the definition of cutoff values of the CAVI for discerning the presence of BSS may be required for every age group. Second, our results may be disease-specific, because the subjects comprised only stroke patients. Thus, validation studies with larger cohorts, methodological improvement, and strictly defined protocols are necessary for future studies.

In conclusion, our results suggest that the CAVI combined with age can clinically serve as an objective tool to diagnose BSS in stroke patients. The present study sheds light on traditional medical concepts from the viewpoint of modern science and medicine.

Acknowledgments

This work was supported by Grant no. KHU-20100672 (2010) from Kyung Hee University, Seoul, Korea.

References

[1] K. Terasawa, "Evidence-based reconstruction of kampo medicine: part ii-the concept of sho," *Evidence-based Complementary and Alternative Medicine*, vol. 1, no. 2, pp. 119–123, 2004.

[2] K. Terasawa, H. Shinoda, A. Imadaya, H. Tosa, M. Bandoh, and N. Satoh, "The presentation of diagnostic criteria for "Yu-xie" (stagnated blood) conformation," *International Journal of Oriental Medicine*, vol. 14, pp. 194–213, 1989.

[3] C. Matsumoto, T. Kojima, K. Ogawa et al., "A proteomic approach for the diagnosis of 'Oketsu' (blood stasis), a pathophysiologic concept of Japanese traditional (Kampo) medicine," *Evidence-based Complementary and Alternative Medicine*, vol. 5, no. 4, pp. 463–474, 2008.

[4] K. Terasawa, K. Toriizuka, H. Tosa, M. Ueno, T. Hayashi, and M. Shimizu, "Rheological studies on "oketsu" syndrome i. The blood viscosity and diagnostic criteria," *Journal of the Medical and Pharmaceutical Society for WAKAN-YAKU*, vol. 3, pp. 98–104, 1986.

[5] J. Ma and J. Q. Chen, "Review of clinical and experimental studies on treatment of atherosclerosis with expelling phlegm and relieving blood stasis principle," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 26, no. 12, pp. 1135–1138, 2006.

[6] Y. Lei, Z. H. Wang, H. Zhao, and J. G. Liu, "Study of the relationship between carotid intima-media thickness and traditional Chinese medicine syndrome of dyslipidemia," *Chinese Journal of Integrative Medicine*, vol. 15, no. 2, pp. 112–116, 2009.

[7] S. Horinaka, A. Yabe, H. Yagi et al., "Comparison of atherosclerotic indicators between cardio ankle vascular index and brachial ankle pulse wave velocity," *Angiology*, vol. 60, no. 4, pp. 468–476, 2009.

[8] K. Ueyama, M. Miyata, T. Kubozono et al., "Noninvasive indices of arterial stiffness in hemodialysis patients," *Hypertension Research*, vol. 32, no. 8, pp. 716–720, 2009.

[9] T. W. Shen, C. H. Wang, Y. H. Lai, B. G. Hsu, H. H. Liou, and T. C. Fang, "Use of cardio-ankle vascular index in chronic dialysis patients," *European Journal of Clinical Investigation*, vol. 41, no. 1, pp. 45–51, 2011.

[10] B. C. K. Choi, "Slopes of a receiver operating characteristic curve and likelihood ratios for a diagnostic test," *American Journal of Epidemiology*, vol. 148, no. 11, pp. 1127–1132, 1998.

[11] H. Goto, Y. Shimada, N. Sekiya et al., "Effects of Keishi-bukuryo-gan on vascular function and hemorheological factors in spontaneously diabetic (WBN/kob) rats," *Phytomedicine*, vol. 11, no. 2-3, pp. 188–195, 2004.

[12] G. S. Ying, M. Maguire, G. Quinn, M. T. Kulp, and L. Cyert, "Roc analysis of the accuracy of noncycloplegic retinoscopy, retinomax autorefractor, and suresight vision screener for preschool vision screening," *Investigative Ophthalmology & Visual Science*, vol. 52, no. 13, pp. 9658–9664, 2011.

[13] Y. Li, K. Chen, and Z. Shi, "Effect of xuefu zhuyu pill on blood stasis syndrome and risk factor of atherosclerosis," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 18, no. 2, pp. 71–73, 1998.

[14] L. Liu, Y. Cheng, and H. Zhang, "Phytochemical analysis of anti-atherogenic constituents of Xue-Fu-Zhu-Yu-Tang using HPLC-DAD-ESI-MS," *Chemical and Pharmaceutical Bulletin*, vol. 52, no. 11, pp. 1295–1301, 2004.

[15] N. Sekiya, M. Kainuma, H. Hikiami et al., "Oren-gedoku-to and Keishi-bukuryo-gan-ryo inhibit the progression of atherosclerosis in diet-induced hypercholesterolemic rabbits," *Biological and Pharmaceutical Bulletin*, vol. 28, no. 2, pp. 294–298, 2005.

[16] D. J. Farrar, M. G. Bond, W. A. Riley, and J. K. Sawyer, "Anatomic correlates of aortic pulse wave velocity and carotid artery elasticity during atherosclerosis progression and regression in monkeys," *Circulation*, vol. 83, no. 5, pp. 1754–1763, 1991.

[17] T. Yambe, M. Yoshizawa, Y. Saijo et al., "Brachio-ankle pulse wave velocity and cardio-ankle vascular index (CAVI)," *Biomedicine and Pharmacotherapy*, vol. 58, supplement 1, pp. S95–S98, 2004.

[18] A. Takaki, H. Ogawa, T. Wakeyama et al., "Cardio-ankle vascular index is superior to brachial-ankle pulse wave velocity as an index of arterial stiffness," *Hypertension Research*, vol. 31, no. 7, pp. 1347–1355, 2008.

[19] T. Okura, S. Watanabe, M. Kurata et al., "Relationship between cardio-ankle vascular index (CAVI) and carotid atherosclerosis in patients with essential hypertension," *Hypertension Research*, vol. 30, no. 4, pp. 335–340, 2007.

[20] K. H. Zou, A. J. O'Malley, and L. Mauri, "Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models," *Circulation*, vol. 115, no. 5, pp. 654–657, 2007.

[21] T. Kubozono, M. Miyata, K. Ueyama et al., "Clinical significance and reproducibility of new arterial distensibility index," *Circulation Journal*, vol. 71, no. 1, pp. 89–94, 2007.

[22] G. F. Mitchell, H. Parise, E. J. Benjamin et al., "Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham heart study," *Hypertension*, vol. 43, no. 6, pp. 1239–1245, 2004.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 120350, 9 pages
doi:10.1155/2012/120350

Research Article

Molecular Mechanisms of Same TCM Syndrome for Different Diseases and Different TCM Syndrome for Same Disease in Chronic Hepatitis B and Liver Cirrhosis

Zhizhong Guo,[1] Shuhao Yu,[2] Yan Guan,[1] Ying-Ya Li,[1] Yi-Yu Lu,[1] Hui Zhang,[1] and Shi-Bing Su[1]

[1] *Research Center for Complex System of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China*
[2] *College of Life Science and Biotechnology, Shanghai Jiaotong University, 800 Dongchuan Road, Shanghai 200240, China*

Correspondence should be addressed to Shi-Bing Su, shibingsu07@163.com

Received 9 February 2012; Revised 2 April 2012; Accepted 5 April 2012

Academic Editor: Aiping Lu

Traditional Chinese medicine (TCM) treatment is based on the traditional diagnose method to distinguish the TCM syndrome, not the disease. So there is a phenomenon in the relationship between TCM syndrome and disease, called Same TCM Syndrome for Different Diseases and Different TCM Syndrome for Same Disease. In this study, we demonstrated the molecular mechanisms of this phenomenon using the microarray samples of liver-gallbladder dampness-heat syndrome (LGDHS) and liver depression and spleen deficiency syndrome (LDSDS) in the chronic hepatitis B (CHB) and liver cirrhosis (LC). The results showed that the difference between CHB and LC was gene expression level and the difference between LGDHS and LDSDS was gene coexpression in the G-protein-coupled receptor protein-signaling pathway. Therein genes GPER, PTHR1, GPR173, and SSTR1 were coexpressed in LDSDS, but not in LGDHS. Either CHB or LC was divided into the alternative LGDHS and LDSDS by the gene correlation, which reveals the molecular feature of Different TCM Syndrome for Same Disease. The alternatives LGDHS and LDSDS were divided into either CHB or LC by the gene expression level, which reveals the molecular feature of Same TCM Syndrome for Different Diseases.

1. Introduction

Traditional Chinese medicine (TCM) is a medical system with at least 3000 years of uninterrupted clinical practice in China. The TCM practice usually requires a TCM syndrome identification based on clinical manifestation followed by the use of individualized treatment that is adapted to address the particular TCM syndrome in patient [1]. Therefore, TCM syndrome, also called ZHENG or TCM pattern, is the core of diagnosis and treatment in TCM [2]. Nowadays, TCM syndrome had been studied in some specific disease such as hypertension [3], coronary heart disease [4], and rheumatoid arthritis [5] or biomedical condition such as neuro-endocrine-immune network [6], suggesting that TCM syndromes are significantly associated with diseases.

Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic disease. Beyond 25% of hepatitis B virus-infected patients would die of severe chronic liver diseases such as liver cirrhosis and liver cancer [7]. Chronic hepatitis B (CHB) and liver cirrhosis (LC) are the intractable diseases that remain a major public health problem worldwide. Although several antiviral drugs had been approved for CHB, they caused significant side effects and drug resistance. In contrast, TCM treatment was regarded as a safe and effective method for CHB and Liver fibrosis [8, 9].

TCM treatment is based on the traditional diagnose method to differentiate the TCM syndrome, not the disease in western medicine. Therefore, TCM syndromes could be classified in CHB as well as in LC. Moreover, different patients, respectively, suffering CHB or LC could also belong to the same TCM syndrome. This phenomenon is called Same TCM Syndrome for Different Diseases and Different TCM syndrome for Same Disease [10–12]. This viewpoint in TCM is very different with Western medicine. The molecular mechanism of this phenomenon is still a mystery.

Previous study reported liver-gallbladder dampness-heat syndrome (LGDHS) and liver depression and spleen deficiency syndrome (LDSDS) are the major syndromes in CHB [13, 14]. In this study, the aim is to demonstrate the molecular mechanism of Same TCM Syndrome for Different Diseases and Different TCM Syndrome for Same Disease by the analysis of whole gene expression in the same syndrome as LGDHS or LDSDS of different diseases as CHB and LC and the same disease as CHB or LC of different syndromes as LGDHS and LDSDS.

2. Material and Methods

2.1. Samples. Blood samples from 92 patients were obtained. Therein 14 samples from 2 LGDHS and 3 LDSDS in CHB patients, 3 LGDHS and 3 LDSDS in LC patients and 3 healthy peoples were used to microarray test, and 78 samples from 20 LGDHS and 18 LDSDS in CHB patients, and 21 LGDHS and 19 LDSDS in LC patients were used to test and verify the accuracy of the result. All patients were from Shanghai Longhua Hospital and have signed an agreement with us. The blood samples were morning fasting venous blood and saved in -20°C with 150 μL EDTA.

2.2. RNA Extraction and Microarrays. Total RNA of leukocyte from the whole blood was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), and a quality control was carried out with NanoDrop ND-1000. The cDNAs were synthesized by the Invitrogen First-Strand cDNA Synthesis kits (Invitrogen, Carlsbad, CA, USA), and RNA polymerase was added to degrade RNA. The cDNA was labeled and hybridized using NimbleGen Homo sapiens 12x135K Arrays (Roche NimbleGen, Madison, WI, USA), according to the manufacturer's protocol.

2.3. Real-Time RT-PCR. Difference-expressed mRNAs were verified by real-time RT-PCR according to SYBR Green Realtime PCR Master Mix kit (TOYOBO, Osaka, Japan) manufacturer. The primer sequences were F: TGGTGTGCGCAGCCATCGTG, R: GCCAGTAACCGGCCACCTCG for DRD5; F: GCTCTGTCAGGGCTCAACCTCC, R: GGCACAAACTTGGAGAGACCGAGC for GABRA; F: GCTACGTGGCCGTGGTGCAT, R: CCGCGGTGCGAGAGAAGACC for SSTR1; F: AGCGAACCCCTCCCACCACA, R: CAGGAAGGCTTGGCTCCGGC for NPFF. F: ACAGAGCCTCGCCTTTGCCG, R: ACATGCCGGAGCCGTTGTCG for ACTB.

2.4. Microarray Data Preprocessing and Statistic Analysis. Microarray data preprocessing was performed using the GenePix software. Raw expression data were log 2 transformed and normalized by quantile normalization. Probes were considered robustly expressed if Signal/Noise (SNR) < 2.

We took the average of 3 healthy people in every probe and let every patient sample *ratio* be this average in every probe. In all the following pages: CHB means chronic hepatitis B versus normal; LC means liver cirrhosis versus normal; LGDHS means liver-gallbladder dampness-heat syndrome versus normal; LDSDS means spleen deficiency syndrome versus normal.

The *t*-test function in R software was used to select difference expressed gene (threshold: P value < 0.01 or P value < 0.05) in diseases between CHB and LC as well as in TCM syndromes between LGDHS and LDSDS. GO enrichment analysis was executed using the selected genes.

Heatmap analysis, also executed in R, was computing the hierarchical clustering in both rows and columns according to the set of gene values and drawing a color image as a visible result.

The correlation analysis was used to analyze the correlation of difference expressed genes between CHB and LC or LGDHS and LDSDS. The level of significance was set at correlation coefficient >0.5.

2.5. Gene Module Analysis and Difference Coexpression Analysis. The Weighted Correlation Network Analysis (WGCNA) R package was used to run the gene module analysis (parameter: networkType = signed, detectCutHeight = 0.97). WGCNA was a systems biology method to describe the correlation patterns among genes across microarray samples. It was used to find clusters (modules) of highly correlated genes and summarizing the clusters using the Module Eigengene (ME) [15].

Furthermore, coXpress R package was used to analyze the difference coexpression (parameter: s = pearson, m = average, h = 0.4). coXpress as a tool has been applied to identify groups of genes that display differential coexpression patterns in microarray datasets and its utility [16].

3. Results and Discussion

3.1. Difference Expression Analysis. At first, to find whether there were some significant genes that could characterize the difference between two disease and two TCM syndromes, *t*-test was used to select difference expression gene in both disease and TCM syndrome levels. The threshold was P value less than 0.01. Remarkably, 6579 in all 14352 genes were differentially expressed between CHB and LC, suggested that the difference in mRNA expression level was very clear, according to CHB and LC that were completely different diseases. In contrast, only 98 genes were differentially expressed between LGDHS and LDSDS. The heatmap of the 98 genes between LGDHS and LDSDS was showed in Figure 1. Moreover, though these genes were obviously differentiated into two syndromes, the 98 genes were in disorder, no significantly related function was found by GO enrichment analysis. It also was tried to change the threshold as P value less than 0.05 and got 830 genes, but still any significantly related GO function was not found.

3.2. Gene Modules Related with Disease or TCM Syndrome. Due to the above result that the molecular mechanisms of the difference between two TCM syndromes could be not commendably explained with the single-gene difference expression method, then the gene module method was used to demonstrate the difference between diseases and TCM

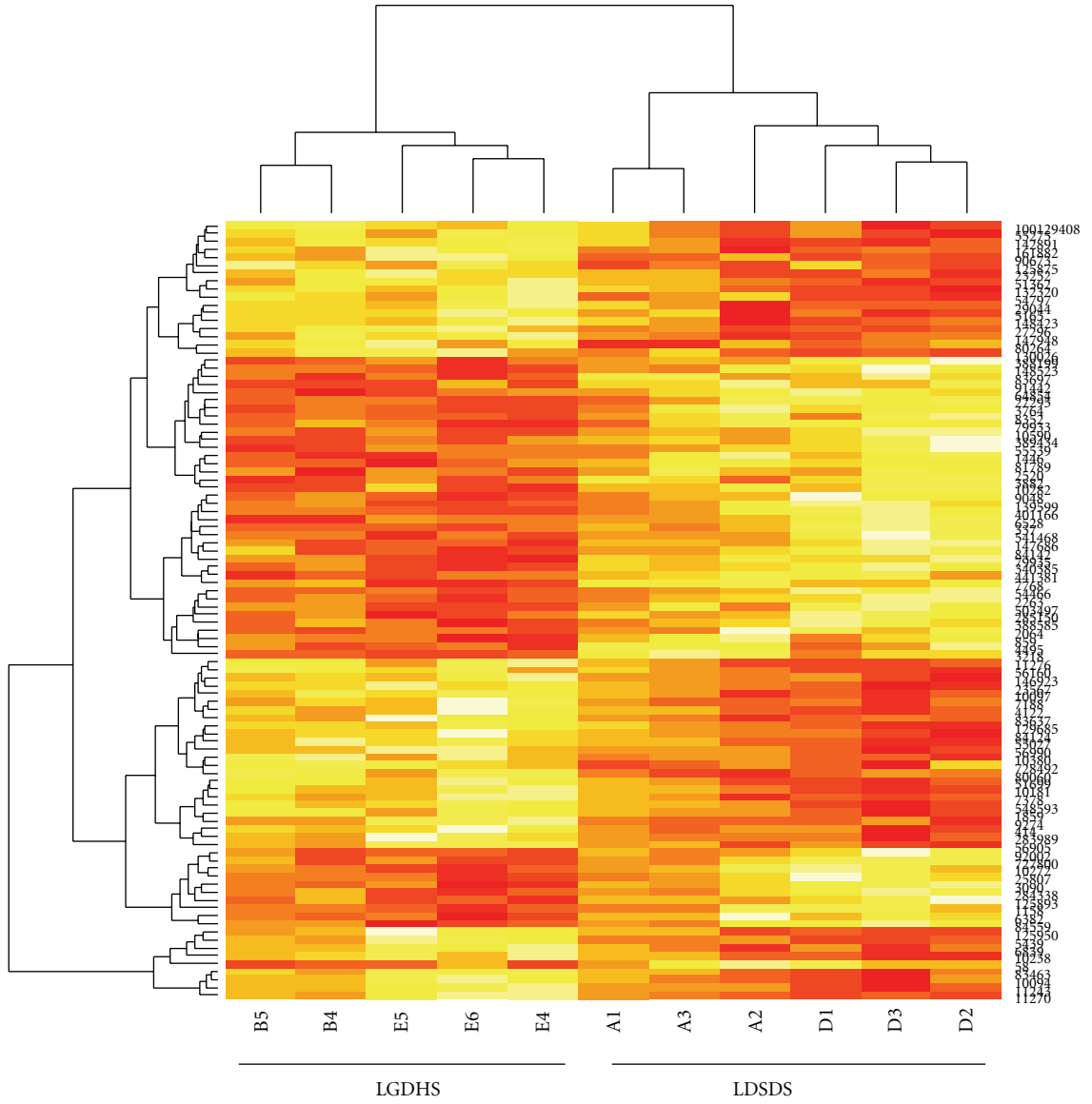


Figure 1: Heatmap of 98 differentially expressed genes between LGDHS and LDSDS. The 98 differentially expressed genes between LGDHS and LDSDS were obviously divided out by Heatmap analysis. Row: genes; column: patient number; deep colour: upexpressed genes; light colour: down-expressed genes; A1–3 and D1–3: LDSDS; B 4, 5 and E4–6: LGDHS.

syndromes. The all 14352 genes were taken into 26 gene modules by the WGCNA R package [15], and each module had a name of color and a ME to identify the gene expression. Among the 26 modules, some significant modules were screened out by correlating the MEs in our disease trail or TCM syndrome trail. In the result, blue, brown, turquoise, and yellow modules were most related with the difference between CHB and LC (Figure 2(a)), and lightgreen module and lightcyan module were most related with the difference between LGDHS and LDSDS (Figure 2(b)).

The above 6 gene modules were used to GO enrichment analysis. The result showed that the blue module was mainly enriched in G-protein-coupled receptor protein-signaling pathway, brown module was mainly enriched in immune system process, yellow module was mainly enriched in cell cycle, and turquoise module was enriched in many basal metabolisms. But it was still hard to understand that ossification function was enriched in lightcyan module, and the lightgreen module did not enrich in any GO function module.

3.3. Comparing Difference Coexpression Network between Two TCM Syndromes. To further demonstrate the mechanism of difference between two TCM syndromes, the correlation of gene expression including difference expression and difference coexpression was analyzed. Figure 3 was a schematic diagram which showed the meaning of difference expression or difference coexpression, respectively. The difference expression meant that there were gene different expression levels between two states. The difference coexpression meant

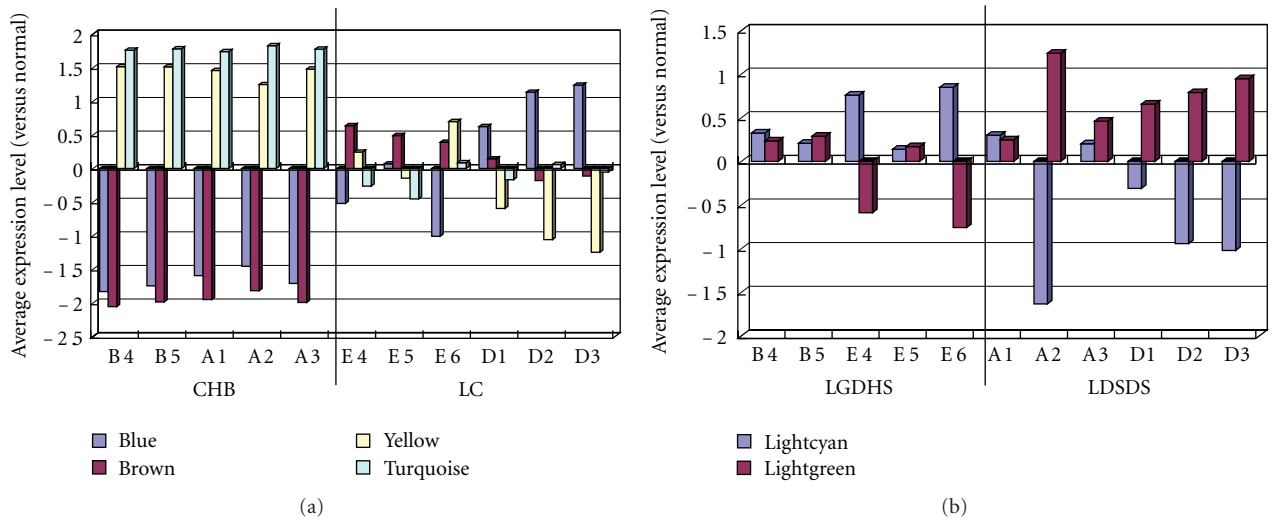


Figure 2: Average gene expression in modules which correlated with diseases or TCM syndromes. In the diseases (a), blue and brown modules both had low expression value in CHB and not consistent in LC. Yellow and turquoise modules both had high expression value in CHB and not consistent in LC. In the TCM syndromes (b), lightcyan modules had low expression value in LDSDS. Lightgreen modules had high expression value in LDSDS. A1–3 and D1–3: LDSDS; B 4, 5 and E4–6: LGDHS.

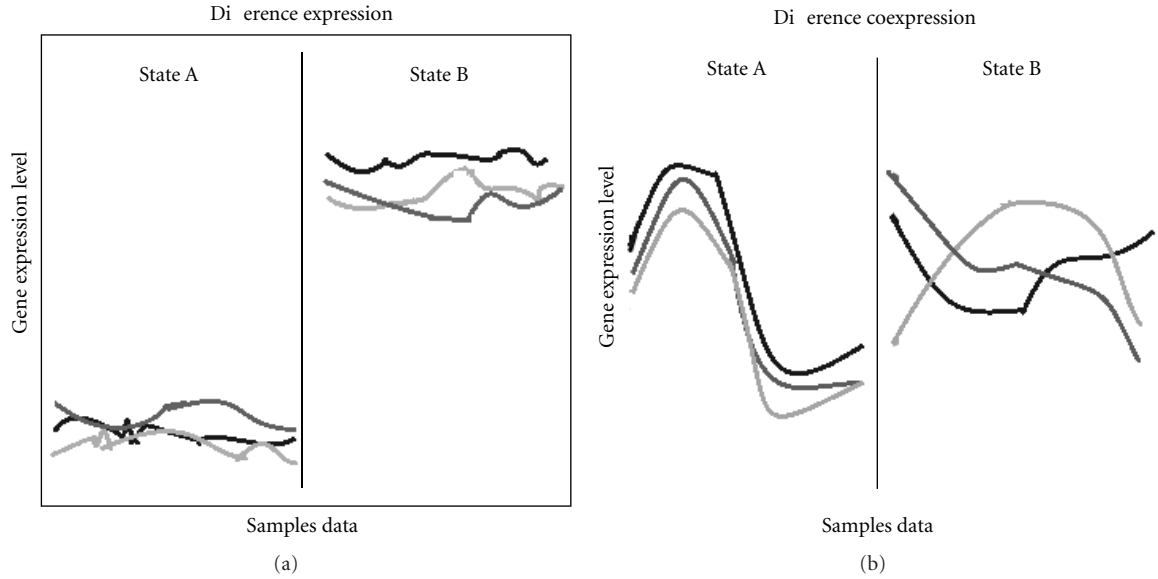


Figure 3: Schematic diagram of difference expression and difference coexpression. Graph of the difference expression (a) represented that there are genes different expression levels between states A and B, and the difference coexpression (b) represented that there is higher correlation in state A and lower correlation in state B. Curves were represented as whichever genes.

that there was higher gene correlation in a state and lower gene correlation in another state.

Then, the difference coexpression groups between LGDHS and LDSDS were analyzed using the advantage of coXpress R package [16]. First, through the analysis using the 830 differential expression genes ($P < 0.05$ in t-test) between the LGDHS and LDSDS, the gene groups whose gene members were coexpressed in LGDHS and not co-expressed in LDSDS were produced by coXpress (A in Table 1). Then we also executed the coXpress again to find the gene groups whose gene members were coexpressed in LDSDS and not coexpressed in LGDHS (B in Table 1). The P values including p.g1 in and p.g2 indicated a gene confusion degree in every group in LGDHS or LDSDS, respectively, ($P > 0.05$ was jumbled or not coexpressed; $P < 0.05$ was order or coexpressed).

It was found that the gene coexpression groups were orderly in LGDHS but jumbled in LDSDS (A in Table 1). Among the groups jumbled in LDSDS, There were the most gene numbers in group 9. The gene confusion degree in group 9 was showed in Figure 4. It was observed that genes of LGDHS in group 9 had similar traces (Figure 4(a)), whereas

Table 1: Comparison of gene coexpression groups in LGDHS and LDSDS.

Group ID	Gene number	P.g1	P.g2
	A LGDHS		
8	6	0.00	0.62
5	10	0.00	0.31
9	***81***	***0.00***	***0.83***
14	18	0.00	0.38
12	34	0.00	0.11
17	15	0.00	0.05
13	45	0.00	0.14
10	58	0.00	0.03
4	19	0.00	0.15
16	27	0.00	0.02
15	55	0.00	0.00
3	48	0.00	0.00
6	16	0.00	0.01
11	92	0.00	0.00
2	11	0.00	0.00
1	234	0.00	0.00
7	61	0.00	0.00
	B LDSDS		
9	6	0.00	0.00
17	10	0.00	0.00
12	13	0.01	0.00
7	297	0.00	0.00
14	5	0.12	0.00
4	90	0.00	0.00
8	5	0.20	0.00
10	12	0.04	0.00
5	69	0.53	0.00
6	26	0.83	0.00
15	3	0.49	0.08
2	***238***	***0.69***	***0.00***
3	21	0.87	0.00
11	8	0.54	0.00
1	8	0.36	0.00
13	4	0.62	0.05
18	6	0.83	0.00
16	9	0.76	0.07

the traces of LDSDS were varied (Figure 4(b)). To further clarify the functional mechanism at molecular level, GO enrichment analysis was taken on the genes in group 9. As Table 2 revealed, LGDHS was involved in electron transport chain function, but LDSDS does not.

Analogously, it was also found that the gene coexpression groups were orderly in LDSDS but jumbled in LGDHS (B in Table 1). Among the groups jumbled in LGDHS, there were the most gene numbers in group 2. Therefore, group 2 were analyzed and showed that the traces of LGDHS were varied (Figure 4(c)) and the traces of LDSDS were in order (Figure 4(d)). Through further studied the molecular functional mechanism by the GO enrichment analysis, it was found that LDSDS was involved in G-protein-coupled receptor protein-signaling pathway (GCRP pathway), but LGDHS does not (Table 2).

3.4. Molecular Mechanism of Difference between Diseases and TCM Syndromes. It was interesting in our result that the genes coexpression in group 2 was enriched in GCRP pathway. Because same situation happened to the genes in blue module, which was related with the difference between CHB and LC by the gene module analysis, these genes in GCRP pathway were differentially expressed between CHB and LC and difference coexpressed between LGDHS and LDSDS. These results were summarized in Figure 5. Interestingly, in GCRP pathway, whether TCM syndrome was LGDHS or LDSDS, the gene expression level was lower in CHB and higher or lower in LC, and whether disease was CHB or LC, the genes in LDSDS had higher correlation than LGDHS. For example, in LDSDS, genes GPER, PTHR1, GPR173, and SSTR1 were connected in a correlation network together, while they, respectively, belong to four correlation networks in LGDHS (Figure 5). These results suggested the different molecular mechanism between diseases (CHB and LC) and TCM syndromes (LGDHS and LDSDS).

3.5. Average Expression and Correlation of DRD5 GABRA SSTR1 and NPFF Genes in Diseases and TCM Syndromes. To test and verify the difference of average expression level and correlation of genes in GCRP pathway, DRD5 GABRA SSTR1 and NPFF mRNAs were expressed by real-time RT-PCR. The average expression levels of these genes in both LGDHS and LDSDS were lower in CHB, and that of LDSDS was more than LGDHS in LC (Figure 6(a)). The correlation coefficient of LDSDS (>0.5) in CHB and LC was more than LGDHS (<0.5) in CHB and LC (Figure 6(b)). These results further confirmed that the gene expression level was lower in CHB and higher or lower in LC. The genes in LDSDS had higher correlation than LGDHS whether disease was CHB or LC.

Previous researches had also found that LC was related with GCRP pathway [17–19], but little literature touched upon the relation between CHB and GCRP. Our result also indicated that genes in GCRP pathway were higher expression in LC and lower expression in CHB. It suggested that LC was a more serious disease than CHB by the activity of GCRP pathway. Further research will clarify the role of genes in GCRP pathway from CHB develop to LC.

Interestingly, our results showed that TCM syndromes, LGDHS and LDSDS did not clearly relate with the gene expression levels in GCRP pathway. The genes correlation or cooperation was more important. As shown in Figure 4, the genes in LDSDS had more connections than LGDHS, so LGDHS and LDSDS constructed different gene network. It incarnated the holistic thought in TCM.

Therefore, our research results suggested that CHB could be divided into LGDHS and LDSDS by the gene correlation as well as LC, which reveals the molecular feature of Different TCM Syndrome for Same Disease. Analogously, LGDHS was being in CHB or LC by the gene expression level as well as LDSDS, which reveals the molecular feature of Same TCM

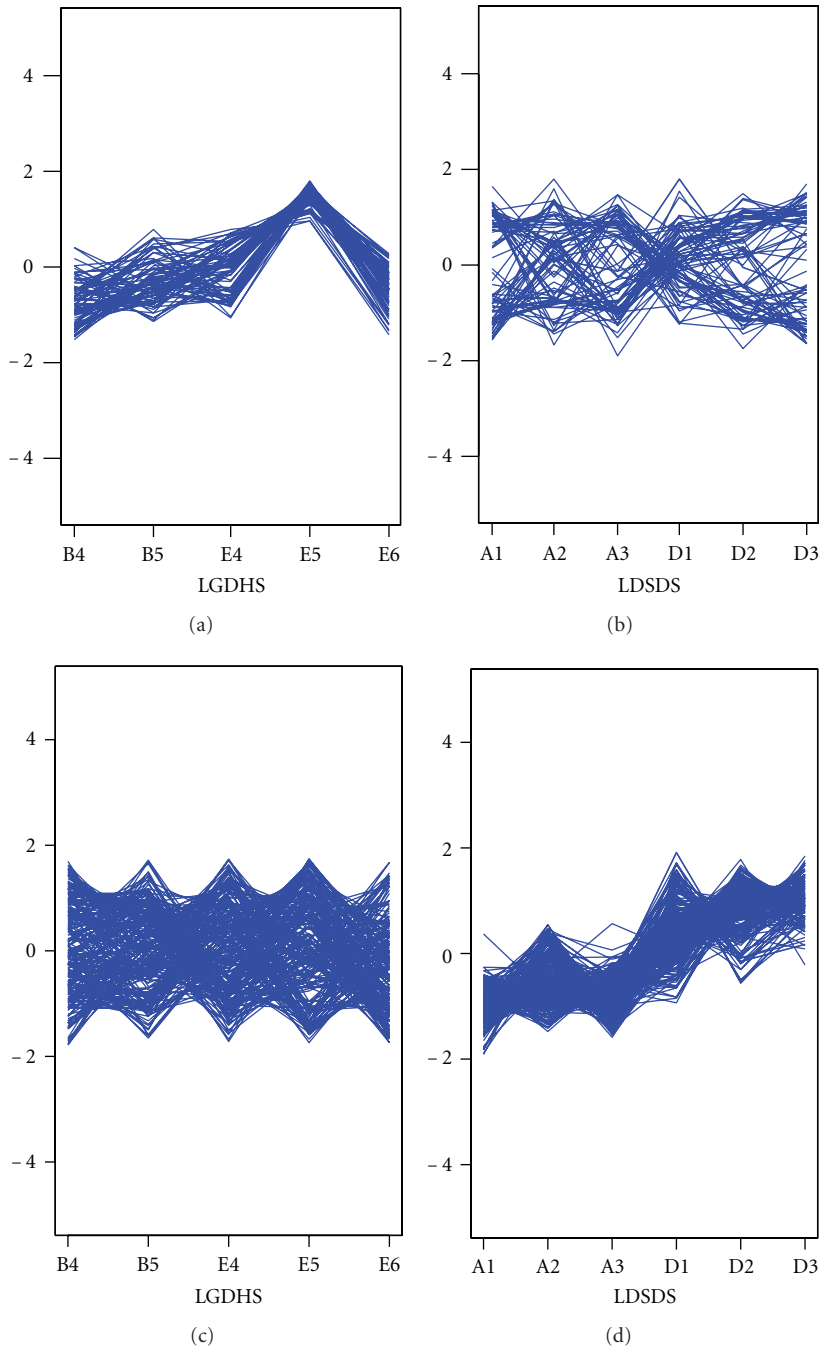


Figure 4: The gene confusion degree of group 2 and 9 in LGDHS and LDSDS. CoXpress was used to find orderly gene groups in LGDHS or LDSDS. The genes in group 9 of orderly gene groups in LGDHS showed good consistency in LGDHS (a) and poor consistency in LDSDS (b). The genes in group 2 of orderly gene groups in LDSDS showed poor consistency in LGDHS (c) and good consistency in LDSDS (d). A1–3 and D1–3: LDSDS; B 4, 5 and E4–6: LGDHS.

Syndrome for Different Diseases. The schematic diagram of the molecular mechanisms was showed in Figure 2.

There are two kinds of therapeutic principles in the TCM syndrome identification and treatment process, called Different treatments for the same disease and same treatment for different diseases. The Different treatments for the same disease means using different prescriptions or Chinese herbal medicines to treat the different TCM syndromes in the same disease process. The Same treatment for different diseases means using the same and prescriptions or Chinese herbal medicines to treat the same TCM syndrome in different disease process. These therapeutic principles are widely used in TCM practice as personalized therapy [12, 20]. Therefore, understanding the molecular mechanisms of Same TCM Syndrome for Different Diseases and Different TCM Syndrome for Same Disease will be primely serving for TCM

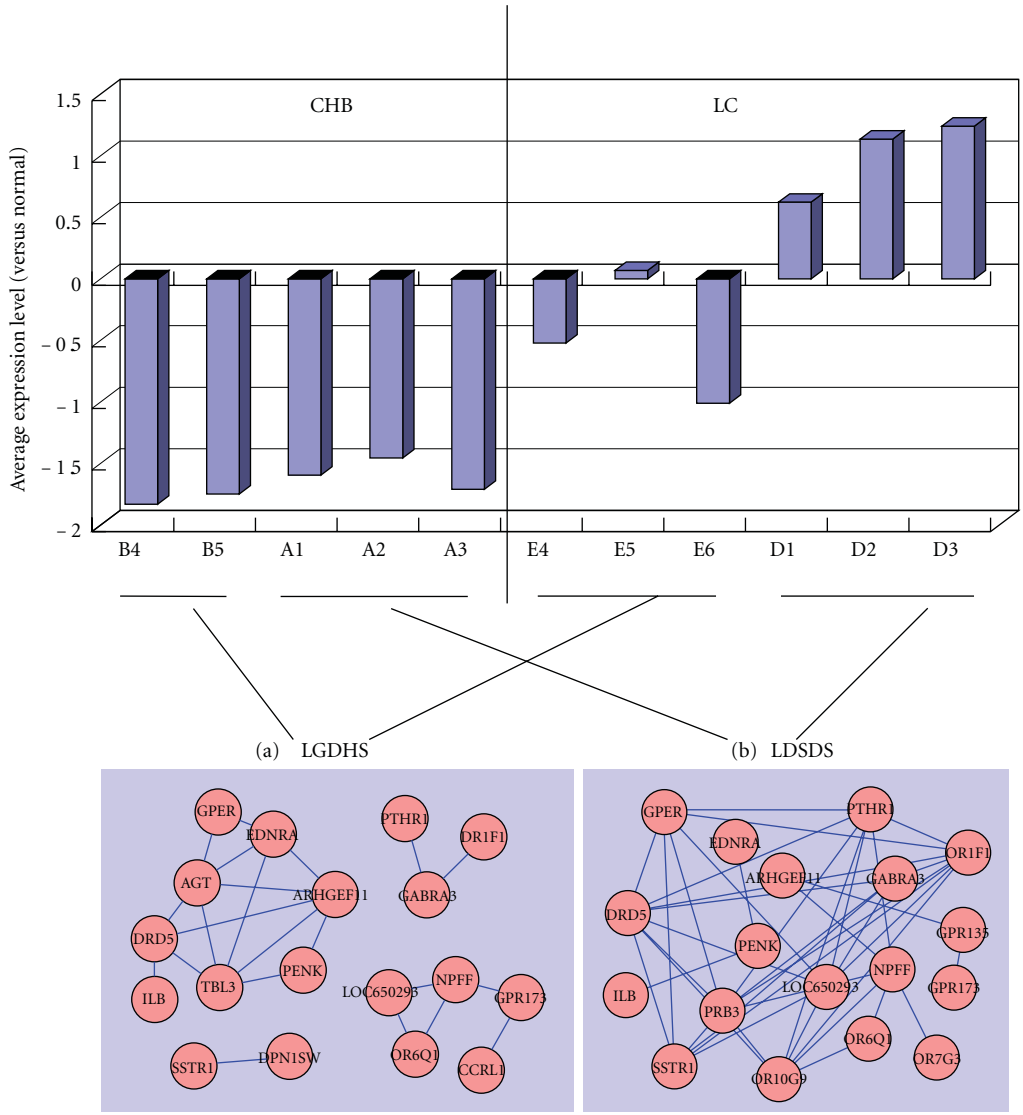


Figure 5: Gene relationships in GCRP pathway in diseases and TCM syndromes. GO enrichment analysis of genes in group 2 was carried out. Whether diseases (CHB or LC) and TCM syndromes (LGDHS or LDSDS) were correlated to GCRP pathway, the gene expression (upper figure) was represented that the gene expression levels were lower in CHB and higher or lower in LC. The gene network ((a), (b)) was represented that the genes connections in LDSDS (b) were more than LGDHS (a).

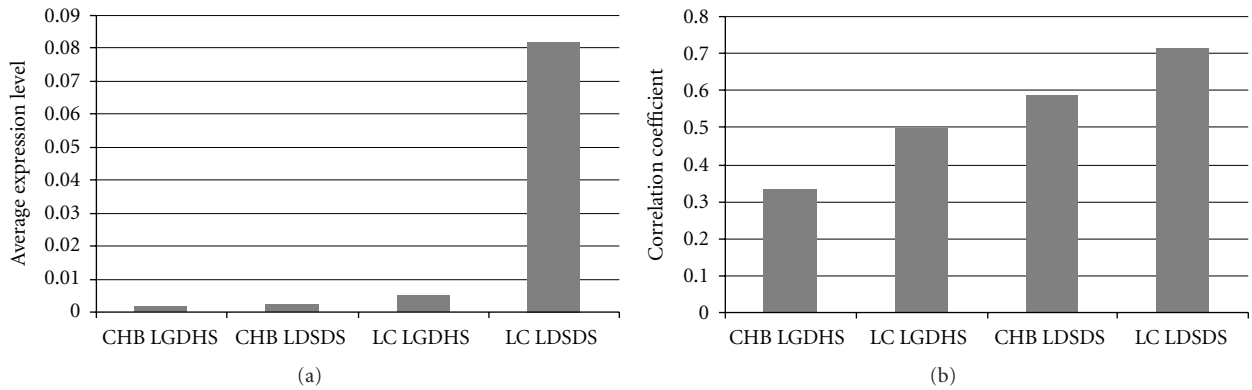


Figure 6: Average expression and correlation of DRD5 GABRA SSTR1 and NPFF mRNAs in diseases and TCM syndromes. The gene expression levels of both LGDHS and LDSDS were lower in CHB and that of LDSDS was more than LGDHS in LC (a). (Gene expression levels were the ratio of each mRNA and ACTB mRNA). The correlation coefficient of LDSDS in CHB and LC was more than LGDHS in CHB and LC (b).

Table 2: GO enrichments of orderly group 2 in LDSDS and group 9 in LGDHS.

GO term ID	Orderly group	Enrichment *P*	Term name
GO:0006120	LGDHS 9	0.022478	Mitochondrial electron transport, NADH to ubiquinone
GO:0022900	LGDHS 9	0.022478	Electron transport chain
GO:0022904	LGDHS 9	0.022478	Respiratory electron transport Chain
GO:0042773	LGDHS 9	0.022478	ATP synthesis coupled electron transport
GO:0042775	LGDHS 9	0.022478	Organelle ATP synthesis coupled electron transport
GO:0006119	LGDHS 9	0.04236	Oxidative phosphorylation
GO:0010468	LGDHS 9	0.048855	Regulation of gene expression
GO:0009987	LGDHS 9	0.049535	Cellular process
GO:0016070	LGDHS 9	0.059695	RNA metabolic process
GO:0006355	LGDHS 9	0.061016	Regulation of transcription, DNA-dependent
GO:0007186	LDSDS2	0.000668	G-protein coupled receptor protein signaling pathway
GO:0007606	LDSDS2	0.004518	Sensory perception of chemical stimulus
GO:0007608	LDSDS2	0.004518	Sensory perception of smell
GO:0007166	LDSDS2	0.014079	Cell surface receptor linked signal transduction
GO:0007586	LDSDS2	0.015106	digestion
GO:0007223	LDSDS2	0.017534	Wnt receptor signaling pathway, calcium modulating pathway
GO:0008203	LDSDS2	0.017534	Cholesterol metabolic process
GO:0016125	LDSDS2	0.017534	Sterol metabolic process
GO:0042157	LDSDS2	0.017534	Lipoprotein metabolic process
GO:0006813	LDSDS2	0.017952	Potassium ion transport

diagnosis and treatment. This research provided firstly the evidence. Further research will be required more samples to proving this evidence.

4. Conclusion

The classification of TCM syndrome is a diagnostic method. TCM syndromes are significantly associated with diseases, which are involved in Same TCM Syndrome for Different Diseases and Different TCM Syndrome for Same Disease. In this study, through analyzing microarray date of LGDHS and LDSDS in patients with CHB and LC, we provided evidence that the difference between CHB and LC was gene expression and the difference between LGDHS and LDSDS was gene coexpression in G-protein-coupled receptor protein-signaling pathway. Therein genes GPER, PTHR1, GPR173, and SSTR1 were coexpressed in LDSDS but not in LGDHS. Either CHB or LC was divided into the alternative LGDHS and LDSDS by the gene correlation, which reveals the molecular feature of Different TCM Syndrome for Same Disease. Either LGDHS or LDSDS was divided into the alternative CHB and LC by the gene expression level, which reveals the molecular feature of Same TCM Syndrome for Different Diseases. These results might be significant for both TCM research and TCM diagnosis and treatment.

Authors' Contribution

Z. Guo and A. Yu equally contributed in this paper.

Acknowledgments

This study was supported by National Science and Technology Major Project of China (no. 2012ZX10005001-004 and no. 2009ZX09311-003), Leading Academic Discipline Project of Shanghai Municipal Education Commission (no. J50301), and E-institutes of Shanghai Municipal Education Commission (no. E 03008).

References

[1] W. Jia, W. Y. Gao, Y. Q. Yan et al., "The rediscovery of ancient Chinese herbal formulas," *Phytotherapy Research*, vol. 18, no. 8, pp. 681–686, 2004.

[2] A. P. Lu and K. J. Chen, "Integrative medicine in clinical practice: from pattern differentiation in traditional Chinese medicine to disease treatment," *Chinese Journal of Integrative Medicine*, vol. 15, no. 2, p. 152, 2009.

[3] Y. H. Lu, H. P. Hao, G. J. Wang, X. H. Chen, X. X. Zhu, and B. R. Xiang, "Metabolomics approach to the biochemical differentiation of Traditional Chinese Medicine syndrome types of hypertension," *Chinese Journal of Clinical Pharmacology and Therapeutics*, vol. 12, no. 10, pp. 1144–1150, 2007.

[4] W. Jian, Z. Yuan, X. Huang et al., "Analysis on urine metabolomics of coronary heart disease patients with the heart blood stasis syndrome," *Journal of Traditional Chinese Medicine*, vol. 51, no. 8, pp. 729–732, 2010.

[5] C. Lu, C. Xiao, G. Chen et al., "Cold and heat pattern of rheumatoid arthritis in traditional Chinese medicine: distinct molecular signatures indentified by microarray expression profiles in CD4-positive T cell," *Rheumatology International*, vol. 32, no. 1, pp. 61–68, 2010.

[6] S. Li, Z. Q. Zhang, L. J. Wu, X. G. Zhang, Y. D. Li, and Y. Y. Wang, "Understanding ZHENG in traditional Chinese medicine in the context of neuro-endocrine-immune network," *IET Systems Biology*, vol. 1, no. 1, pp. 51–60, 2007.

[7] WHO Media Centre and B. Hepatitis, http://www.who.int/mediacentre/factsheets/fs204.

[8] X. Cui, Y. Wang, N. Kokudo, D. Fang, and W. Tang, "Traditional Chinese medicine and related active compounds against hepatitis B virus infection," *Bioscience Trends*, vol. 4, no. 2, pp. 39–47, 2010.

[9] . C. Liu, Y. Hu, L. Xu, and P. Liu, "Effect of Fuzheng Huayu formula and its actions against liver fibrosis," *Chinese Medicine*, vol. 4, p. 12, 2009.

[10] Z. Y. Li, X. X. Zhang, and Z. C. Xu, "Study on integrative point of traditional and western medicine—from "integrative disease and syndrome" to "integrative pathological process and syndrome"," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 25, no. 3, pp. 259–262, 2005.

[11] X. Q. Li, H. Zhang, and X. W. Li, "Study on reference laboratory diagnostic index of liver-fire ascending syndrome," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 21, no. 3, pp. 190–192, 2001.

[12] J. Li, "Thinking on syndrome differentiation treatment and personalized therapy for tumor," *Zhong Xi Yi Jie He Xue Bao*, vol. 7, no. 4, pp. 306–308, 2009.

[13] X. X. Zeng, Z. X. Bian, T. X. Wu, S. F. Fu, E. Ziea, and W. T. Woon, "Traditional chinese medicine syndrome distribution in chronic hepatitis B populations: a systematic review," *The American Journal of Chinese Medicine*, vol. 39, no. 6, pp. 1061–1074, 2011.

[14] Y. A. Ye, F. Jiang, Z. M. Zhao et al., "Chinese medical pattern distribution of chronic type hepatitis B," *Journal of Traditional Chinese Medicine*, vol. 48, no. 3, pp. 256–258, 2007.

[15] P. Langfelder and S. Horvath, "WGCNA: an R package for weighted correlation network analysis," *BMC Bioinformatics*, vol. 9, article 559, 2008.

[16] M. Watson, "CoXpress: differential co-expression in gene expression data," *BMC Bioinformatics*, vol. 7, article 509, 2006.

[17] T. Y. Chen, T. L. Hwang, C. Y. Lin et al., "EMR2 receptor ligation modulates cytokine secretion profiles and cell survival of lipopolysaccharide-treated neutrophils," *Chang Gung Medical Journal*, vol. 34, no. 5, pp. 468–477, 2011.

[18] C. Rancoule, J. P. Pradère, J. Gonzalez et al., "Lysophosphatidic acid-1-receptor targeting agents for fibrosis," *Expert Opinion on Investigational Drugs*, vol. 20, no. 5, pp. 657–667, 2011.

[19] H. E. Wasmuth and R. Weiskirchen, "Pathogenesis of liver fibrosis: modulation of stellate cells by chemokines," *Zeitschrift fur Gastroenterologie*, vol. 48, no. 1, pp. 38–45, 2010.

[20] Y. Liang, Z. Lu, N. Zhang, and L. Shen, "Evaluation of multidimensional outcomes of chronic diseases: a clinical example from China," *Archives of Gerontology and Geriatrics*, vol. 52, no. 3, pp. e106–e109, 2011.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 626320, 10 pages
doi:10.1155/2012/626320

Research Article

Classification of Traditional Chinese Medicine Syndromes in Patients with Chronic Hepatitis B by SELDI-Based ProteinChip Analysis

Ya-Nan Song,[1] Hui Zhang,[1] Yan Guan,[1] Jing-Hua Peng,[2] Yi-Yu Lu,[1] Yi-Yang Hu,[2] and Shi-Bing Su[1]

[1] *Research Center for Traditional Chinese Medicine Complexity System, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Pudong, Shanghai 201203, China*
[2] *Institute of Liver Diseases, Shuguang Hospital, Key Laboratory of Liver and Kidney Diseases of Ministry of Education, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*

Correspondence should be addressed to Yi-Yang Hu, yyhuliver@163.com and Shi-Bing Su, shibingsu07@163.com

Received 12 January 2012; Accepted 28 March 2012

Academic Editor: Shao Li

Traditional Chinese medicine (TCM) syndrome, also called ZHENG, is the basis concept of TCM theory. It plays an important role in TCM practice. There are excess and deficiency syndromes in TCM syndrome. They are the common syndromes in chronic hepatitis B (CHB) patients. Here we aim to explore serum protein profiles and potential biomarkers for classification of TCM syndromes in CHB patients. 24 healthy controls and two cohorts of CHB patients of excess syndrome ($n = 25$) or deficiency syndrome ($n = 19$) were involved in this study. Protein profiles were obtained by surface-enhanced laser desorption ionization time-flight mass spectrometry (SELDI-TOF/MS) and multiple analyses were performed. Based on SELDI ProteinChip data, healthy controls and CHB patients or excess and deficiency syndromes in CHB patients were obviously differentiated by orthogonal partial least square (OPLS) analysis. Two significant serum proteins (m/z 4187 and m/z 5032) for classifying excess and deficiency syndromes were found. Moreover, the area under the receiver operating characteristic (ROC) curve was 0.887 for classifying excess and nonexcess syndrome, and 0.700 for classifying deficiency and nondeficiency syndrome, respectively. Therefore, the present study provided the possibility of TCM syndrome classification in CHB patients using a universally acceptable scientific approach.

1. Introduction

Traditional Chinese medicine (TCM) syndrome classification (also defined as Zheng differentiation) and treatment is the basis concept of TCM theory. TCM syndrome, a profile of symptoms and signs as a series of clinical phenotypes, plays an important role in understanding the human homeostasis and guiding the applications of Chinese herbs and acupuncture. Heat, cold, excess, and deficiency are the four basic syndromes of maladjustment nature in TCM [1]. Damp heat stasis syndrome and liver and kidney Yin deficiency syndrome, classified as excess syndrome and deficiency syndrome, respectively, are the common syndromes in chronic hepatitis B (CHB) patients [2]. Excess syndrome refers to the accumulation or stagnation of metabolic waste, body fluids, and blood, whereas deficiency syndrome means to "overcatabolism" and "overconsumption", the deficiency of nutrients, and weakness [1].

So far, an experiential diagnosis approach has been always used to classify excess syndrome and deficiency syndrome in CHB patients. TCM practitioners with rich experience in TCM diagnosis and treatment are often able to improve the symptoms of CHB patients, which may be considered to be untreatable by conventional medicine [3]. Lu et al. [4] mentioned that for coronary heart patients with different TCM syndromes, if herbal medicine was appropriate to TCM syndrome, the effective rate would increase. It was suggested that syndrome classification acts as a pivot in the therapeutic process and directly affects the therapeutic result of a specific disease. Instead of experiential diagnosis,

therefore, it is necessary to standardize the diagnosis criteria for classification of excess and deficiency syndromes in patients with CHB by using a universally acceptable scientific approach.

Proteomics, a rapidly evolving tool in systems biology of analyzing protein expression in a comprehensive degree, is widely applied for disease diagnosis and prognosis, such as brain injury [5], appendicitis [6], liver fibrosis [7], and esophageal cancer [8]. Surface-enhanced laser desorption ionization time-flight mass spectrometry (SELDI-TOF/MS), a powerful tool for global analysis of protein expression, provides an efficient and sensitive method for biomarker discovery. It can obtain the spectra composed of hundreds of protein peaks, each characterized by its mass-to-charge ratio (m/z) and each area represented by its amount [9]. Considering the features of measuring in a high-throughput way and analyzing with a small amount of materials, SELDI-TOF/MS has become an attractive tool for clinical application. The technology has successfully led to the discovery of new biomarkers for diagnosis and treatment of various diseases, for example, accurate diagnosis of early hepatocellular carcinoma [10] and laryngeal carcinoma [11], and identification of treatment efficacy-related host factors in chronic hepatitis C [12].

CHB is a kind of global infective disease induced by hepatitis virus B (HBV). It is estimated that about 400 million people are suffering from HBV infection worldwide [13, 14]. And HBV leads to 500,000 to 1.2 million deaths every year because of turning into liver cirrhosis and hepatocellular carcinoma (HCC) [15]. With 120 million people infected with HBV, China has the largest population in the world. And among them, about 30 million people are suffering from CHB [3]. TCM is widely used in the treatment of CHB and was found to be effective in China [16–18], and conventional medicine hardly heals CHB patients completely, so more and more people therefore turn to get help from TCM. In the present study, we aim to use SELDI-TOF/MS analysis and related data processing methods to find the protein profiles of excess and deficiency syndromes and the promising protein biomarkers to classify these TCM syndromes in patients with CHB.

2. Material and Methods

2.1. Study Population. The study has been approved by Shuguang Hospital, the affiliated hospital of Shanghai University of TCM. Serum samples were collected from November 2009 to July 2010. The experiment involved 24 healthy controls and two cohorts of CHB patients of excess syndrome ($n = 25$) or deficiency syndrome ($n = 19$). The demographic and clinicopathological data about the participants were showed in Table 1. The differences of gender and age have no statistical significance among three groups ($P > 0.05$). The selected 44 patients with CHB must be in accordance with the following criteria: (1) all patients were diagnosed according to both CHB and TCM syndromes and confirmed by chief physicians; (2) the diagnosis of CHB was based on the guideline defined by the Chinese Society of Hepatology and Chinese Society of Infectious Diseases in 2005 [19]; (3) the TCM syndrome differentiation was referred to the viral hepatitis diagnostic standard described by the Internal Medicine Hepatopathy Committee of Chinese Traditional Medicine Association in December, 1991 [20]. An informed consent was signed by each of the participants, and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki (1964).

The fasting blood samples were collected from two experimental groups of patients with CHB and healthy controls in the morning and allowed to stand for 30 min at room temperature and then centrifuged at 1,5000 rpm for 10 min. All the serum samples were stored at -80°C until further analysis.

2.2. Protein Profiling by SELDI-TOF/MS. CM10 (Ciphergen Biosystems, Fremont, CA, USA) was used to further serum differential protein spectrum analysis. First, 5 μL of the cleared serum was mixed with 10 μL of U9 solution containing 9 mol/L urea, 2% CHAPS, 50 mmol/L Tris/HCl, and 1% DTT (pH 9.0; Sigma, USA). Subsequently, the previous sample was diluted with 185 μL CM10-binding buffer (50 mmol/L sodium acetate, pH 4.0; Sigma, USA) to give a final dilution of 40-fold. In addition, the array spots should be preactivated twice with 200 μL of binding buffer for 5 min. And then, 100 μL of diluted serum samples was loaded on each array spot and incubated with shaking for 1 h at 4°C. Two washes with binding buffer and one quick rinse with HPLC grade water were continued to remove nonselectively bound proteins. After air-drying, 0.5 μL of freshly prepared sinapinic acid solution in 0.5% trifluoroacetic acid and 50% acetonitrile was added on each spot for twice. The chips were ready for MS detection when dried.

Mass accuracy was calibrated externally by using the all-in-one peptide molecular mass standard. After calibration passed, the chips were scanned by SELDI-TOF/MS in a PBS-IIc ProteinChip reader (Ciphergen Biosystems) to measure the masses and intensities of the protein peaks. According to experience, many parameters were optimized for getting more protein peaks and separating these peaks better. At last, the reader was set up as follows: laser intensity, 100; laser sensitivity, 8; optimized mass range, 2,000–15,000 Da; focus mass, 8,500 Da; high mass, 50,000 Da; and data acquisition parameters, 25 delta to 5 transients per to 10 ending position to 75. Data were processed automatically using the Ciphergen Protein-Chip Software (version 3.1.1, Ciphergen Biosystems). Spectra were normalized, calibrated, and aligned.

2.3. Data Processing. Protein spectra were automatically generated after all raw data were collected. The profiling spectra of serum samples were first normalized using total ion current by Ciphergen ProteinChip Software 3.1.1. Peak selection was carried out by the Biomarker Wizard program. Protein peaks were selected based on a first pass of signal-to-noise ratio of 5. This process was completed with a second pass of signal-to-noise ratio of 2, and peak selection at

Table 1: Clinical parameters and TCM syndromes in CHB patients and controls.

Clinical parameters	Excess syndrome ($n = 25$)	Deficiency syndrome ($n = 19$)	Healthy control ($n = 24$)
gender (M/F)	21/4	14/5	15/9
age (year)	38.0 ± 13.4	38.1 ± 11.1	36.4 ± 11.6
BMI (Kg/m^2)	23.2 ± 3.0	22.1 ± 2.7	21.3 ± 2.1
ALT (U/L)	91.8 ± 116.8	57.4 ± 41.7	20.7 ± 8.7
AST (U/L)	59.8 ± 54.4	50.5 ± 29.1	19.9 ± 5.5
GGT (U/L)	47.8 ± 47.7	56.8 ± 72.0	21.5 ± 9.8
ALP (U/L)	84.2 ± 21.4	90.2 ± 34.7	58.0 ± 20.2
ALB (g/L)	45.2 ± 4.1	44.2 ± 3.5	43.9 ± 5.7
TG (mmol/L)	1.1 ± 0.4	1.4 ± 0.7	0.8 ± 0.3
BA (μmol/L)	10.3 ± 15.9	13.0 ± 18.2	8.0 ± 1.8
TBIL (μmol/L)	19.8 ± 8.3	18.9 ± 5.2	15.0 ± 3.8
PT (s)	13.4 ± 2.0	13.2 ± 1.8	12.7 ± 0.8
HbsAg (+/−)	25/0	19/0	0/24
HBV DNA (+/−)	19/6	11/8	0/24

0.3% of the mass window, and the estimated peaks were added. After the preliminary analysis of protein spectra, these selected protein peaks were exported to other commercially available software for further analysis.

The statistical analysis was performed by SPSS software (version 15.0, Chicago, IL, USA). Values are expressed as the mean ± SD. The baseline characteristics were compared using appropriate method. For continuous variables, one-way factorial analysis was used, or the Wilcoxon rank-sum test was used because of the skewed distributions. And for categorical variables, x^2 test was used. Multivariate analysis was carried out to determine the independent variables associated with differentiation of syndromes. Two-sided P value < 0.05 for one-way factorial analysis or adjusted P value < 0.0167 for Wilcoxon rank-sum test was considered statistically significant. SELDI-TOF/MS-measured variables showing statistical significance on univariate analysis were subjected to binary logistic regression to determine significant independent factors. After the regression, the values of the prediction probability were applied to the classification of the samples. Then receiver operating characteristic curve (ROC) was made by using the SPSS software.

The preprocessed data obtained by Ciphergen ProteinChip Software were also exported and analyzed by principle component analysis (PCA) and orthogonal partial least squares (OPLSs) using the SIMCA-P software (version 11.5, Umetrics AB, Umea, Sweden).

3. Results

3.1. Clinical Characteristics of Study Population. Clinical characteristics and TCM syndromes in CHB patients and healthy controls are shown in Table 1. Data including body mass index (BMI), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-Glutamyltransferase (GGT), alkaline phosphatase (ALP), albumin (ALB), triglyceride (TG), bile acid (BA), total bilirubin (TBIL), prothrombin time (PT), Hepatitis B surface antigen (HbsAg), and HBV DNA were expressed as the mean ± SD. According to the statistical analysis, no clinical factors were significantly different between excess syndrome and deficiency syndrome, indicating that the two TCM syndromes could not been classified by the general clinical parameters of CHB.

3.2. Serum Protein Profiling by SELDI-TOF/MS. Using the SELDI ProteinChip system, we analyzed the serum protein profiling from 24 healthy controls, 25 excess syndrome patients with CHB, and 19 deficiency syndrome patients with CHB. Peaks were detected automatically after baseline subtraction. 184 protein peaks were detected and these peaks were overlapping among 3 groups. Figure 1(a) displays the representative protein profiling obtained by SELDI-TOF/MS analysis showing the protein peaks of healthy controls and CHB patients of two different TCM syndromes. As shown, the SELDI technology was effective in separating low molecular weight proteins and polypeptides between m/z 2,000 and m/z 15,000.

3.3. Classification of TCM Syndromes by Pattern Recognition Analysis. To explore whether the serum protein profiles could help to classify excess syndrome and deficiency syndrome in CHB patients, pattern recognition analysis was carried out to analyze the data generated by SELDI-TOF/MS. Principle component analysis (PCA) was first used as an unsupervised statistical method to study the protein differences among the three groups. The result showed that there was not a trend of separation between control group and CHB group or excess syndrome and deficiency syndrome groups (Figure 2(a)). Then a supervised statistical method, that is orthogonal partial least squares (OPLSs) analysis, was performed as mentioned before. As OPLS score plots were displayed, a tendency of separation was observed among the three groups (Figure 2(b)), and an obvious separation exists between excess syndrome group and deficiency syndrome group (Figure 2(c)), indicating that the whole protein expression was different not only between

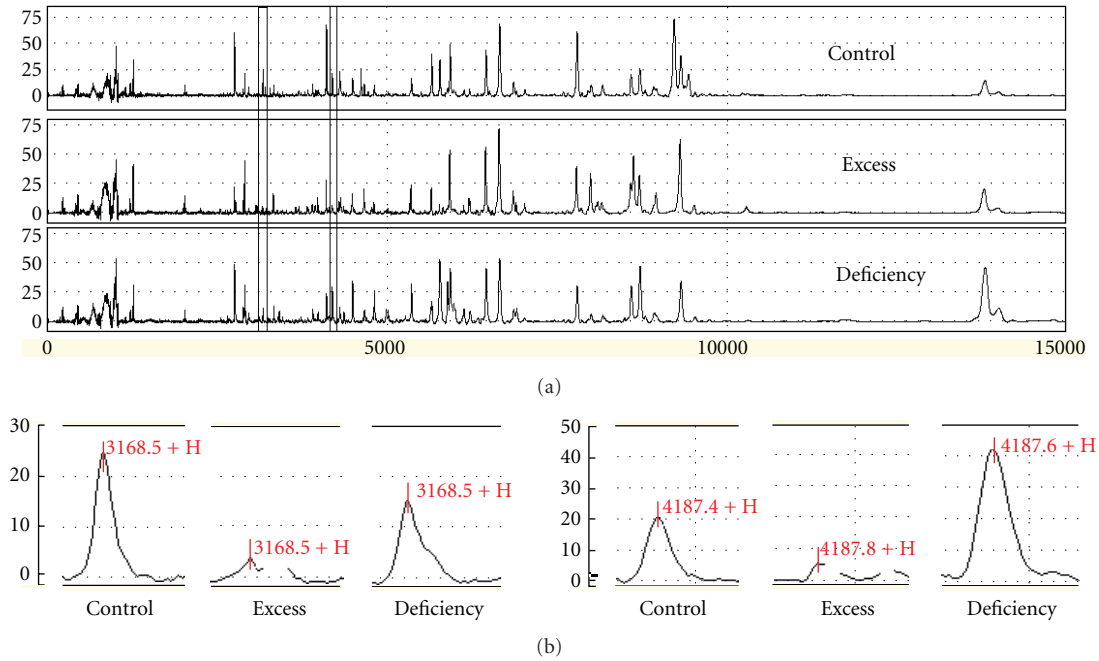


Figure 1: Representative protein profiles of serum samples of healthy controls and patients with CHB of excess symptom and deficiency syndrome. Protein peak spectrum of serum was analyzed by the SELDI-TOF/MS system, and representative protein peaks within m/z 0–1,5000 of three groups are shown (a). Statistically significantly different peaks between excess syndrome and deficiency syndrome are shown in the enlarged view, m/z 3168 on the left and m/z 4187 on the right (b).

healthy controls and CHB patients but also between excess and deficiency syndromes in CHB patients.

On the other hand, to investigate whether clinical parameters had influence on classification, the PCA model comparing three groups was constructed using clinicopathological data alone. But the result was not satisfying and the groups could not be differentiated from each other (not shown). And then the OPLS model was carried out. As shown in Figure 2(d), only the control group could be separated from the two others, whereas the TCM syndrome groups could not be separated from each other. It was suggested that the general clinical data were good at classifying health and HBC, while the data from SELDI-TOF/MS could be used for TCM syndrome classification.

3.4. Serum Protein Potential Biomarkers of TCM Syndromes. Among a total of 184 protein peaks detected, 4 significantly different peaks were observed between excess and deficiency syndromes according to Wilcoxon rank-sum test. Three of four protein peaks were in lower abundance in excess syndrome group (Figures 3(a), 3(b), and 3(c)), and the remaining one was in higher abundance (Figure 3(d)). These statistically significant differences can be displayed clearly in the box-plots. Also, an enlarged view of m/z 3168 and m/z 4187 is shown in Figure 1(b). So they may be potential biomarkers for classifying excess syndrome and deficiency syndrome with CHB.

3.5. Logistic Regression Analysis. To identify the variables independently associated with TCM syndromes in CHB patients and to compare the value of SELDI data and clinical parameters in classifying TCM syndromes, logistic regression analysis was performed including SELDI-TOF/MS-measured four significantly different variables displayed in Figure 3 and some clinical parameters listed in Table 1. As shown in Table 2, two protein peaks were independent factors that were associated with TCM syndromes and no clinical parameters were selected. Just as mentioned in Section 3.3, it was proven again that the general clinical data were only good at classifying health and HBC, while the method of SELDI-TOF/MS could be used for TCM syndrome classification. Then peak m/z 4187 and peak m/z 5032 were applied to the classification of different TCM syndrome. And 88% of excess syndrome patients and 73.7% of deficiency syndrome patients were correctly discriminated (cutoff value: 0.5, Figure 4).

3.6. Sensitivity and Specificity of Serum Protein Markers for TCM Syndrome Classification. To determine the sensitivity and specificity of serum protein potential biomarkers and the usefulness of protein peak quantifications as classification of different TCM syndromes, ROC analysis was conducted. To increase the performance of the classification, the most efficient peak combination was determined using regression analysis. Control group and deficiency syndrome group were put together and defined as the nonexcess syndrome group, so ROC analysis was carried out for discriminating excess syndrome with nonexcess syndrome. The area under the ROC curve for the combination of m/z 4187 and m/z 5032 was 0.887 (Figure 5(a)). In the same way, Control

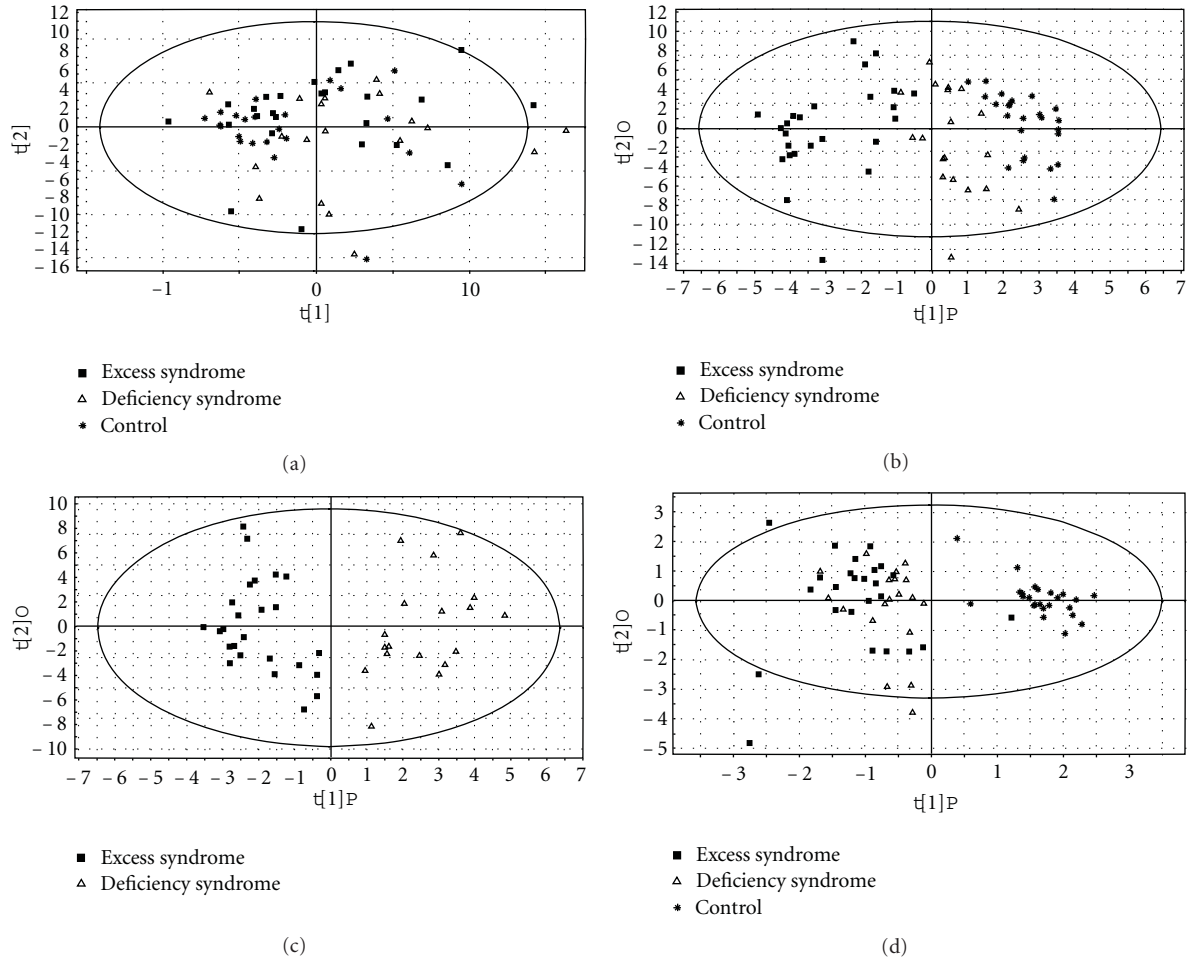


Figure 2: PCA score plot and OPLS score plots of 25 CHB patients of excess syndrome (■), 19 CHB patients of deficiency syndrome (△), and 24 healthy controls (∗) based on the serum protein profiling detected from SELDI-TOF/MS or the clinicopathological data of each individuals. (a) PCA score plot among the control group and CHB groups of excess syndrome and deficiency syndrome; OPLS score plots (b) among the control group and CHB groups of excess syndrome and deficiency syndrome and (c) between excess syndrome group and deficiency syndrome group. (a)–(c) Models of score plots were constructed by the data from SELDI-TOF/MS. (d) Another OPLS score plot among the three groups using clinical parameters.

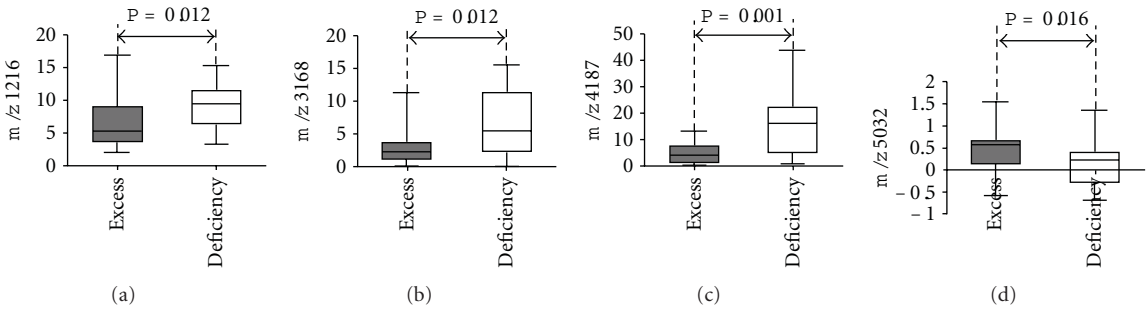


Figure 3: Box-plots for protein peak comparison between TCM syndrome groups. Proteins m/z 1216 (a), m/z 3168 (b), and m/z 4187 (c) were in lower abundance in excess syndrome group than those in deficiency syndrome one, while protein m/z 5032 (d) was in higher abundance.

Table 2: Logistic regression analysis for TCM syndrome classification in CHB patients.

Factors	Odds ratio	95% CI	*P* value
m/z 4187	1.349	1.100–1.655	0.004
m/z 5032	0.054	0.005–0.597	0.017
m/z 1216	—	—	0.894
m/z 3168	—	—	0.097
BMI (Kg/m^2)	—	—	0.301
ALT (U/L)	—	—	0.544
AST (U/L)	—	—	0.452
GGT (U/L)	—	—	0.074
ALP (U/L)	—	—	0.779
ALB (g/L)	—	—	0.093
TG (mmol/L)	—	—	0.262
BA (μmol/L)	—	—	0.206
TBIL (μmol/L)	—	—	0.901
PT (s)	—	—	0.150

group and excess syndrome group were put together and defined as the nondeficiency syndrome group, and then ROC analysis was performed to discriminate excess syndrome with nonexcess syndrome. The area under the ROC curve was 0.700 (Figure 5(b)). It was suggested that the quantification of these variables by SELDI-TOF/MS was useful to classify excess and deficiency syndromes (Figure 5).

4. Discussion

TCM practitioners classify biomedical maladjustments into different syndromes, and each syndrome has its own suitable treatment protocol. Also, considering that the mechanism of disease might not be identical in different people, that is to say, one disease could display several different syndromes, so the same disease may be treated by different therapeutic approaches. The syndrome classification-based individualized therapy is commonly applied in the TCM practice. So we have sufficient reasons to believe that the therapeutic effect will be influenced if excess syndrome and deficiency syndrome of CHB patients were not classified correctly. Therefore, much attention should be paid to the accuracy and the standard of syndrome classification. However, people often argue that the diagnostic approach of TCM practitioners does not meet requirements of objectivity and reproducibility. And TCM diagnosis studies have proved that there exists considerable variability across different practitioners, even when the same patient was diagnosed [21, 22]. So it is essential to find a kind of scientific and persuasive approach for the application of TCM syndrome classification.

Proteomics is playing an important role in improving our understanding of biologic systems by observing the different interactions among hundreds of proteins simultaneously and aims at studying proteins of human body in the level of integrity. It happens to be in accordance with the viewpoint of TCM, which has always been emphasized on the integrity of human body and the close relationship between human and its environment [3]. In addition, the characteristics of proteomics make it possible to integrate various proteins [23] and easy to study TCM syndrome classification. Comparing with the traditional method that syndromes are classified into groups based on TCM theory and clinical experiences, they can be clustered into specific groups using the approaches of proteomics and bioinformatics. Matsumoto et al. found several proteins for the diagnosis of "Oketsu", a pathophysiologic concept of Japanese traditional medicine, and differentiated "Oketsu" with "non-Oketsu" successfully [24]. Obviously, it is more scientific and more persuasive. As described in this paper, a proteomics approach was applied, which aimed to provide a kind of accurate and reliable method for TCM syndrome classification.

In this study, we used the ProteinChip system to analyze and compare the serum protein profiles of excess and deficiency syndromes in CHB patients to define the new potential protein biomarkers for syndrome classification. According to pattern recognition analysis, excess and deficiency syndromes were observed to be clustered into different groups. And four protein peaks were found statistically significant when both groups were compared. On the other hand, syndrome groups could not be classified using general clinical data, and no clinical data were found significantly different between TCM syndrome groups. Among those four possible protein markers, three (m/z 1216, m/z 3168, and m/z 4187) were overexpressed in the deficiency syndrome group and one (m/z 5032) was increased in the group of excess syndrome. Multivariate regression analysis performed by using four significantly different protein peaks from SELDI-TOF/MS data and laboratorial serum markers from clinical data showed the usefulness of two protein peaks (peak m/z 4187 and peak m/z 5032) for excess and deficiency syndromes classification. To observe the sensitivity and specificity of the two proteins, ROC curve analysis was conducted to differentiating excess with nonexcess syndromes and deficiency with nondeficiency syndromes. The area under the ROC curve was 0.887 and 0.700, respectively, suggesting that they could be applied for the classification of TCM syndromes in CHB patients.

Since one disease could display multiple syndromes in TCM theory, this study focused on several subgroups of CHB patients. It would make protein profiles of different patients keep in the same level of a specific disease and eliminate the interference of diseases for looking for biomarkers classifying different syndromes.

Also, comparing healthy controls with CHB patients of excess syndrome or deficiency syndrome, significant variables were supposed to represent the potential biomarkers about CHB disease and excess syndrome or deficiency syndrome, and the common variables were supposed to represent the potential biomarkers between CHB and healthy group. So in order to find out potential biomarkers for classifying TCM syndromes, those about CHB disease should be eliminated from the significant variables comparing excess syndrome with deficiency syndrome. Therefore, 27 significantly different serum proteins between healthy controls and excess syndrome might be the potential biomarkers for CHB disease and excess syndrome. In the same way,

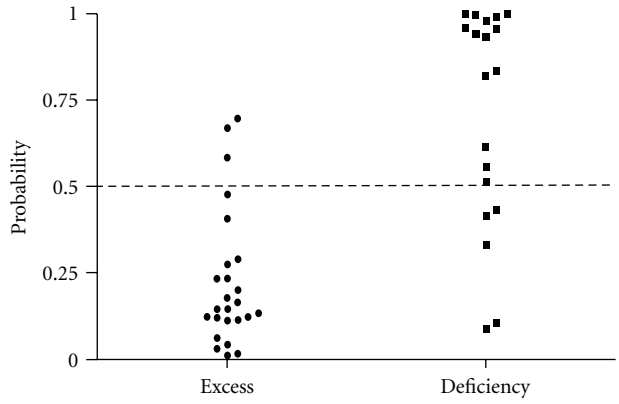


Figure 4: Diagnostic potential of the two marker proteins (m/z 4187 and m/z 5032) using binary logistic regression method with the data from different TCM syndromes in CHB patients. 88% of excess syndrome patients and 73.7% of deficiency syndrome patients were correctly discriminated (cutoff value: 0.5).

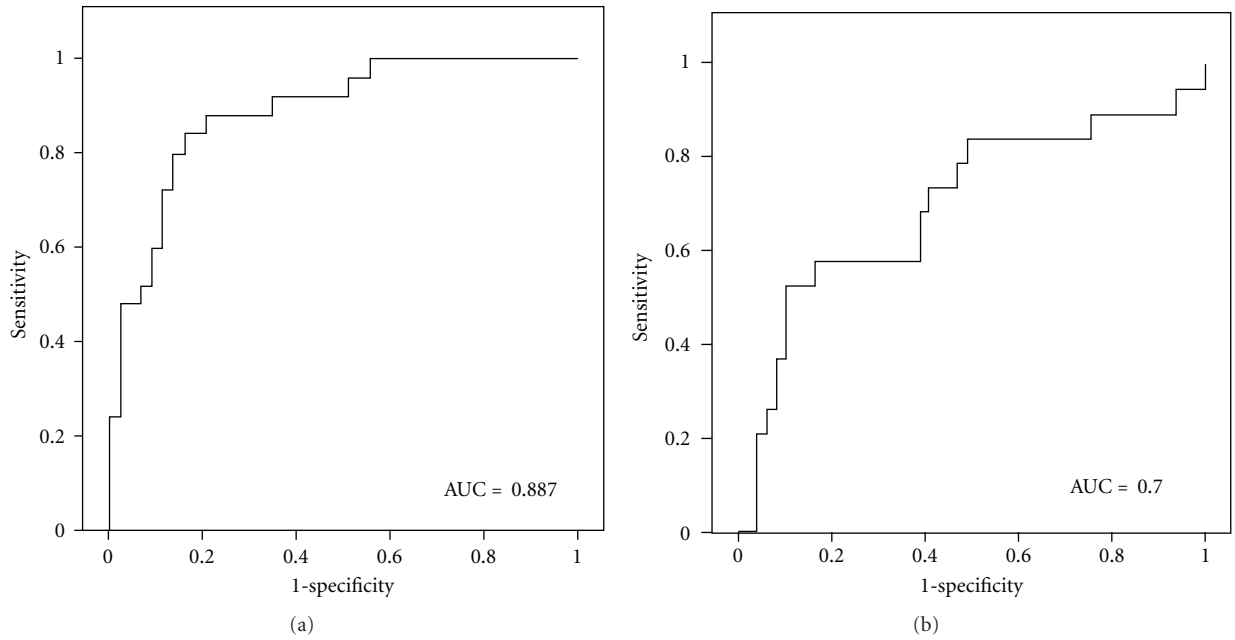


Figure 5: ROC curve for classification of two different TCM syndromes in CHB patients. It was generated combining the peak values of m/z 4187 and m/z 5032. (a) ROC curve for classification of excess syndrome and non-excess syndrome. AUC (area under the curve) = 0.887. (b) ROC curve for classification of deficiency syndrome and nondeficiency syndrome. AUC = 0.700.

28 significantly different ones between healthy controls and deficiency syndrome might be the potential biomarkers for CHB disease and deficiency syndrome (Table 3). And 9 common proteins (marked in bold in Table 3) were supposed to represent the potential biomarkers between CHB and healthy group, which should be eliminated from those significantly different proteins between excess syndrome and deficiency syndrome. However, these 9 proteins were totally different with those 4 ones found when comparing between TCM syndrome groups. So it was demonstrated that the interference of diseases to biomarkers had been eliminated.

Most importantly, this study is the first time to classify TCM syndromes in CHB patients by an objective and scientific approach instead of a subjective and experiential one. Our work found the characteristic markers in biochemistry associated with specific TCM syndromes and it will facilitate the development of syndrome classification. Also, it provides an important direction for the understanding and acceptance of TCM theory all around the world. Furthermore, the incorporation of SELDI-based ProteinChip technology into TCM syndrome classification will lead to a new era in the development of TCM to improve treatment efficacy. Our

Table 3: Significantly different peaks between healthy controls and excess or deficiency syndromes.[a]

m/z	Healthy controls	Excess or deficiency syndrome	Change[b]	*P* value
Healthy control versus excess syndrome				
1174	0.20 ± 0.49	0.69 ± 1.17	↑	0.011
2037	**1.71 ± 0.71**	**3.10 ± 2.24**	↑	**0.010**
2269	**0.86 ± 0.38**	**1.62 ± 1.25**	↑	**0.011**
2592	0.49 ± 0.27	−0.04 ± 0.34	↓	0.002
3203	2.38 ± 1.22	0.13 ± 0.42	↓	0.001
3408	1.71 ± 0.80	0.21 ± 0.51	↓	0.000
4104	**32.07 ± 13.66**	**11.11 ± 6.43**	↓	< 0.001
4187	11.66 ± 4.61	4.08 ± 3.33	↓	0.004
429	7.98 ± 2.88	3.64 ± 2.53	↓	0.001
4311	4.79 ± 1.46	1.97 ± 2.70	↓	< 0.001
5032	−0.05 ± 0.33	0.48 ± 0.56	↑	< 0.001
5497	**1.75 ± 0.79**	**0.37 ± 0.39**	↓	< 0.001
5650	**18.09 ± 6.50**	**9.10 ± 5.46**	↓	**0.001**
7027	2.10 ± 0.75	3.33 ± 1.97	↑	0.007
7587	1.19 ± 0.45	2.07 ± 1.43	↑	0.015
11732	**0.52 ± 0.26**	**1.04 ± 0.68**	↑	**0.001**
14070	0.55 ± 0.23	0.98 ± 0.65	↑	0.008
15167	1.95 ± 1.45	4.50 ± 4.42	↑	0.002
15354	0.49 ± 0.43	1.22 ± 1.24	↑	0.002
22862	**1.09 ± 0.63**	**2.31 ± 1.47**	↑	< 0.001
23481	**2.25 ± 1.36**	**4.78 ± 2.65**	↑	< 0.001
28118	1.72 ± 0.65	2.31 ± 1.02	↑	0.013
33516	0.13 ± 0.28	0.37 ± 0.68	↑	0.004
38571	0.04 ± 0.02	0.08 ± 0.08	↑	0.005
38814	0.04 ± 0.02	0.08 ± 0.08	↑	0.007
46804	**0.03 ± 0.03**	**0.09 ± 0.08**	↑	< 0.001
47818	0.02 ± 0.01	0.04 ± 0.04	↑	0.015
Healthy control versus deficiency syndrome				
1074	0.49 ± 0.48	0.13 ± 0.37	↓	0.013
1210	1.89 ± 1.42	2.64 ± 1.02	↑	0.006
1216	6.03 ± 3.50	9.26 ± 3.39	↑	0.002
1261	21.14 ± 9.18	29.16 ± 10.80	↑	0.014
1440	0.79 ± 1.03	1.36 ± 0.95	↑	0.004
2003	1.97 ± 1.04	4.32 ± 2.74	↑	<0.001
2018	6.92 ± 3.50	13.81 ± 7.86	↑	0.001
2037	**1.71 ± 0.81**	**3.62 ± 2.51**	↑	**0.001**
2269	**0.86 ± 0.47**	**1.93 ± 1.08**	↑	< 0.001
3331	3.78 ± 2.69	6.43 ± 3.33	↑	0.006
4104	**32.07 ± 18.72**	**16.01 ± 11.01**	↓	**0.005**
5260	0.80 ± 1.44	1.96 ± 1.79	↑	0.004
5346	9.05 ± 13.75	21.53 ± 15.00	↑	0.007
5497	**1.75 ± 1.37**	**0.49 ± 0.50**	↓	**0.001**
5558	0.97 ± 1.40	1.85 ± 1.32	↑	0.007
5650	**18.09 ± 10.43**	**10.39 ± 7.67**	↓	**0.015**
5919	23.56 ± 16.61	40.84 ± 22.76	↑	0.014
5947	2.98 ± 3.47	6.79 ± 5.24	↑	0.010
6128	4.83 ± 5.95	9.72 ± 6.92	↑	0.003
8176	2.82 ± 2.70	4.31 ± 2.73	↑	0.010
9723	0.37 ± 0.31	0.83 ± 0.51	↑	0.002
10292	1.17 ± 0.98	2.40 ± 1.22	↑	0.001

Table 3: Continued.

m/z	Healthy controls	Excess or deficiency syndrome	Change[b]	*P* value
11732	**0.52 ± 0.36**	**0.90 ± 0.42**	↑	**0.004**
15009	0.07 ± 0.09	0.60 ± 1.86	↑	0.002
22572	0.30 ± 0.23	0.93 ± 1.18	↑	<0.001
22862	**1.09 ± 0.61**	**2.39 ± 1.35**	↑	< 0.001
23481	**2.25 ± 1.53**	**4.74 ± 2.62**	↑	< 0.001
46804	**0.03 ± 0.02**	**0.08 ± 0.06**	↑	< 0.001

[a]Protein peaks marked in bold were the common biomarkers for CHB disease. [b] "↑" and "↓" represent the protein was up- and downregulated in CHB patients compared with the control, respectively.

researched results also suggest that TCM syndromes really have their own biological fundament.

5. Conclusion

The SELDI-based proteomics found some promising protein profiles and potential biomarkers to classify excess and deficiency syndromes in CHB patients, and it provided an evidence for objective TCM syndrome classification. However, there also exist some limitations in the study, such as the small amount of study population and lack of identification of candidate biomarkers, which would be researched in future study.

Acknowledgments

This study was supported by the National Science and Technology Major Project of China (nos. 2012ZX10005001-004 and no. 2009ZX09311-003), Leading Academic Discipline Project of Shanghai Municipal Education Commission (no. J50301), and E-Institutes of Shanghai Municipal Education Commission (no. E 03008). Research Center for Traditional Chinese Medicine Complexity System and Shanghai Shuguang Hospital are contributed equally.

References

[1] W. Y. Jiang, "Therapeutic wisdom in traditional Chinese medicine: a perspective from modern science," *Trends in Pharmacological Sciences*, vol. 26, no. 11, pp. 558–563, 2005.

[2] Y. Liu, P. Liu, R. Dai et al., "Analysis of plasma proteome from cases of the different traditional Chinese medicine syndromes in patients with chronic hepatitis B," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 59, no. 1, pp. 173–178, 2012.

[3] W. Xu, L. Q. Su, and H. J. Li, "Research advances and clinical evaluation of drugs for hepatitis B," *Chinese Journal of Hospital Pharmacy*, vol. 28, no. 9, pp. 737–739, 2008.

[4] A. P. Lu, H. W. Jia, C. Xiao, and Q. P. Lu, "Theory of traditional chinese medicine and therapeutic method of diseases," *World Journal of Gastroenterology*, vol. 10, no. 13, pp. 1854–1856, 2004.

[5] A. Zuberovic, M. Wetterhall, J. Hanrieder, and J. Bergquist, "CE MALDI-TOF/TOF MS for multiplexed quantification of proteins in human ventricular cerebrospinal fluid," *Electrophoresis*, vol. 30, no. 10, pp. 1836–1843, 2009.

[6] N. Johannesson, L. Olsson, D. Bäckström, M. Wetterhall, R. Danielsson, and J. Bergquist, "Screening for biomarkers in plasma from patients with gangrenous and phlegmonous appendicitis using CE and CEC in combination with MS," *Electrophoresis*, vol. 28, no. 9, pp. 1435–1443, 2007.

[7] R. K. T. Kam, T. C. W. Poon, H. L. Y. Chan, N. Wong, A. Y. Hui, and J. J. Y. Sung, "High-throughput quantitative profiling of serum N-glycome by MALDI-TOF mass spectrometry and N-glycomic fingerprint of liver fibrosis," *Clinical Chemistry*, vol. 53, no. 7, pp. 1254–1263, 2007.

[8] G. Zhou, H. Li, D. DeCamp et al., "2D differential in-gel electrophoresis for the identification of esophageal scans cell cancer-specific protein markers.," *Molecular & Cellular Proteomics*, vol. 1, no. 2, pp. 117–124, 2002.

[9] T. W. Hutchens and T. T. Yip, "New desorption strategies for the mass- spectrometric analysis of macromolecules," *Rapid Communications in Mass Spectrometry*, vol. 7, no. 7, pp. 576–580, 1993.

[10] L. Chen, D. W. Y. Ho, N. P. Y. Lee et al., "Enhanced detection of early hepatocellular carcinoma by serum SELDI-TOF proteomic signature combined with alpha-fetoprotein marker," *Annals of Surgical Oncology*, vol. 17, no. 9, pp. 2518–2525, 2010.

[11] C. Liu, C. Pan, H. Wang, and L. Yong, "Effect of surface-enhanced laser desorption/ionization time-of-flight mass spectrometry on identifying biomarkers of laryngeal carcinoma," *Tumor Biology*, pp. 1–7, 2011.

[12] N. Fujita, M. Nakanishi, J. Mukai et al., "Identification of treatment efficacy-related host factors in chronic hepatitis C by proteinchip serum analysis," *Molecular Medicine*, vol. 17, no. 1-2, pp. 70–78, 2011.

[13] W. F. Leemans, H. L. A. Janssen, and R. A. de Man, "Future prospectives for the management of chronic hepatitis B," *World Journal of Gastroenterology*, vol. 13, no. 18, pp. 2554–2567, 2007.

[14] D. Lavanchy, "Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention," *Journal of Clinical Virology*, vol. 34, no. 1, pp. S1–S3, 2005.

[15] D. C. Valla, R. De Franchis, A. Hadengue et al., "EASL International Consensus Conference on Hepatitis B. 13-14 September, 2002 Geneva, Switzerland. Consensus statement (long version)," *Journal of Hepatology*, vol. 39, no. S1, pp. S3–S25, 2003.

[16] S. L. Wang, N. L. Yao, and W. L. Lv, "Advances in studies on effect superiorities of traditional Chinese medicine on chronic hepatitis B," *China Journal of Chinese Materia Medicasource*, vol. 32, no. 23, pp. 2468–2470, 2007.

[17] L. L. Tang, J. F. Sheng, C. H. Xu, and K. Z. Liu, "Clinical and experimental effectiveness of Astragali compound in the treatment of chronic viral hepatitis B," *Journal of International Medical Research*, vol. 37, no. 3, pp. 662–667, 2009.

[18] B. E. Wang, "Treatment of chronic liver diseases with traditional Chinese medicine," *Journal of Gastroenterology and Hepatology*, vol. 15, pp. E67–E70, 2000.

[19] Chinese Society of Hepatology and Chinese Society of Infectious Diseases, *Guide to Prevention and Treatment of Chronic Hepatitis B*, 2005.

[20] "The standards of TCM differential syndromes of viral hepatitis," Defined in December 1991 by Internal Medicine Hepatopathy Committee of Chinese Traditional Medicine Association.

[21] C. J. Hogeboom, K. J. Sherman, and D. C. Cherkin, "Variation in diagnosis and treatment of chronic low back pain by traditional Chinese medicine acupuncturists," *Complementary Therapies in Medicine*, vol. 9, no. 3, pp. 154–166, 2001.

[22] B. Zell, J. Hirata, A. Marcus, B. Ettinger, A. Pressman, and K. M. Ettinger, "Diagnosis of symptomatic postmenopausal women by traditional Chinese medicine practitioners," *Menopause*, vol. 7, no. 2, pp. 129–134, 2000.

[23] J. F. Cui, Y. K. Liu, H. J. Zhou et al., "Screening serum hepatocellular carcinoma-associated proteins by SELDI-based protein spectrum analysis," *World Journal of Gastroenterology*, vol. 14, no. 8, pp. 1257–1262, 2008.

[24] C. Matsumoto, T. Kojima, K. Ogawa et al., "A proteomic approach for the diagnosis of 'Oketsu' (blood stasis), a pathophysiologic concept of Japanese traditional (Kampo) medicine," *Evidence-Based Complementary and Alternative Medicine*, vol. 5, no. 4, pp. 463–474, 2008.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 496575, 7 pages
doi:10.1155/2012/496575

Research Article

Comparative Study of TCM Syndrome Scale for Liver Disease and Chronic Liver Disease Questionnaire Based on Assessment of Posthepatitic Cirrhosis

Hua Zhang,[1] Hua Lv,[2] Pin-Xian Huang,[3] Yan Lin,[4] Xin-Cai Hu,[4] and Ping Liu[5]

[1] *Key Laboratory of Liver and Kidney Diseases (Ministry of Education), Institute of Liver Diseases, Shuguang Hospital-Shanghai University of Traditional Chinese Medicine, 528 Zhangheng Road, Shanghai 201203, China*
[2] *Center for Clinical Effect Evaluation, Shuguang Hospital-Shanghai University of Traditional Chinese Medicine, 528 Zhangheng Road, Shanghai 201203, China*
[3] *Department of Preventive Medicine, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China*
[4] *Institute of Liver Diseases, Shuguang Hospital-Shanghai University of Traditional Chinese Medicine, 528 Zhangheng Road, Shanghai 201203, China*
[5] *E-Institute of Traditional Chinese Internal Medicine, Shanghai Municipal Education Commission, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China*

Correspondence should be addressed to Ping Liu, liuliver@vip.sina.com

Received 14 February 2012; Accepted 1 April 2012

Academic Editor: Shi-Bing Su

Objective. To compare and analyze the relevance and applied value of chronic liver disease questionnaire (CLDQ) and Traditional Chinese Medicine liver disease questionnaire (TCMLDQ) in patients with posthepatitic cirrhosis. *Methods*. The data of 146 patients' scales of CLDQ and TCMLDQ which based on the characteristics of chinese medical symptoms were collected. We made comparative analysis of the relationship between these two scales by the linear regression model and canonical correlation method and evaluated the advantages and disadvantages of two scales about its items setting and dimension definition. *Result*. There is a negative correlation in total scores between the two scales and the linear regression equation: CLDQ = 239.38 − 1.232TCMLDQ. The further canonical correlation analysis was used to analyze the two extracted canonical correlative variables with significances ($P < 0.05$), and the results showed that the overall negative correlation between the two scales mainly came from contributions of both the four dimensions of TCMLDQ (CS, GSYX, GYPX, and OS) and the five dimensions of CLDQ (AS, FA, SS, AC, and EF). *Conclusion*. These two scales have good consistency in the evaluation of severity and life quality of liver cirrhosis patients, so we suggested that TCMLDQ can be used to evaluate the severity and life quality of patients with posthepatitic cirrhosis.

1. Background

The questionnaire widely used for assessment of quality of life has been considered as an effective method for quantification, objectification, and standardization of clinical data by World Health Organization, widely recognized by experts, which could be also introduced into the study on quantification of Traditional Chinese Medicine (TCM) symptoms and signs [1]. But how to make the scale design in accordance with TCM theory and its thinking ways accepted by domestic and foreign counterparts and well applied is the key problem to be solved. With selected patients of posthepatitic cirrhosis as research subjects, referring to the basic ideas from assessment quality of life questionnaire, combining with clinical practice and the results based on the study of laws of symptoms and signs classification [2], our task group had preliminarily established TCM liver disease questionnaire (TCMLDQ). Then, through the assessment of both patients and healthy people, TCMLDQ had been confirmed with high reliability, validity, and good sensitivity.

TCM syndromes are the conclusions to the current pathological state of disease made on the basis of synthesis and analysis of information (the body's own feelings and the external appearance) obtained by doctor through the four examinations—inspection, hearing and smelling, inquiring, and palpating. This puts emphasis on the role of individual subjective symptoms in the individualized process of occurrence, development, diagnosis, and treatment of the disease, grasping life and health overall, which has common characteristics with quality of life assessment questionnaire, in order to reflect the advantages and thinking ways of the design of TCM questionnaire and discuss the value and significance of the questionnaire in life quality assessment. In this study, linear regression and canonical correlation analysis methods were used to analyze the comparison of self-developed TCMLDQ and internationally accepted chronic liver disease questionnaire [3] (CLDQ) to explore the relevance between two questionnaires in the evaluation of patient's quality of life and subjective clinical information and provide evidence for recognition and application in counterparts.

2. Materials and Methods

2.1. Questionnaire

2.1.1. CLDQ (Chinese Version) (See [4]). The questionnaire consists of 6 major categories, 29 questions, and six dimensions as fatigue (FA), activity (AC), emotional function (EF), abdominal symptoms (ASs), systemic symptoms (SSs), and worry (WO) (Table 1). Severities ranged from very serious to no symptoms are divided into 7 classes (1 to 7 points score), and the higher score means the higher quality of life.

2.1.2. TCMLDQ. The questionnaire was self-developed by task group, based on the entry pool constituted preliminary analysis of clinical data of 900 patients with posthepatitis cirrhosis [5]. By pretesting to a little portion of the patients, entries which are repeated, unclearly described, unreadable, or with frequency below 5% were modified or deleted. By reasoning with experts and referring to the CLDQ, TCMLDQ including 38 entries was formed, of which severities ranked from no symptoms to continuous lasting were divided into 7 class (1 to 7 points score), and the higher score indicated the more severe symptoms. By extracting the characteristics of property related to TCM syndromes (similarity analysis to the clinical data of 437 patients with posthepatitis cirrhosis), and combining with the clinical practice and ensuring the uniqueness of the dimension of each entry, five dimensions were classified as common syndromes (CSs, which show commonalities of disease), yin deficiency of liver and kidney (GSYX), yang deficiency of spleen and kidney (PSYX), liver depression, and spleen deficiency (GYPX) and the other syndromes (OSs, symptoms which have no specificities for classification of syndromes) (Table 1).

2.1.3. Evaluating Method for Questionnaire. TCMLDQ and CLDQ were evaluated simultaneously. The investigators are trained in the same way and to unify filling methods and clarify requirement. The two questionnaires are all self-rating scale completed by the patients themselves, and the investigators had given the necessary guidance and instructions to the patients. Score points were marked according to the scoring instruction.

2.2. Clinical Data. All patients were outpatients and inpatients from Shuguang Hospital and Longhua Hospital affiliated to Shanghai University of Traditional Chinese Medicine, Putuo District Center Hospital, and the Shanghai Public Health Clinical Center during the period from 2007 to 2008.

2.2.1. Recruitment

Inclusion Criteria. These include (1) patients who meet the diagnostic criteria of liver cirrhosis (according to "Guide to prevention and treatment of chronic hepatitis B" [6] revised by Liver Diseases Institute, Infectious Diseases institute of Chinese Medical Association in 2005), age 18 to 70 years old, male or female; (2) patient's willingness to participate in scale tests; they can fully understand the significance of scale in all the entries; (3) no previous mental illness history and other psychosomatic disease currently.

Exclusion Criteria. These include (1) patients complicated with severe diseases of heart, brain, kidney, lung, endocrine, and hematopoietic system; patients complicated with liver cancer and other serious hepatobiliary diseases and mental illness; (2) patients complicated II degree or above hepatic encephalopathy and severe spontaneous bacterial peritonitis, gastrointestinal bleeding, and hepatorenal syndrome; (3) unclear history of viral infection and other liver diseases related with alcohol, drug, genetic, autoimmune, and so on; (4) women in the period of pregnancy or lactation.

2.2.2. Collection of Clinical Information. A total of 146 patients (average age 46.54 ± 12.54 years) with posthepatitic cirrhosis had been adopted, including 76 inpatients and 70 outpatients; 105 males (average height 171.99 ± 5.25 cm, average weight 67.00 ± 10.82 Kg) and 41 female (average height 159.85 ± 3.96 cm, average weight 59.58 ± 8.85 Kg); 25 cases with a past history of upper gastrointestinal track bleeding; 72 cases with a history of ascites; 77 cases with child-pugh A grade; 45 cases with child-pugh B grade; 24 cases with child-pugh C grade (Table 2).

2.3. Statistical Methods. With SPSS17.0 statistical package, the reliability and validity of the TCMLDQ were analyzed by using Cronbach's α-coefficient and factor analysis. We carried out an analysis for dependencies between total scores of two scales by using linear regression analysis and introduced the canonical correlation analysis into studying correlation of the two sets of variables (i.e., two scales consisting of different dimensions) and giving a quantitative description of the correlation between two scales.

Table 1: The questionnaire dimensionality consists of TCMLDQ and CLDQ.

Dimensionality	Variable	Items	Questions
CLDQ total score	CLDQ	29	AS + FA + SS + AC + EF + WO
Abdominal symptoms (ASs)	$Y1$	3	1, 5, 17
Fatigue (FA)	$Y2$	5	2, 4, 8, 11, 13
Systemic symptoms (SSs)	$Y3$	5	3, 6, 21, 23, 27
Activity (AC)	$Y4$	3	7, 9, 14
Emotional function (EF)	$Y5$	8	10, 12, 15, 16, 19, 20, 24, 26
Worry (WO)	$Y6$	3	18, 22, 25, 28, 29
TCMLDQ total score	TCMLDQ	38	CS + GSYX + GYPX + PSYX + OS
CS	$X1$	18	1, 5, 17, 18, 20, 22, 24, 25, 26, 27, 28, 29, 30, 31, 35, 36, 37, 38
GSYX	$X2$	5	2, 3, 11, 12, 15
PSYX	$X3$	1	33
GYPX	$X4$	6	4, 6, 7, 8, 19, 34
OS	$X5$	8	9, 10, 13, 14, 16, 21, 23, 32

Table 2: The general information of patients with posthepatitic cirrhosis.

	Characteristic	Count	Proportion (%)
Patients source	Shuguang Hospital	78	53.42
	Longhua Hospital	56	38.36
	Putuo District Center Hospital	8	5.48
	Shanghai Public Health Clinical Center	4	2.74
Section	Outpatient/inpatient	70/76	47.95/52.05
Sex	Male	105	71.9
	Female	41	28.1
Age (years)	<40	14	9.58
	40–60	105	71.92
	≥60	27	18.49
Virus infection	Hepatitis B virus	143	97.95
	Hepatitis C virus	3	2.05
Splenectomy	Yes	16	10.95

3. Results

3.1. The Reliability and Validity of the TCMLDQ. This scale was tested by Cronbach's analysis the α-coefficient is 0.844 (more than 0.80), which shows that the internal consistency of entries is good and with high reliability; the assessment of structural validity of the scale was analyzed by factor analysis, the KMO and Bartlett's test showed that P value <0.01, so these data were fit for the factor analysis. According to whether the latent root being greater than 1, 14 factors were extracted from 38 entries; the accumulative contribution rate of total variance is 69.45%. The results show that the scale has good structural validity.

3.2. Linear Regression Analysis for Total Scores of TCMLDQ and CLDQ Scale. Linear regression analysis was carried out for total scores of 146 patients in two scales to establish regression equation (Table 3, Figure 1).

From Table 3, the linear regression equation can be drawn: $\widehat{\text{CLDQ}} = 239.38 - 1.232\text{TCMLDQ}$ shows that the total scale score between the two linear correlations was significantly negatively correlated.

We predicted the total score of CLDQ with that of TCMLDQ. Individual 95% confidence intervals is a statistic which reflects the prediction effect of regression equation. It has lower and upper bounds (two predicted total scores of CLDQ) for the prediction interval of the CLDQ for every

Table 3: Linear regression equation of total scores of TCMLDQ and CLDQ.

Model	Unstandardized coefficients		Standardized coefficients	t	P value	95% confidence interval for β	
	β	Std. error	Beta			Lower bound	Upper bound
Constant	239.38	6.750		35.462	0.000	226.039	252.724
TCMLDQ	−1.232	0.094	−0.737	−13.069	0.000	−1.418	−1.046

Note: dependent variable: CLDQ total score; TCMLDQ: TCMLDQ total score.

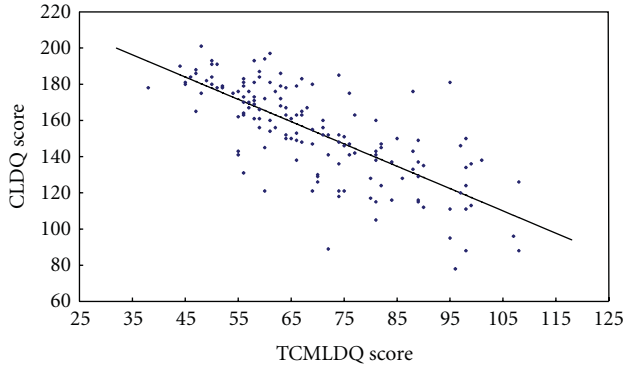


Figure 1: Linear regression plot of total scores of TCMLDQ and CLDQ.

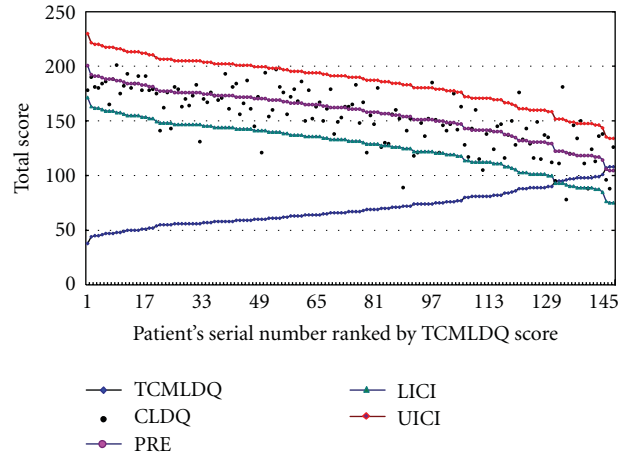


Figure 2: CLDQ total score, predicted values and individual 95% confidence intervals, and TCMLDQ total score line graph. Note: TCMLDQ: TCMLDQ actual measured total score; CLDQ: CLDQ actual measured total score; PRE: CLDQ scores predicted by TCMLDQ score; UICI: upper bounds of predicted CLDQ individual 95% confidence intervals; LICI: lower bounds of predicted CLDQ individual 95% confidence intervals.

single case. Through the equation, we can estimate every patient's individual 95% confidence interval of CLDQ and verify whether the actual observation of CLDQ falls in its individual 95% confidence interval. The result has shown that 91.8% of patient's measured values of the CLDQ fall in their corresponding intervals, which means there is a good consistency between TCMLDQ and CLDQ (Figure 2).

3.3. Canonical Correlation Analysis between TCMLDQ and CLDQ. We carried out canonical correlation analysis between two sets of dimensions, five dimensions of TCMLDQ as CS ($X1$), GSYX ($X2$), PSYX ($X3$), GYPX ($X4$), and OS ($X5$) and six dimensions of CLDQ as AS ($Y1$), FA ($Y2$), SS ($Y3$), AC ($Y4$), EF ($Y5$), and WO ($Y6$).

3.3.1. Correlation Analysis between Various Dimensions of TCMLDQ and CLDQ. In addition to having no correlation between $X3$ and $Y2$, $Y4$, $Y5$, $Y6$, TCMLDQ, and CLDQ, the results show negative correlations among the other dimensions ($P < 0.05$) (Table 4).

3.3.2. Extraction of Canonical Correlation Coefficient and Test. This is to discuss whether there is significant correlation in various canonical variables, that is to extract canonical correlation coefficients among canonical variables and carry out hypothesis testing for each pair of canonical correlation coefficients. The results show that there are five pairs of canonical correlation variables; first and second pairs have statistical significant correlation ($P < 0.05$), so these two pairs of canonical correlation variables are selected for analysis (Table 5).

3.3.3. Standardized Correlation Coefficients between Canonical Correlation Variables and Variables of X and Y Groups. These are Standardized correlation coefficients between U canonical correlation variables and various dimensions of TCMLDQ ($X1$ to $X5$), and between V canonical correlation variables and various dimensions of CLDQ ($Y1$ to $Y6$) (Table 6). The conversion formula of canonical correlation variable could be written according to 1st to 2nd pairs of canonical variables.

The formula reflects that the contribution of original variables on canonical variable is determined by the canonical correlation coefficients (i.e., canonical variable loads) between original variables and canonical variables, that is to say, the greater the load capacity, the more impacts on canonical variable by original variable. In accordance with contribution rate to the first pair canonical variable, the original variables follow in the order of $X4$, $X1$, $Y1$, $Y4$, and $Y5$, $Y2$, which means liver depression and spleen deficiency syndrome, common syndrome, and other syndrome in TCMLDQ have the largest contribution to the first pair of

Table 4: Correlation coefficients between various dimensions of TCMLDQ and CLDQ.

Dimensions	$Y1$	$Y2$	$Y3$	$Y4$	$Y5$	$Y6$
$X1$	−0.5426**	−0.5711**	−0.5904**	−0.5118**	−0.5578**	−0.3695**
$X2$	−0.2283**	−0.4891**	−0.3352**	−0.2653**	−0.2798**	−0.2001*
$X3$	−0.2171**	−0.0839	−0.2145**	−0.1552	−0.0502	−0.1244
$X4$	−0.6688**	−0.5714**	−0.4349**	−0.6409**	−0.4110**	−0.3430**
$X5$	−0.2416**	−0.3234**	−0.3532**	−0.2115*	−0.3203**	−0.1936*

** Correlation is significant at the 0.01 level (2 tailed).
* Correlation is significant at the 0.05 level (2 tailed).

Table 5: Canonical correlation coefficients of variables of TCMLDQ and CLDQ.

Canonical variable	Coefficient	Wilk's	Chi-square	df	P value
1 ($U1$ and $V1$)	0.812	0.212	215.318	30	0.000
2 ($U2$ and $V2$)	0.532	0.624	65.619	20	0.000
3 ($U3$ and $V3$)	0.324	0.870	19.418	12	0.079
4 ($U4$ and $V4$)	0.166	0.972	3.988	6	0.678
5 ($U5$ and $V5$)	0.027	0.999	0.103	2	0.950

Note: U ($U1$ to $U5$) stands for extracted canonical correlation variables from a group of X variables (TCMLDQ); V ($V1$ to $V5$) stands for extracted canonical correlation variables from Y (CLDQ).

Table 6: Standardized U and V of canonical correlation variables coefficient table.

Variable 1	Standardized correlation coefficients (U)					Variable 2	Standardized correlation coefficients (V)				
	$U1$	$U2$	$U3$	$U4$	$U5$		$V1$	$V2$	$V3$	$V4$	$V5$
$X1$	0.497	0.749	0.718	0.213	0.814	$Y1$	0.487	−0.674	0.190	0.111	−0.975
$X2$	0.038	0.474	0.972	0.522	0.211	$Y2$	0.222	0.563	−1.233	−0.119	−0.333
$X3$	0.054	0.122	0.453	0.932	0.030	$Y3$	0.094	0.712	0.653	−0.708	0.126
$X4$	0.639	1.056	0.423	0.158	0.341	$Y4$	0.296	−0.792	0.115	0.073	1.055
$X5$	0.135	0.324	0.282	0.314	1.157	$Y5$	0.244	0.438	0.321	1.115	−0.054
						$Y6$	−0.076	0.030	0.122	−0.588	0.372

(1) $U1 = 0.497X1 + 0.038X2 + 0.054X3 + 0.639X4 + 0.135X5$,
$V1 = 0.487Y1 + 0.222Y2 + 0.094Y3 + 0.296Y4 + 0.244Y5 - 0.076Y6$.
(2) $U2 = 0.749X1 + 0.474X2 + 0.122X3 + 1.056X4 + 0.324X5$,
$V2 = -0.674Y1 + 0.563Y2 + 0.712Y3 - 0.792Y4 + 0.438Y5 + 0.030Y6$.

extracted canonical correlation variable, while abdominal symptoms, activity, emotional function, and fatigue in CLDQ have the largest contribution to the second pair of extracted canonical correlation variable, and the original variables follow the order of $X4$, $X1$, $X2$, $X5$, and $Y4$, $Y3$, $Y1$, $Y2$, and $Y5$ (correlation coefficient greater than 0.2 [7]), which means liver depression and spleen deficiency syndrome, common syndrome, and yin deficiency syndrome of liver and kidney in TCMLDQ have the larger weight to the second pair of extracted canonical correlation variable, while activity, systematic symptoms, abdominal symptoms, fatigue, and emotional function in CLDQ have larger contribution.

4. Discussion

Due to the features of chronic liver diseases—long term, persistent, and recurrent—the therapeutic effects can not simply be evaluated by cure, improvement of laboratory makers, or restoration of normal function, and so forth, in clinic, so comprehensive evaluations of patients' subjective feeling and quality of life were needed. Rating scale or questionnaire is an effective tool for the assessment of respondents' subjective feelings. Subjective symptoms (i.e., the patient's self-feelings) are also the important factors in TCM syndrome differentiation process, which play a main role in identification of TCM syndromes and evaluation of TCM clinical efficacy. But so far, a set of objective methods and standards of evaluating therapeutic effect which can be in line with TCM laws have not been established by TCM. Therefore, Chinese version western scales such as SF-36 [8, 9] and CLDQ [4] were used in evaluation of chronic liver diseases.

However, the introduction of foreign scale to evaluate the quality of life of Chinese people may cause some misunderstandings due to different cultural background and living habits and could not achieve the goal of syndrome classification in the thinking way of traditional Chinese medicine.

For this reason, TCM scholars began referring to psychometric principles and methods to design questionnaires or scale. But no one of scales had gotten the recognition of counterparts in clinical practice. Therefore, we had tried to design TCMLDQ to meet TCM theory and way of thinking and reflect the symptom information and characteristics of syndromes clas-sification of posthepatitic cirrhosis, in order to achieve quan-titative assessment of TCM syndromes in posthepatitic cir-rhosis.

TCMLDQ involves a total of five dimensions and 38 entries, common symptoms include 18 entries—fatigue, hypochondriac pain, bitter mouth, halitosis, nausea, yellowish urine, loose stools, difficulty in falling asleep, easy to wake up, dreamfulness, nocturnal enuresis, irritability, depression, skin itching, edema, gum bleeding, epistaxis, and muscle bleeding; other symptoms include headache, dizziness, eye soreness, redden and swollen eyes and throat, dry mouth, belching, dry stool, and night sweating; yin deficiency syndromes of liver and kidney have backache, limb weakness, dry eyes, blurred vision, and tinnitus; liver depression and spleen deficiency syndrome consists of hypochondriac discomfort, abdominal distension, chest and hypochondriac distension, lower abdominal distension, anorexia, and heavy body and limbs; spleen-kidney yang deficiency includes syndrome of aversion to cold and cold limbs.

At the beginning of this century, CLDQ was introduced to evaluate quality of life and clinical effects for patients with chronic liver disease [10–12], and became a domestic and international accepted specific scale for chronic liver disease, which is used as a reference for the control study with TCMLDQ. CLDQ includes six dimensions and 29 questions. To test different aspects of life quality of patients with chronic liver diseases, its fatigue dimensions consist of sense of fatigue, daytime drowsiness, decreased physical strength, and so forth. Abdominal symptoms include abdominal distension, abdominal pain, abdominal discomfort; activity includes appetite, general weakness, and diet restriction; systemic symptoms include body pain, chest distress, shortness of breath, muscle cramps, dry mouth, and skin itching; emotional function dimension includes anxiety, unhappiness, depression, irritability, sleep disorders, and distraction; worry dimension mainly concentrates on patient's worry with the disease. The different dimensions or categories have a certain degree of overlap, of which the differences in individual experience had been fully taken into account.

CLDQ is used to evaluate the quality of life, and therefore the higher score means the higher quality of life and the milder symptoms. TCMLDQ is used to evaluate the severity of clinical symptoms; the higher score means the more severe symptoms. So considering the results of linear dependencies between total scores of the two scales indicated that there was a significantly negative correlated relationship between the two scales. According to linear relationship between the total score of the two scales, we use the total score of TCMLDQ as independent variables to predict the total score of CLDQ (dependent variable) and make a comparison between predicted and measured scores. The results indicated that the predicted and measured scores had a good match, and almost all observation points were in range of the upper and lower limits of the fitted values. It means that there is a good consistency between TCMLDQ and CLDQ in evaluating the severity of symptoms and quality of life of posthepatitis cirrhosis.

For further analyzing contribution degree of each dimension to overall correlation of the two scales, we introduced the canonical correlation analysis into study of the linear correlation between two scales. The canonical correlation analysis is used to study the correlation between two sets of multivariables and takes each group of variables as a whole rather than analyzing internal situation in each group of variables. It includes two groups of variables as a whole to find one or more comprehensive variables (linear combination of actual observed variables) to replace original variables, thereby turning the relationship between two sets of variables into the relationship of a few comprehensive variables (canonical variables), which can fully explore the related information between two groups of indicators.

Canonical correlation analysis was used to analyze the correlation between five dimensions in TCMLDQ and six dimensions in CLDQ. By analyzing the correlation of two groups' dimensions of intersection (interrelations in single dimension), in addition to spleen-kidney yang deficiency and fatigue, activity, emotional function, worry having no correlation, the other showed a negative correlation ($P < 0.05$). Further extracting five pairs of canonical correlation variables, the whole relationship of two groups of dimensions in two scales was analyzed; the overall negative linear correlation mainly comes from negative correlation between the four dimensions of TCMLDQ as common symptoms, yin deficiency syndromes of liver and kidney, liver depression and spleen deficiency syndrome, other symptoms, and five dimensions of CLDQ as abdominal symptoms, fatigue, systemic symptoms, activity, and emotional function (in order of the priority according to the contribution). However, dimension of spleen and kidney yang deficiency syndrome in TCMLDQ and dimension of worry in CLDQ have little or no significant contribution to the overall correlation between the two scales.

According to the entries and dimensions of two scales, it was believed that there are two aspects of the main factor leading to the results above. First, dimension of spleen-kidney yang deficiency syndrome in TCMLDQ has only one entry of "chills and cold limbs"; there is no such concepts of cold feeling in modern medicine, which are unique evaluation indictors of TCM. So there is no corresponding entry of dimension in CLDQ study, and it is reasonable that this dimension has no contribution to the negative correlation between the two scales. Second, we had a lack of attention on mental, social, and psychological factors in initially prepared TCMLDQ, did not set up the entries to judge the degree of anxiety, and only had two entries associated with irritability and depression. Thus, just like dimension of spleen-kidney yang deficiency syndrome, it is reasonable and realistic that this dimension has no contribution to the negative correlation between the two scales. Therefore, it can also be proved that canonical correlation analysis could be applied into comparison among dimensions of two different scales and

could be promoted in the comparison studies of scales in the future.

5. Conclusion

According to the results of comparisons between self-developed TCMLDQ and accepted CLDQ scale, TCMLDQ could cover most of the CLDQ's study. They are comparable in dimensions and consistent in the internal structure. That means they could explain and reflect each other to some extent, which had also confirmed that there was a certain rationality for the classification of TCM syndromes based on clinical practice. TCMLDQ described by TCM terms could reflect the quantification of TCM syndromes with TCM characteristics and could also replace CLDQ for the evaluation of severity and life quality of patients with chronic liver disease by continuous improvement and amendments. With improvement of TCM symptoms and signs scale and development and application of instruments and equipment such as tongue diagnosis and pulse-taking diagnosis, it will further improve the quality and level of TCM syndrome evaluation.

The study focused on analyzing the relationship between the two scales and aimed at laying the methodological foundation for international counterparts.

Acknowledgments

This research was sponsored by China 973 Project (National Key Basic Research and Development Program, 2006 CB 504800); Leading Academic Discipline of Hepatology of State Administration of TCM China (no. 2010sh); Innovative Research Team in Universities, Shanghai Municipal Education Commission (first stage); Shanghai Leading Academic Discipline Project (no. Y0302); Shanghai Key Laboratory of Traditional Chinese Clinical Medicine.

References

[1] H. Zhang and P. Liu, "Probe into the thinking and methods of quantification of TCM Syndromes," *Chinese Journal of Basic Medicine in Traditional Chinese Medicine*, vol. 15, no. 8, pp. 574–576, 2009.

[2] P. Liu, "Thinking and exploration of disease-syndrome-efficacy integrated research," *Acta Universitatis Traditionis Medicalis Sinensis Pharmacologiaeque Shanghai*, vol. 21, no. 1, pp. 4–6, 2007.

[3] Z. M. Younossi, G. Guyatt, M. Kiwi, N. Boparai, and D. King, "Development of a disease specific questionnaire to measure health related quality of life in patients with chronic liver disease," *Gut*, vol. 45, no. 2, pp. 295–300, 1999.

[4] Y.-Z. Ni, J.-X. Zhang, X.-H. Li et al., "Chronic liver disease patients health related quality of life of the scale evaluation," *Modern Rehabilitation*, vol. 5, no. 8, pp. 18–19, 2001.

[5] Q. Zhang, P. Liu, and H. W. Zhang, "Study on the patterns of TCM syndrome differentiation of 900 patients with posthepatitic cirrhosis," *Chinese Journal of Integrated Traditional and Western Medicine*, vol. 26, no. 8, pp. 694–697, 2006.

[6] Chinese Society of Hepatology and Chinese Society of Infectious Diseases, Chinese Medical Association, "The guideline of prevention and treatment for chronic hepatitis," *Chinese Hepatology*, vol. 10, pp. 348–357, 2005.

[7] T.-H. Xu and Q. Liu, *China Medical Statistics Encyclopedia, Volume of Multivariate Statistical*, People's Medical Publishing House, Beijing, China, 2004.

[8] Y. Yang, "Related factors for the quality of life of patients with chronic hepatitis B," *Chinese Journal of Clinical Rehabilitation*, vol. 8, no. 24, pp. 4960–4961, 2004.

[9] G. C. Wu, W. P. Zhou, Y. R. Zhao et al., "Long-term health-related quality of life in chronic hepatitis B patients," *Chinese Journal of Hepatology*, vol. 11, no. 5, pp. 275–277, 2003.

[10] W.-D. Liu, J.-Z. Liu, and L.-H. Xin, "Chronic hepatitis patients for clinical comprehensive treatment the influence on the quality of survival," *Modern Jorunal of Integrated Traditional Chinese and Western Medicine*, vol. 13, no. 12, pp. 1574–1575, 2004.

[11] C.-H. Wu, Q.-W. Deng, X.-S. Ji et al., "Preliminary use of the CLDQ in chronic hepatitis B patients," *Chinese Journal of Clinical Psychology*, vol. 11, no. 1, pp. 60–62, 2003.

[12] X.-F. Guo, Q. Guo, W.-H. Lv et al., "Primary application of Chronic Liver Disease Questionnaire in HBsAg carriers," *Jilin Medical Journal*, vol. 31, no. 4, pp. 1472–1474, 2010.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 247012, 9 pages
doi:10.1155/2012/247012

Research Article

Nonlinear Analysis of Auscultation Signals in TCM Using the Combination of Wavelet Packet Transform and Sample Entropy

Jian-Jun Yan,[1] Yi-Qin Wang,[2] Rui Guo,[2] Jin-Zhuan Zhou,[1] Hai-Xia Yan,[2] Chun-Ming Xia,[1] and Yong Shen[1]

[1] *Center for Mechatronics Engineering, East China University of Science and Technology, Shanghai 200237, China*
[2] *Syndrome Laboratory of TCM, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*

Correspondence should be addressed to Jian-Jun Yan, jjyan@ecust.edu.cn and Yi-Qin Wang, wangyiqin2380@sina.com

Received 12 January 2012; Accepted 28 March 2012

Academic Editor: Shao Li

Auscultation signals are nonstationary in nature. Wavelet packet transform (WPT) has currently become a very useful tool in analyzing nonstationary signals. Sample entropy (SampEn) has recently been proposed to act as a measurement for quantifying regularity and complexity of time series data. WPT and SampEn were combined in this paper to analyze auscultation signals in traditional Chinese medicine (TCM). SampEns for WPT coefficients were computed to quantify the signals from qi- and yin-deficient, as well as healthy, subjects. The complexity of the signal can be evaluated with this scheme in different time-frequency resolutions. First, the voice signals were decomposed into approximated and detailed WPT coefficients. Then, SampEn values for approximated and detailed coefficients were calculated. Finally, SampEn values with significant differences in the three kinds of samples were chosen as the feature parameters for the support vector machine to identify the three types of auscultation signals. The recognition accuracy rates were higher than 90%.

1. Introduction

TCM is considered a unique medical system because of its basic theories describing the physiology and pathology of the human body, disease etiology, diagnosis, and differentiation of symptom complexes. The zang-fu organs, according to TCM theories, comprise the core of the human body as an organic entity in which tissues and sense organs are connected through a network of channels and collaterals (blood vessels). In traditional Chinese medicine the zang and fu organs more importantly represent the generalization of the physiology and pathology of certain systems of the human body instead of simply anatomical substances, but Zang fu is comprised of the five zang and six fu organs. The five zang include heart, liver, spleen, lung, and kidney. The six Fu are the gallbladder, stomach, large intestine, small intestine, bladder, and triple burner. When one falls ill, a dysfunction in the zang-fu organs may be reflected on the body's surface through the channels and their collaterals. At the same time, diseases involving body surface tissues may also affect their related zang or fu organs. Furthermore, the affected zang or fu organs may influence each other through internal connections [1]. In addition, auscultation, one of the auscultation and olfaction methods in TCM diagnosis, is used to detect vocal changes reflecting the functional activities of zang-fu organs and abundance or decline of the qi, blood, and body fluid.

Auscultation was clearly illustrated as early as in the Internal Classic of Huang Di [2], which provided the theoretical basis for clinical diagnosis in terms of listening to the vocal change. However, complete acoustic diagnostic methods have not been formulated. After the Ming and Qing Dynasties, auscultation gradually attracted the attention of the medical field with both theoretical content and clinical application considerably developed. Thus, a considerable distinctive step-by-step diagnostic method was formed. People around the world made substantial progress in the objective research of auscultation in the recent years with the development of computer and signal processing technology.

Mo made a frequency spectral analysis on the voice of cough patients using digital sonograph [3]. Wang and Yan performed a number of studies on the nonlinearity

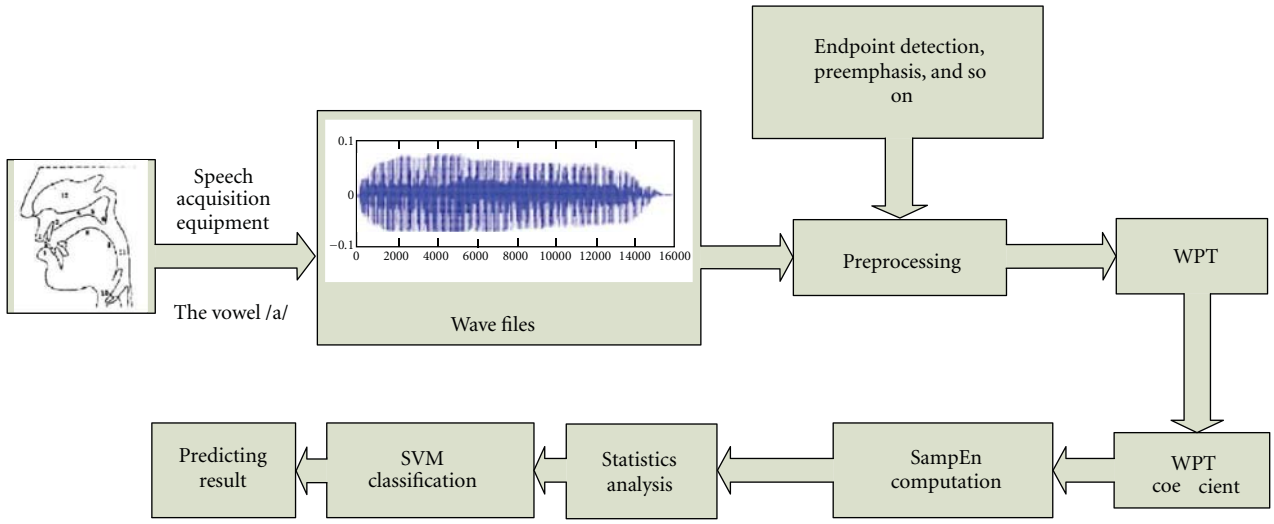


Figure 1: Analytic process of auscultation signals.

of the vowel /a/ signals of healthy persons and patients with deficiency syndrome by applying delay vector variance [4, 5]. These studies were effective attempts on the objective auscultation research. Chiu et al. proposed four novel acoustic parameters, such as the average number of zero crossings, variations in local peaks and valleys, variations in first and second formant frequencies, and the spectral energy ratio, to analyze and identify the characteristics among non-, qi-, and yin-deficient subjects [6].

There are several other studies on auscultation around the world [7–11]. These methods have provided a good basis for objective auscultation in clinical diagnosis. However, auscultation signal analysis and recognition are still in the initial stage. The experiment are conducted on a small sample database. Thus the recognition is not satisfactory such that further investigation is necessary to be carried out based on these studies.

The variations in energy imply corresponding changes in signal characteristics considering the changes in the normal and abnormal voice signals corresponding with the changes in the spatial distribution of the voice signal energy. In other words, the different signal frequency components can represent the different physical properties of the measured signal [12, 13]. Compared with the traditional Fourier transform time-frequency analytical method, the wavelet transform (WT) can reveal more information on signals based on multiscale and multiresolution decomposition. Wavelet packets have recently been applied to analyse auscultation signals because of their capability of partitioning both low- and high-band frequencies unlike the WT that often fails to capture accurately high-frequency information [14–16].

Both approximate entropy (ApEn) and sample entropy (SampEn) can represent the signal complexity which can be used in many biomedical fields. ApEn was proposed by Pincus and Goldberg [17] to compute the quantitative information for the experimental data. However, there are some weak points in the ApEn computation process because its computation in irregular times is affected by a bias, in addition to the inconsistency of ApEn in some cases. SampEn, compared with ApEn, does not count self-matches and shows better relative consistency and less dependence on data length.

Daubechies 4 (db4) wavelet is selected in this paper as the wavelet packet function to decompose the auscultation signals into 5-level wavelet packet coefficients. Then, SampEn is proposed as a feature parameter extracted from these coefficients to analyze quantitatively the auscultation signals. Furthermore, statistical analysis is conducted to obtain the effective feature parameters with significant differences for the recognition of the voice signals. Finally, these feature values are used as input vectors of the support vector machine (SVM) classifier for automatic identification for qi- and yin-deficient, as well as healthy, subjects.

2. Materials and Methods

Feature parameters of auscultation signals were extracted using a combined WPT and SampEn (Figure 1). Traditional signal processing methods, including the Fourier transform (FT), fast Fourier transform (FFT), and short-time Fourier transform (STFT), cannot reveal the nonlinear information contained in the nonstationary signal. The non-linear information of the auscultation signal can be extracted under different time-frequency resolutions with this scheme.

2.1. WPT. Wavelets are generally well crafted to have specific properties that make them available for signal processing. WT has the capability of time-frequency analysis and can draw different frequency bands of the signal. However, with increasing scale, the higher the space resolution ratio of the wavelet functions, the lower the frequency resolution ratio will be. This phenomenon is a drawback of the wavelet function. WPT was developed to adapt the underlying wavelet bases to the contents of a signal. The basic idea

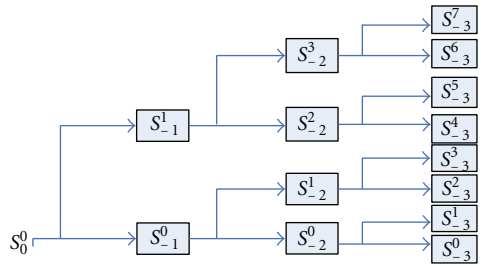


Figure 2: Wavelet packet decomposition tree.

is to allow subband decomposition to select adaptively the best basis for a particular signal. The WPT characteristic of narrowing wide window of frequency spectrum with increasing scale overcomes the shortcoming of the WT.

Given a finite energy signal whose scaling space is assumed as S_0^0, WPT can decompose S_0^0 into small subspaces S_j^n in a dichotomous way (Figure 2).

S_j^{n-1} shows the nth subspace in the jth resolution level.

The dichotomous way is realised by the following recursive scheme:

$$S_{j+1}^n = S_j^{2n} \oplus S_j^{2n+1}, \quad j \in Z; n \in Z_+, \tag{1}$$

where $j \le 0$ is the resolution level and $\oplus$ denotes orthogonal decomposition. S_{j+1}^n, S_j^{2n}, and S_j^{2n+1} are three close spaces corresponding to $S_n(t)$, $S_{2n}(t)$, and $S_{2n+1}(t)$, respectively. $S_n(t)$ satisfies the following equations:

$$\begin{aligned} S_{2n}(t) &= \sqrt{2}\sum_{k\in Z} h(k) S_n(2t-k), \\ S_{2n+1}(t) &= \sqrt{2}\sum_{k\in Z} g(k) S_n(2t-k), \end{aligned} \tag{2}$$

where $h(k)$ and $g(k)$ are the coefficients of the low- and the high-pass filters, respectively. The sequence of function $\{S_n\}(n = 0, 1, \ldots, \infty)$ generated from a given function S_0 is called the wavelet packet basis function.

The voice signal is a kind of transient, non-stationary, and random signal. Therefore, db wavelets have been widely implemented because of their advantage in matching the transient components in voice signals. Moreover, another main issue in wavelet analysis is the vanishing moment determined by trial-and-error methods. More points that can be neglected will emerge in the high frequencies if the degree of vanishing moment increases. Therefore, db wavelets with vanishing moments of 4, 6, 8, and 10 were chosen to decompose and reconstitute the voice signals in this study. The db4 wavelet function was selected after analysing the different effects of the wavelet functions to decompose and reconstitute the voice signals because the rate of decay and less point can be neglected.

The signal is decomposed into two subbands in the first level, namely, low- and high-frequency sub-bands. Then, the low-frequency subbands are further decomposed into lower- and higher-frequency parts in the following level, which was also performed in the high-frequency sub-bands. The same decomposition goes on repeatedly. Then, frequency sub-bands can be partitioned to be consistent with the signal features.

2.2. SampEn. SampEn examines time series for similar epochs and assigns a nonnegative number to the sequence, with larger values corresponding to greater complexity or irregularity in the data [18]. Self-matches in the SampEn algorithm are not included in calculating the probability, in contrast to the ApEn algorithm. The time series and similar patterns in parameter m and tolerance window r are used as two input parameters, which must be set before computation. For a time series $x(n)$, N is the length of the time series. SampEn (m, r, N) is computed as follows [18].

(1) The m vectors $X_m(1), \ldots, X_m(N-m+1)$ defined by $X_m(i) = [x(i), x(i+1), \ldots, x(i+m-1)]$, for $1 \le i \le (N-m+1)$, are formed. These vectors represent m consecutive x values starting with the ith point.

(2) The distance between vectors $X_m(i)$ and $X_m(j)$, $d[X_m(i), X_m(j)]$, as the absolute maximum difference between their components is defined:

$$d[X_m(i), X_m(j)] = \max_{k=0,\ldots,m-1} (|x(i+k) - x(j+k)|). \tag{3}$$

(3) For a given $X_m(i)$, the number of $j\,(1 \le j \le N-m, j \ne i)$, denoted as B_i, is counted such that the distance between $X_m(i)$ and $X_m(j)$ is less than or equal to r. Then, for $1 \le j \le N-m$,

$$B_i^m(r) = \frac{1}{N-m-1} B_i. \tag{4}$$

(4) $B_i^m(r)$ is defined as

$$B^m(r) = \frac{1}{N-m} \sum_{i=1}^{N-m} B_i^m(r). \tag{5}$$

(5) The dimension is increased to $m+1$, and $B^{m+!}(r)$ was calculated.

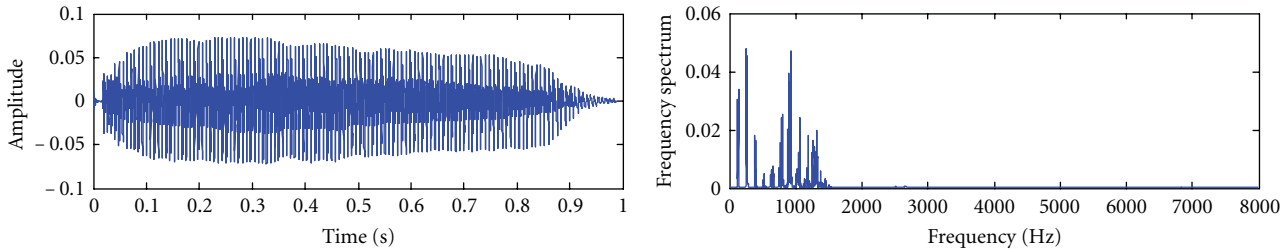
Thus, $B^m(r)$ is the probability that two sequences will match m points, whereas $B^{m+!}(r)$ is the probability that two sequences will match $m+1$ points. Finally, SampEn can be defined as

$$\text{SampEn}(m, r, N) = \sum_{N\to\infty} -\ln\left[\frac{B^{m+1}(r)}{B^m(r)}\right]. \tag{6}$$

This value is estimated by the statistics:

$$\text{SampEn}(m, r, N) = -\ln\left[\frac{B^{m+1}(r)}{B^m(r)}\right]. \tag{7}$$

2.3. SVM. SVM is a useful machine learning technique that has been successfully applied in the classification area. Classifying data is a common task in machine learning. In most cases, the data to be classified is linearly non-separable but nonlinearly separable in which the nonlinear



(a) (b)

Figure 3: Original signal and amplitude spectrum for it.

support vector classifier can then be used. The main idea is to transform the original data into a high-dimensional feature space. Thus, it may be nonlinear in the original input space even though the classifier is a hyperplane in the high-dimensional feature space [19].

The product (x, y) is replaced by a kernel function $K(x, y)$ to construct a nonlinear support vector classifier. The following are some commonly used kernel functions:
polynomial (homogenous)

$$k(x, x') = (x \cdot x')^d, \tag{8}$$

polynomial (inhomogeneous)

$$k(x, x') = (x \cdot x' + 1)^d, \tag{9}$$

radial basis function

$$k(x, x') = \exp\left(-\gamma ||x - x'||^2\right), \quad \text{for } \gamma > 0, \tag{10}$$

Gaussian radial basis function

$$k(x, x') = \exp\left(-\frac{\|x - x'\|^2}{2\sigma^2}\right), \tag{11}$$

hyperbolic tangent

$$k(x,x') = \tanh(\kappa x \cdot x' + c), \quad \text{for some (not all) } \kappa > 0,\ c < 0. \tag{12}$$

The goal of SVM is to produce a model that predicts target values of data instances in the test set for which only the attributes are given. The following decision function is applied to determine which class the sample belongs to:

$$f(x) = \operatorname{sgn}\left(\sum_{i=1}^{l} y_i a_i^* k\left(x_i, x_j\right) + b^*\right). \tag{13}$$

The parameters a_i^* and b^* are the optimum solutions for specificity.

2.4. Clinical Data. Qi-deficient patients, based on TCM theory and clinical practice, exhibit the following characteristics: dispirited spirit, lack of qi and no desire to speak, discouraged, small voice; giddy dazzled, palpitations, sweaty, qualitatively weak tongue, tender, and feeble pulse. By contrast, yin-deficient patients are characterised as follows: emaciation, feverish sensation over the five centres, hot flushes, night sweats, and dry stool, among others. The subjects comprised voice signals from people of different age and sex. The detailed information is listed in Tables 1 and 2.

Table 1: The groups and sex of all samples in the experiments.

	Healthy	Qi deficiency	Yin deficiency	Head count
Sample number	27	116	38	181
Man	9	39	11	59
Woman	18	77	27	122

Table 2: The ages of three groups' samples in the experiments.

	Age (year)		
	Healthy	Qi deficiency	Yin deficiency
Max. age	54	76	80
Min. age	19	6	18
Average age	24.9	42.4	52.1

All these data are collected by our research partner the TCM Syndrome Laboratory of the Shanghai University of Traditional Chinese Medicine in its affiliated hospitals including the Longhua Hospital and the Shuguang Hospital. The voice is recorded using a high-performance microphone (the band is AKG model HSD171) and a 16-bit A/D converter connected to a computer. The frequency response range of the microphone is 60 Hz to 17 kHz. Its sensitivity is 1 mv/Pa (−60 dBV) with an impedance of 600 ohms. In addition, the sample frequency is 16 kHz. All the voice samples were collected by the acquisition system developed based on Visual C++ 6.0. The endpoint detection algorithm was applied to remove the nonvoice portions of the leading and trailing of each utterance.

The vowel /a/ was chosen as the utterance. Each subject produced a stable phonation of a sustained English vowel /a/ lasting about one second. This vowel is chosen because both patients and healthy subjects can easily pronounce this vowel. In addition, the vocal organ is not abuttal, and there is no obstacle in the cavity when this vowel is pronounced [20]. The pronunciation flow is unblocked, and a periodical waveform can be produced. Therefore, the vowel /a/ was mainly recently chosen as the utterance. The time-domain plot and spectrum of the vowel /a/ are shown in Figure 3.

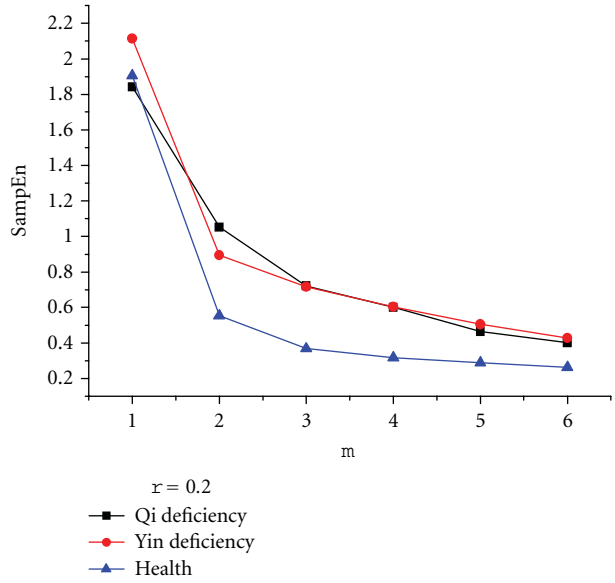


Figure 4: Influence of m on the separability among three classes using SampEn. The maximum separability is achieved with $m = 2$.

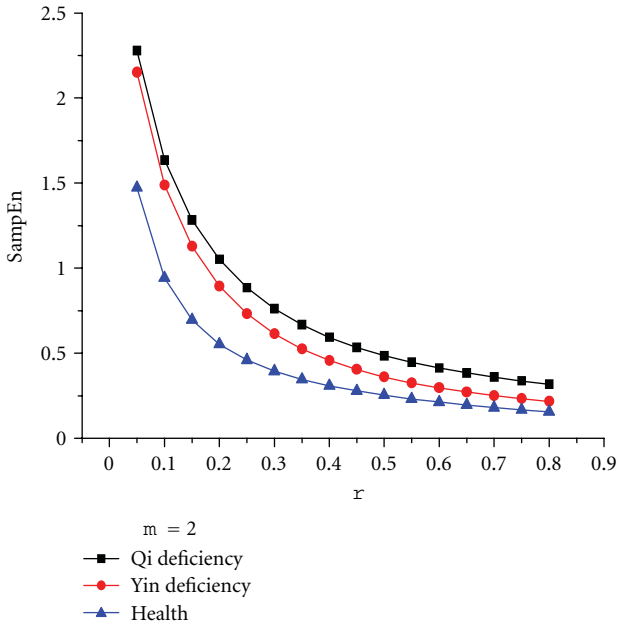


Figure 5: Influence of r on the separability among three classes using SampEn. The maximum separability is achieved with $r = 0.1 \sim 0.25$.

2.5. Processing of Voice Signal Using WPT. The voice signals including three kinds of samples were analyzed using WPT in the first stage of processing of sample identification. Five levels of wavelet packet decomposition were applied as the preprocessing step for all subjects. The maximum frequency in high-frequency bands of the original signal is 8 kHz under the sample frequency 16 kHz, then the frequency interval of the coefficients for the frequency bands is 250 Hz in fifth level.

2.6. The SampEn Computation. In the second stage, SampEn values of approximation and detailed coefficients at each level of the wavelet decomposition were computed for the voice signals of the healthy subjects, as well as yin- and qi-deficient patients. In choosing the optimum parameters m and r, Pincus suggested $m = 2$ and $r = 0.1\ \delta$ to $0.25\ \delta$, where δ is the standard deviation of the original signal $u(i)$, $i = 1, \ldots, N$. One of the original signals was chosen and analysed using different m and r values to better illustrate the advantages of the choice. The results are shown in Figures 4 and 5. We can easily see that the difference in the SampEn values was the largest among the signals of the three kinds of samples (shown in Figure 5). This condition indicates that the choice of the value $m = 2$ is appropriate. We can also see that the SampEn value decreased as the parameter increased, although in a lower degree. Therefore, r is selected as $0.2\ \delta$ appropriately.

3. Results and Discussion

3.1. Results on SampEn Values for WPT Coefficients. Voice signals from qi- and yin-deficient, as well as healthy, subjects were decomposed into sub-bands using WPT. The frequency bands for these sub-bands were as follows: S_{-1}^n (the frequency interval is 4 kHz, $n = 0, 1$), S_{-2}^n (the frequency interval is 2 kHz, $n = 0, 1, 2, 3$), S_{-3}^n (the frequency interval is 1 kHz, $n = 0, 1, 2, \ldots, 7$), S_{-4}^n (the frequency interval is 0.5 kHz, $n = 0, 1, 2, \ldots, 15$), and S_{-5}^n (the frequency interval is 0.25 kHz, $n = 0, 1, 2, \ldots, 31$). SampEn values of the approximated and detailed coefficients under fifth-level WPT decompositions were computed using the selected parameters in Section 2.6.

The average SampEn values for the coefficients of the 1–5 levels are illustrated in Figures 6(a)–6(e). The differences between healthy and qi- or yin-deficient samples are relatively high, except in 0–0.5 kHz and 7.5–8 kHz of the forth level and 0.25–0.0.5 kHz, 7.5–7.75 kHz and 7.75–8 kHz of fifth level. However, the differences between the qi- and yin-deficient samples are relatively low apart from the following frequency ranges: 0 kHz to 8 kHz in the 1–5 levels.

We also can see in Figures 6(a)–6(e) that, with increasing wavelet packet levels, the frequency bands become more subtle. At the same time, more feature information contained in the voice signal is represented. Slight changes that cannot be reflected in low scales will be represented in high scales. Furthermore, the overall trend of SampEn values for qi-deficient, yin-deficient and healthy samples tends to be higher as frequency increases. The SampEn values of qi-deficient samples are lower than those of yin-deficient samples in most of frequency bands of 0–4 kHz in 1–5 levels, while the SampEn values for qi- and yin-deficient samples are intertwined in 4–8 kHz.

3.2. Statistical Analysis. Statistical analysis software, SPSS 20, was applied to analyse the differences among the samples. All SampEn values of the WPT coefficients from the first to the fifth levels were analyzed to obtain the features with significant differences among the three groups of samples. Tables 3, 4, and 5 shows there were 47 frequency bands

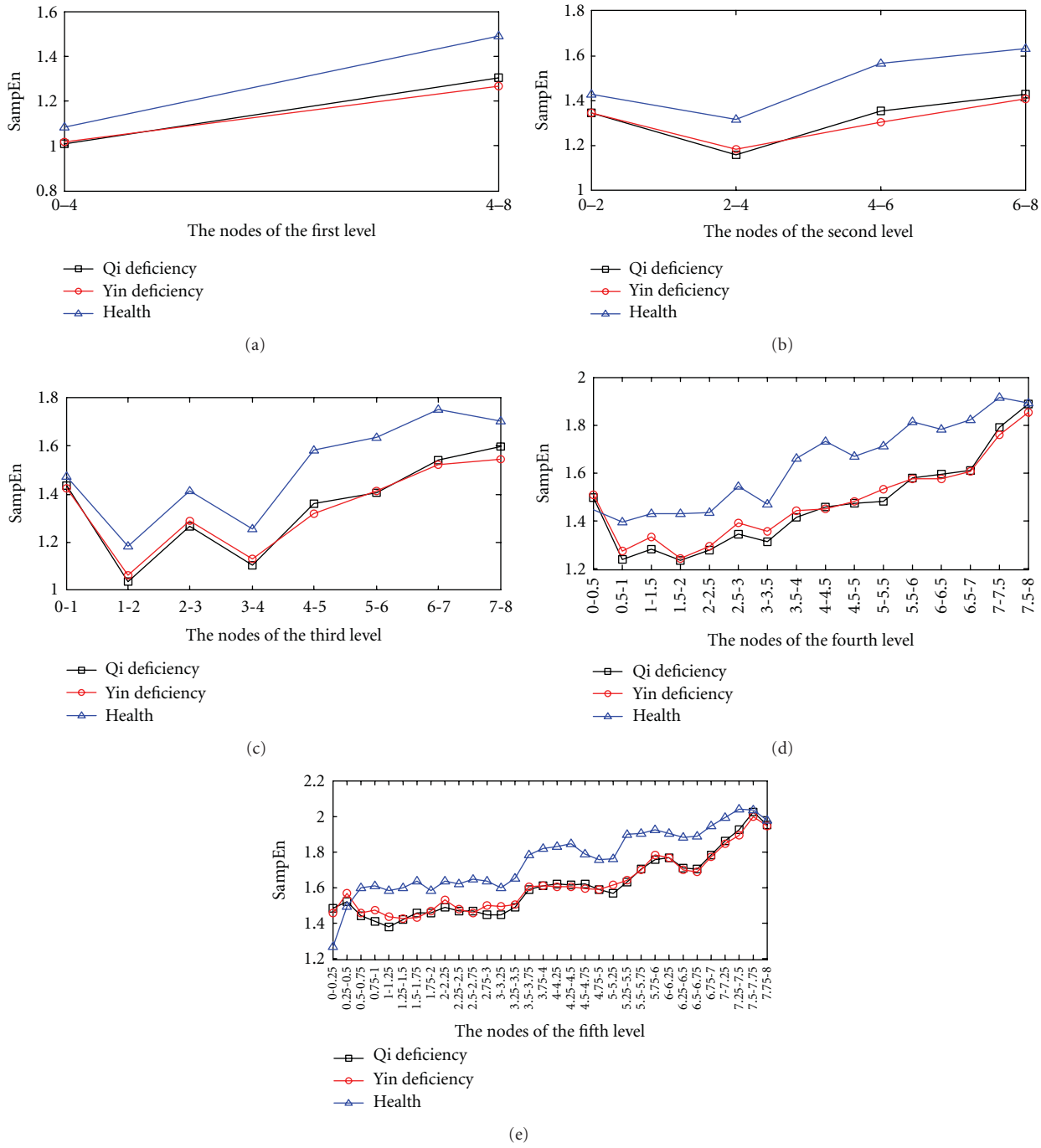


FIGURE 6: The SampEn values for the coefficients of WPT: (a)–(e) SampEn values for the first to the fifth level coefficients.

having SampEn values with significant differences from 1 to 5 level.

3.3. Classification Analysis. LibSVM 2.93 was used to identify the auscultation signal. The feature parameters with remarkable differences (47 features in different bands) were chosen as the input vectors consistent with the format of the LibSVM. The SVM type is C-SVC, and the RBF function was chosen as the kernel function for nonlinear training and testing after numerous experiments. The optimum parameters c and g were obtained as 0.25 and 0.0625 using cross-validation (c is the penalty factor, and g is the parameter for kernel function). Table 6 shows the classification results using SVM, in which a good result for classifying the samples (up to 96%) was obtained. This finding proves that the method applied in this paper is impressive.

Table 3: SampEn values for the subbands' coefficients in first, second and third levels with significant differences.

Frequency band (kHz)	Qi deficiency		Yin deficiency		Healthy		P value
	Mean	SD	mean	SD	Mean	SD	
4–8	1.303	0.346	1.266	0.346	1.490	0.374	0.011
4–6	1.356	0.356	1.304	0.358	1.567	0.357	0.005
6–8	1.428	0.312	1.410	0.289	1.632	0.220	0.002
3-4	1.104	0.266	1.131	0.306	1.256	0.296	0.035
4-5	1.359	0.364	1.319	0.406	1.582	0.362	0.005
5-6	1.406	0.334	1.412	0.339	1.636	0.332	0.003
6-7	1.541	0.292	1.524	0.313	1.755	0.251	0.001
7-8	1.600	0.266	1.544	0.279	1.703	0.269	0.044

Table 4: SampEn values for the subbands' coefficients in the fourth level with significant differences.

Frequency band (kHz)	Qi deficiency		Yin deficiency		Healthy		P value
	mean	SD	mean	SD	mean	SD	
0.5–1	1.240	0.220	1.276	0.289	1.397	0.293	0.005
1–1.5	1.284	0.263	1.334	0.310	1.434	0.324	0.029
1.5–2	1.238	0.307	1.246	0.352	1.431	0.303	0.021
2.5–3	1.346	0.290	1.392	0.342	1.544	0.287	0.009
3–3.5	1.317	0.277	1.359	0.325	1.472	0.306	0.048
3.5–4	1.418	0.348	1.444	0.355	1.661	0.351	0.002
4–4.5	1.459	0.373	1.452	0.406	1.733	0.358	0.001
4.5–5	1.476	0.351	1.481	0.385	1.670	0.386	0.014
5–5.5	1.482	0.337	1.532	0.346	1.712	0.313	0.004
5.5–6	1.582	0.284	1.578	0.329	1.815	0.283	0.001
6–6.5	1.596	0.296	1.576	0.335	1.782	0.306	0.005
6.5–7	1.610	0.274	1.608	0.321	1.824	0.224	0.002
7–7.5	1.793	0.232	1.759	0.242	1.914	0.219	0.015

3.4. Discussion. The quantitative analysis of the speech of healthy persons and deficient patients is one of the important task in the objectification and modernization of auscultation of TCM. The voices of healthy people are natural, gentle, clear, fluent, and understandable, while the patients with deficient syndrome speak feebly in low voice and discontinuously. The SampEn values of healthy samples are higher than qi- or yin-deficient samples in most of frequency bands. It may demonstrate that healthy persons have more physiological adaptabilities than the patients with deficiency syndrome. The variation trend of the SampEn values in the qi- and yin-deficient samples were almost similar, perhaps because both qi- and yin-deficient subjects belong to the deficiency syndrome, and the differences of voice signal characteristic between them are not remarkably significant. The classification result demonstrated that the SVM classifier was effective for the identification of the auscultation signals. Therefore auscultation analysis based on WPT-SampEn-SVM was suitable for the identification among qi- and yin-deficient, as well as healthy, subjects.

4. Conclusions

In this paper, we proposed a new method in identifying the auscultation signals in TCM including three kinds of samples, namely, qi- and yin-deficient, as well as healthy, samples. Instead of solely using traditional time or frequency domain features, we applied nonlinear dynamic parameter SampEn together with time and frequency analysis method to come up with the wavelet packet to obtain our feature parameters. Wavelet packets are specifically used because of their capability to partition both low- and high-frequency signals. At the same time, SampEn, a statistics parameter used to measure the predictability of the current amplitude values of a physiological signal, is adopted in our research to analyze the signals from three kinds of samples. Experimental results illustrated that WPT-SampEn-SVM-based analysis was suitable for the identification among qi- and yin-deficient, as well as healthy, subjects. Our future research will improve the performance of indentifying deficient patients by analyzing the SampEn variability of the signals

Table 5: SampEn values for the subbands' coefficients in fifth level with significant differences.

Frequency band (kHz)	Qi deficiency		Yin deficiency		Healthy		P value
	mean	SD	mean	SD	mean	SD	
0.00–0.25	1.487	0.304	1.459	0.275	1.269	0.386	0.020
0.50–0.75	1.446	0.242	1.457	0.250	1.601	0.238	0.008
0.75–1.00	1.410	0.273	1.475	0.326	1.612	0.321	0.004
1.00–1.25	1.380	0.295	1.436	0.327	1.584	0.323	0.005
1.25–1.50	1.423	0.301	1.429	0.344	1.603	0.374	0.025
1.50–1.75	1.459	0.323	1.433	0.378	1.635	0.322	0.041
2.50–2.75	1.470	0.302	1.459	0.366	1.647	0.328	0.020
2.75–3.00	1.448	0.306	1.501	0.358	1.638	0.286	0.015
3.25–3.50	1.489	0.287	1.508	0.323	1.652	0.315	0.025
3.50–3.75	1.587	0.335	1.608	0.342	1.784	0.336	0.008
3.75–4.00	1.611	0.383	1.613	0.367	1.823	0.354	0.007
4.00–4.25	1.622	0.379	1.607	0.371	1.832	0.387	0.005
4.25–4.50	1.617	0.349	1.605	0.369	1.847	0.333	0.002
4.50–4.75	1.624	0.321	1.594	0.381	1.791	0.346	0.016
4.75–5.00	1.588	0.337	1.588	0.377	1.759	0.359	0.028
5.00–5.25	1.567	0.346	1.614	0.353	1.761	0.332	0.008
5.25–5.50	1.631	0.300	1.642	0.355	1.897	0.216	0.000
5.50–5.75	1.703	0.277	1.700	0.315	1.903	0.254	0.002
5.75–6.00	1.760	0.255	1.784	0.266	1.924	0.205	0.005
6.00–6.25	1.767	0.268	1.767	0.278	1.902	0.245	0.020
6.25–6.50	1.712	0.272	1.702	0.336	1.884	0.257	0.006
6.50–6.75	1.706	0.287	1.690	0.349	1.891	0.242	0.005
6.75–7.00	1.783	0.252	1.776	0.305	1.945	0.223	0.007
7.00–7.25	1.863	0.230	1.848	0.251	1.991	0.193	0.018
7.25–7.50	1.923	0.223	1.896	0.249	2.040	0.161	0.010
7.50–7.75	2.026	0.164	1.997	0.149	2.034	0.251	0.037

Table 6: Prediction accuracies using SVM.

Group numbers	Accuracy for each class	Overall accuracy
Qi deficiency	99%	
Yin deficiency	89%	96%
Healthy	93%	

of reconstructed coefficients in different frequency bands of each level. In addition, the clinical sample size will be extended for the verification of our methods.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grants no. 30701072, 81173199, and 30901897) and the Shanghai 3rd Leading Academic Discipline Project (Grant no. S30302).

References

[1] Y. Q. Wang, *Diagnostics of Traditional Chinese Medicine*, Higher Education Press, Beijing, China, 2006.

[2] S. G. Zhao, "A modem research overview on the auscultation diagnosis of TCM," *Chinese Journal of the Practical Chinese with Modern Medicine*, vol. 14, pp. 1218–1220, 2008.

[3] X. M. Mo, "Preliminary study on making use of sound ring instrument for the diagnosis of deficiency syndrome of the lung with cough," *Journal of Traditional Chinese Medicine Research*, vol. 3, pp. 43–44, 1987.

[4] H. J. Wang, J. J. Yan, Y. Q. Wang, F. Li, and R. Guo, "Digital technology for objective auscultation in traditional Chinese medical diagnosis," in *Proceedings of the International Conference on Audio, Language and Image Processing (ICALIP '08)*, pp. 1100–1104, July 2008.

[5] J. J. Yan, Y. Q. Wang, H. J. Wang et al., "Nonlinear analysis in TCM acoustic diagnosis using delay vector variance," in *Proceedings of the 2nd International Conference on Bioinformatics and Biomedical Engineering (iCBBE '08)*, pp. 2099–2102, May 2006.

[6] C. C. Chiu, H. H. Chang, and C. H. Yang, "Objective auscultation for traditional Chinese medical diagnosis using novel acoustic parameters," *Computer Methods and Programs in Biomedicine*, vol. 62, no. 2, pp. 99–107, 2000.

[7] X. M. Mo and Y. S. Zhang, "The current situation and prospect of auscultation research in TCM," *Foundation Medical Journal of Traditional Chinese Medicine*, vol. 4, no. 1, 1998.

[8] X. H. Zhang, *Concise Sound Medical*, People's Health Publishing House, 1985.

[9] A. H. Tewfik, D. Sinha, and P. Jorgensen, "On the optimal choice of a wavelet for signal representation," *IEEE Transactions on Information Theory*, vol. 38, no. 2, pp. 747–765, 1992.

[10] C. H. Horng, *The Principles and Methods of Diagnostics*, The Illustrations of Chinese Medicine, chapter 4, Lead Press, Taipei, Taiwan, 1993.

[11] X. L. Wang, *First exploration of the smell diagnosis on tuberculosis*, Summary of Graduate Student Thesis, 1992.

[12] G. Cui, X. Cao, and X. Zhang, "Analysis of biological data with digital signal processing," in *Proceedings of the IEEE 7th Workshop on Multimedia Signal Processing (MMSP '05)*, Shanghai, China, November 2005.

[13] C. Roberto, *Modern Digital Signal Process*, Tshinghua University Press, Beijing, China, 2004.

[14] B. C. Li and J. S. Luo, *Wavelet Analysis and Its Applications*, Electronics Engineering Press, Beijing, China, 2003.

[15] X. H. Tang and Q. L. Li, *Time-Frequency Analysis and Wavelet Transform*, Science Press, Beijing, China, 2008.

[16] L. H. Yang, D. Q. Dai, and W. L. Huang, *Wavelet Tour to Signal Processing*, China Machine Press, Beijing, China, 2002.

[17] S. M. Pincus and A. L. Goldberger, "Physiological time-series analysis: what does regularity quantify?" *American Journal of Physiology*, vol. 266, no. 4, pp. H1643–H1656, 1994.

[18] J. S. Richman and J. R. Moorman, "Physiological time-series analysis using approximate and sample entropy," *American Journal of Physiology*, vol. 278, no. 6, pp. H2039–H2049, 2000.

[19] V. N. Vapnik, *Statistics Learning Theory*, Wiley, New York, NY, USA, 1998.

[20] L. Z. Hou and D. M. Han, "Selection of the vowel sound in the throat sound detection," *Journal of Audiology and speech Diseases*, vol. 10, p. 16, 2002.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 453503, 8 pages
doi:10.1155/2012/453503

Research Article

Metabonomic Evaluation of ZHENG Differentiation and Treatment by Fuzhenghuayu Tablet in Hepatitis-B-Caused Cirrhosis

Shujun Sun,[1] Jianye Dai,[1] Wenyu Wang,[1] Huijuan Cao,[1] Junwei Fang,[1] Yi Yang Hu,[2] Shibing Su,[3] and Yongyu Zhang[1]

[1] *Research Center for Traditional Chinese Medicine and Systems Biology, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Pudong, Shanghai 201203, China*
[2] *Institute of Liver Diseases, Shuguang Hospital, Key Laboratory of Liver and Kidney Diseases of Ministry of Education, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*
[3] *Research Center for Traditional Chinese Medicine Complexity System, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Pudong, Shanghai 201203, China*

Correspondence should be addressed to Shibing Su, shibingsu07@163.com and Yongyu Zhang, dryyz@sina.com

Received 13 February 2012; Accepted 28 March 2012

Academic Editor: Shao Li

In Traditional Chinese Medicine (TCM), treatment based on ZHENG (also called TCM syndrome and pattern) differentiation has been applied for about 3 thousand years, while there are some difficulties to communicate with western medicine. In the present work, metabonomic methods were utilized to differentiate ZHENG types and evaluate the therapeutic efficiency of Fuzhenghuayu (FZHY) tablet in hepatitis-B-caused cirrhosis (HBC). Urine samples of 12 healthy volunteers (control group, CG) and 31 HBC patients (HBCG) were analyzed by gas chromatography mass spectrometry (GC/MS) and multivariate statistical analysis. The significantly changed metabolites between CG and HBCG were selected by PLS-DA loading plot analysis. Moreover, 4 ZHENGs were differentiated mutually, suggesting that there was urine metabolic material basis in ZHENG differentiation. The efficiency of FZHY tablet on subjects with spleen deficiency with dampness encumbrance syndrome (SDDES) and liver-kidney yin deficiency syndrome (LKYDS) was better than that of other syndromes. The efficiency of FZHY treatment based on ZHENG differentiation indicated that accurately ZHENG differentiating could guide the appropriate TCM treatment in HBC.

1. Introduction

Cirrhosis and its complications are one of the main causes of mortality [1] especially for individuals aged 45 to 54 years [2]. Hepatitis B virus (HBV) infection is one of the most common viral infections in humans. Approximately 350 million people have been chronically infected by HBV [3], and around 20% to 30% of them will result in cirrhosis [4]. To date, the definite and indefinite duration treatments with interferon and nucleotide analogue, respectively, are two first line strategies in western medicine [4]. Facing the sustained high morbidity and mortality, new effective therapeutic protocols of HBC are imperative. As a holistic and multitarget approach, TCM has shown some advantages on the treatment of those complicated chronic diseases [5–7].

With increasing attentions paid to TCM, many researches [5–7] about the curative effect of Chinese medicinal and formulas were conducted but lack direction of TCM theory. TCM is a large and complex system, and ZHENG differentiation and treatment (Bian Zheng Lun Zhi) is one of its essences. To further explaination, the diagnosis that guides treatment of TCM is called ZHENG (TCM syndrome or pattern), a temporary state at one time and which is defined by symptoms and signs. It could be assessed by four diagnostic methods (looking, listening and smelling, asking, and touching) [8]. And the same disease can usually manifest in different syndromes, so patients with different ZHENG

types may be treated by different rules and therapeutic regimen. Based on the holistic and systemic characteristics of ZHENG, metabonomics [9], genomics [10], proteomics [11], and the integration of them [12] were introduced to the research of ZHENG differentiation.

The marketed Fuzhenghuayu tablet is a TCM prescription including *Radix Salvia miltiorrhizae, Cordyceps mycelia extract, Semen Persicae, Gynostemma pentaphyllum Mak, Pollen Pini* and *Fructus schisandrae chinensis*. The recipe composition was directed by the therapeutic method of invigorating blood transforming stasis and boosting essence supplementing deficiency [13]. In the present study, urine Metabonomics [14] based on gas chromatography mass spectrometry (GC/MS) and multivariate statistical techniques was utilized to differentiate four ZHENG types of HBC in the molecular level and evaluate the therapeutic effects of FZHY tablet for different ZHENG types. To our knowledge, this study is the first report of urinary Metabonomics method used to investigate the therapeutic effects of FZHY tablet for different ZHENGs.

2. Materials and Methods

2.1. Subjects and Experiment Design. Twelve healthy volunteers and 31 patients from Shanghai Shuguang Hospital (Shanghai, China) were enrolled in the study. The healthy volunteers without any treatment were considered as CG. All of patients were affected with hepatitis-B-caused cirrhosis (HBC) and regarded as disease group (HBCG). The patients were classified into 4 ZHENG types, including spleen deficiency with dampness encumbrance syndrome (SDDES, $n = 7$), liver-gallbladder dampness-heat syndrome (LGDHS, $n = 7$), liver-kidney yin deficiency syndrome (LKYDS, $n = 10$), and blood stasis syndrome (BSS, $n = 7$). All of them were treated with the same formula by oral administration. Then metabonomic detection and analysis was performed to evaluate the therapeutic effect on HBC patients with different ZHENG types. The clinical study was approved by the local ethics committee and all of the recruited persons were given informed consent. Diagnosis standard of cirrhosis is referred to "Chronic hepatitis B prevention and treatment guidelines." [15]. And all cases of HBC caused by other factors such as hepatitis C infection, alcohol consumption, and usage of drugs with hepatotoxicity were ruled out before all the subjects entered the study. The TCM ZHENG types were identified by three chief or deputy physicians, according to "evaluation criteria of the clinical diagnosis, drug efficacy, and ZHENG differentiation for cirrhosis (pilot program)" [16]. The study was performed in accordance with the principles contained in the Declaration of Helsinki.

2.2. Chemicals and Drugs. Ethyl chloroformate (ECF), pyridine, anhydrous ethanol, sodium hydroxide, chloroform, and anhydrous sodium sulfate were analytical grade from China National Pharmaceutical Group Corporation (Shanghai, China). L-2-chlorophenylalanine (Shanghai Intechem Tech. Co. Ltd., China) was used as an internal quality standard which was prepared in the ultrapure water from a Milli-Q system (Millipore, USA). FZHY tablets were provided by Shanghai Huanghai Pharmaceutical Co., Ltd.

2.3. Sample Collection and Preparation. Urina sanguinis was collected from 12 healthy subjects and 31 HBC patients when they were enrolled in the study. And after 12 and 24 weeks of treatment the patients were asked for urina sanguinis again. Urine samples were stored at -80°C until GC-MS assay.

All these samples were thawed in ice water bath and vortex-mixed before analysis. Each 600 μL aliquot of standard mixture or urine sample was added to a screw tube. After adding 100 μL of l-2-chlorophenylalanine (0.1 mg mL^{-1}), 400 μL of anhydrous ethanol, and 100 μL of pyridine to the urine sample, 50 μL of ECF was added for first derivatization at $20.0 \pm 0.1^\circ$C. The pooled mixtures were sonicated at 40 kHz for 60 s. Subsequently, extraction was performed using 300 μL of chloroform, with the aqueous layer pH carefully adjusted to 9-10 using 100 μL of NaOH (7 mol L^{-1}). The derivatization procedure was repeated with the addition of 50 μL ECF into the aforementioned products. After the two successive derivatization steps, the overall mixtures were vortexed for 30 s and centrifuged for 3 min at 3000 rpm. The aqueous layer was aspirated off, while the remaining chloroform layer containing derivatives was isolated and dried with anhydrous sodium sulfate and subsequently subjected to GC-MS. The derivatization method referred to [17].

2.4. Data Acquisition. All GC-MS analyses were performed by a mass spectrometer 5975B (Agilent technologies, USA) coupled to an Agilent 6890 (Agilent technologies, USA) gas chromatography instrument. In the gas chromatographic system, a catabletary column (Agilent J&W DB-5 ms Ultra Inert 30 m $\times$ 0.25 mm, film thickness 0.25 μm) was used. Helium carrier gas was used at a constant flow rate of 1.0 mL $*$ min^{-1}. One μL of derivatized samples was injected into the GC/MS instrument, and splitless injection mode was used. To acquire a well separation, the column temperature was initially maintained at 80°C for 2 min and then increased from 80 to 140°C at the rate of 10°C/min for 6 min. Then, the column temperature was increased to 240°C at the rate of 4°C/min for 25 min. After that, the column temperature was increased to 280°C at the rate of 10°C/min for 4 min and held for 3 min. The temperatures of the injection port, the interface, and source temperature were set at 280°C, 260°C, and 230°C, respectively. The measurements were made with electron impact ionization (70 eV) in the full scan mode (m/z 30–550). The solvent posttime was set to 5 min.

2.5. Data Analysis. Due to experimental variations and column aging, shifts in retention time between fingerprints occur. When the total ion current chromatograms (TICs) were obtained, peak-alignment or warping techniques are commonly applied to compensate for minor shifts in retention times. Thus, in the subsequently data processing, the same variable manifested synchronous information in every profile. So all the GC-MS raw files after being converted to CDF format via the software come with Agilent

MSD workstation, and were subsequently processed by the XCMS toolbox (http://metlin.scripps.edu/download/) using XCMS's default settings with the following exceptions: xcmsSet (full width at half-maximum: fwhm = 5; S/N cutoff value: snthresh = 10, max = 15), and group (bw = 5). The resulting table (CSV file) was exported into Microsoft Excel (Microsoft Inc., USA), where normalization was performed prior to multivariate analyses. The resulting three-dimensional matrix involving peak index (RT-m/z pair), sample names (observations), and normalized peak area percent was introduced into Simca-P 11.5 Software package (Umetrics, Umea, Sweden) for partial least squares-discriminate analysis (PLS-DA). Differential variables between CG and HBCG were generated from loadings plot. To find the influential metabolites responsible for the separation, we calculated the variable importance for the projection (VIP) values [18]. Variables with VIP values exceeding 1.5 were first selected. In a second step, those variables were further compared by Mann-Whitney *U*-test to confirm the changed metabolites in SPSS 17.0 (SPSS, Chicago, IL, USA) with the threshold of *P* value set at 0.05. Those variables, then, were identified by searching in NIST 2005 database and verified by standards. References and the Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.ad.jp/kegg/) were based to give the biochemical interpretation of changed metabolites affected by HBC.

3. Results

3.1. Metabolic Profiles of Cirrhosis Patients and Healthy Control. One μL aliquots of supernatants of all the urine samples, after a two-step derivatization, extraction and dryness, were injected into GC/MS for analysis with the method described previously. PLS-DA analysis was employed to discriminate HBCG and CG, and the score plot with $R^2Y = 0.888$ and $Q^2Y = 0.792$ is shown in Figure 1(a). In this map, HBCG could be absolutely separated from healthy group. The results might demonstrate that the urine metabolic profiles had changed significantly.

A loading plot was constructed to indicate the most influential variables according to their respective contributions to the discrimination between the 2 groups (Figure 1(b)). The further away from the main cluster, the greater influence the variables have on the PLS-DA scores plot. Every variable could be identified by the measured m/z value and NIST database. The metabolites' names, corresponding VIP values, and changed trend compared with the healthy group are presented in Table 1, simultaneously.

3.2. Biochemical Interpretation

3.2.1. Disorder of Immunity. Alanine and tyrosine, which are the substrates of alanine transaminase (ALT) and aspartate aminotransferase (AST), respectively, were upregulated in HBCG. And ALT and AST will increase when activated $CD4^+$ and $CD8^+$ lymphocytes recognize various HBV-derived proteins located on the surface of infected hepatocytes [19] (summarized in Figure 2). So the increase of alanine and tyrosine might suggest that HBC was correlated with the disorder of immune system, which was in agreement with the previous report that chronic HBV infection develops in the setting of impaired immune reactions or a relatively tolerant immune system status [20].

Table 1: Identification results and the changed trend of differential metabolites of hepatitis-B-caused Cirrhosis subjects compared to healthy group.

Number	Metabolites	RT (min)	VIP	Changing trend compared with HG
1	Butanoic acid	5.29	2.25	↑**
2	Propanedioic acid	5.98	1.81	↑**
3	Hexanedioic acid	6.03	1.97	↓**
4	L-Alanine	8.06	2.28	↑**
5	Thiourea	9.75	1.73	↓**
6	acrylic acid	12.91	1.55	↓**
7	L-Proline	16.9	2.12	↑**
8	Methionine	17.8	1.57	↑**
9	Phenol	27.87	2.08	↑**
10	Benzoic acid	31.71	1.59	↓**
11	Benzenamine	31.79	1.97	↑**
12	Tyrosine	32.49	1.75	↑**
13	1,4-Butanedioic acid	34.41	1.92	↑**
14	DL-Tryptophan	34.44	1.83	↑**
15	L-Lysine	34.75	2.11	↑**
16	Isothiourea	34.78	2.04	↑**
17	Benzyl alcohol	35.08	1.61	↑**
18	Indole	36.21	2.03	↑**
19	Propanoic acid	36.4	1.64	↑**
20	Hexanoic acid	36.71	2.26	↑**
21	n-Butylamine	36.78	1.88	↑**
22	Acetic acid	36.81	1.69	↑**
23	Pentanoic acid	36.89	1.64	↑**
24	Tyrosyl-cysteine methyl ester	37.37	2.04	↑**

The levels of differential metabolites were labeled with (↓) downregulated and (↑) upregulated (**$P < 0.01$).

3.2.2. Energy Metabolism. Alanine and proline are precursors of pyruvate which can convert to acetyl-coenzyme A (Acetyl-CoA) and is the main input for a series of reactions known as TCA cycle. The increased level of alanine and proline in HBCG might indicate that HBCG relieves the inhibition of proline iminopeptidase (PIP) and activates the biosynthesis of pyruvate to increase carbohydrate catabolism [20].

3.2.3. ABC Transporters. The significantly changed metabolites in this study, proline, lysine, and alanine, participated the pathway of ABC transporters, which was found in database KEGG. Liver is the most active site of cholesterol metabolism, and the content of cholesterol is closely correlated with cirrhosis [21]. However, ABC transporters play

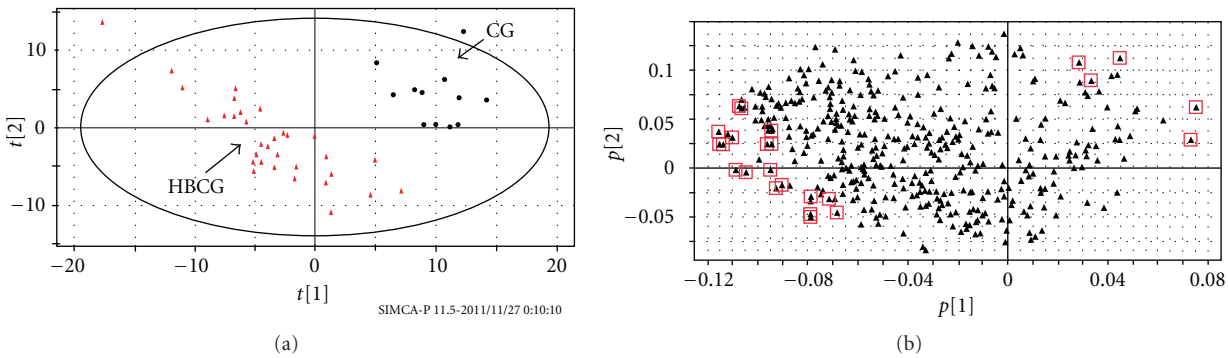


Figure 1: (a) PLS-DA score plot between CG and HBCG. Black dots and red triangles refer to healthy subjects and hepatitis-B-caused Cirrhosis subjects, respectively. (b) PLS-DA loading plot from HBCG and CG.

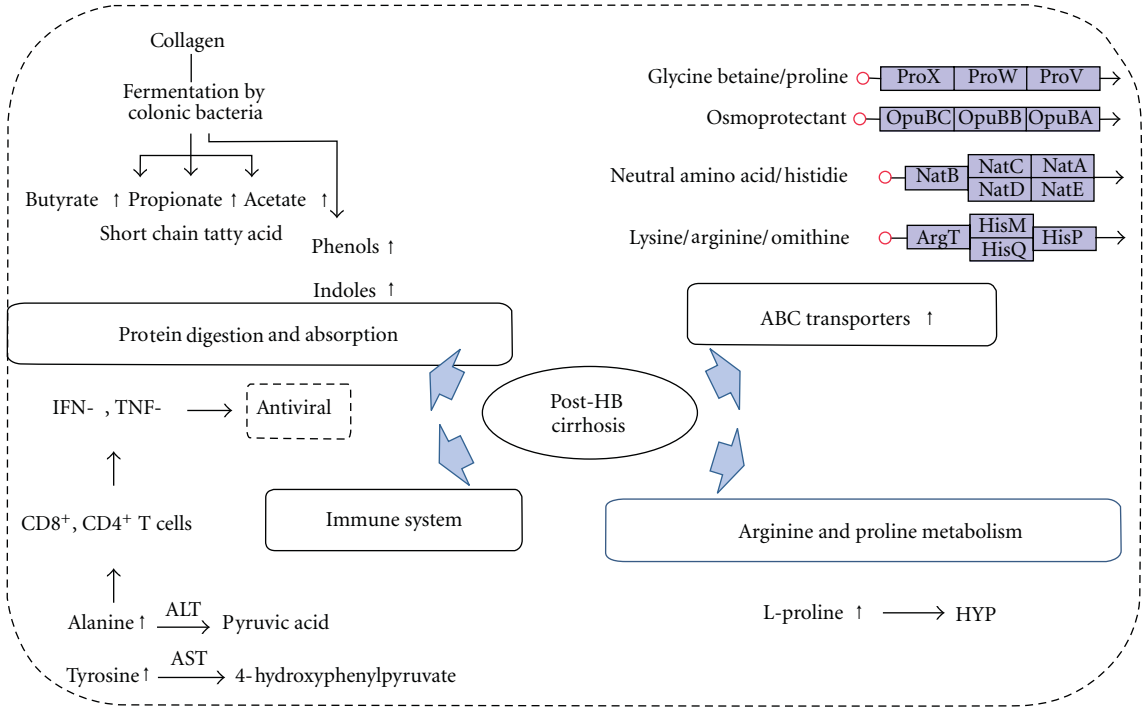


Figure 2: HBC-related pathway observed in this research.

an important role in secretion of cholesterol from liver into bile [22]. The three changed amino acids might suggest that HBC would be correlated with the dysfunction of ABC transporters, which was in accordance with the literature [23].

3.2.4. Protein Digestion and Absorption. The contents of precollagen type III and collagen type IV in cirrhosis subjects are higher than normal ones, and they were reported as two of the main factors for hepatitis fibrosis and cirrhosis [23]. Butyrate, propionate, acetate, phenol, and indole are the products of collagens after fermentation by colonic bacteria. Those compounds that were detected increased compared with healthy group in this research. Among them butyrate, propionate, and acetate were retrieved in form of their acid which were listed as butanoic acid, propanoic acid, and acetic acid in Table 1. The results might prompt that the collagens in subjects of this research have been improved, meaning that they may have been affected with HBC.

In addition, Hydroxyproline, a product of proline hydroxylation, is a common used biomarker of fibrosis or cirrhosis in animal experiments. In tissue of animals with cirrhosis, the content of hydroxyproline is much higher than healthy group [5, 6], which manifested in the increase of proline in urine samples in this test. We have detected several small molecules including alanine, tyrosine, butanoic acid, propanoic acid, and acetic acid dovetailing with clinical

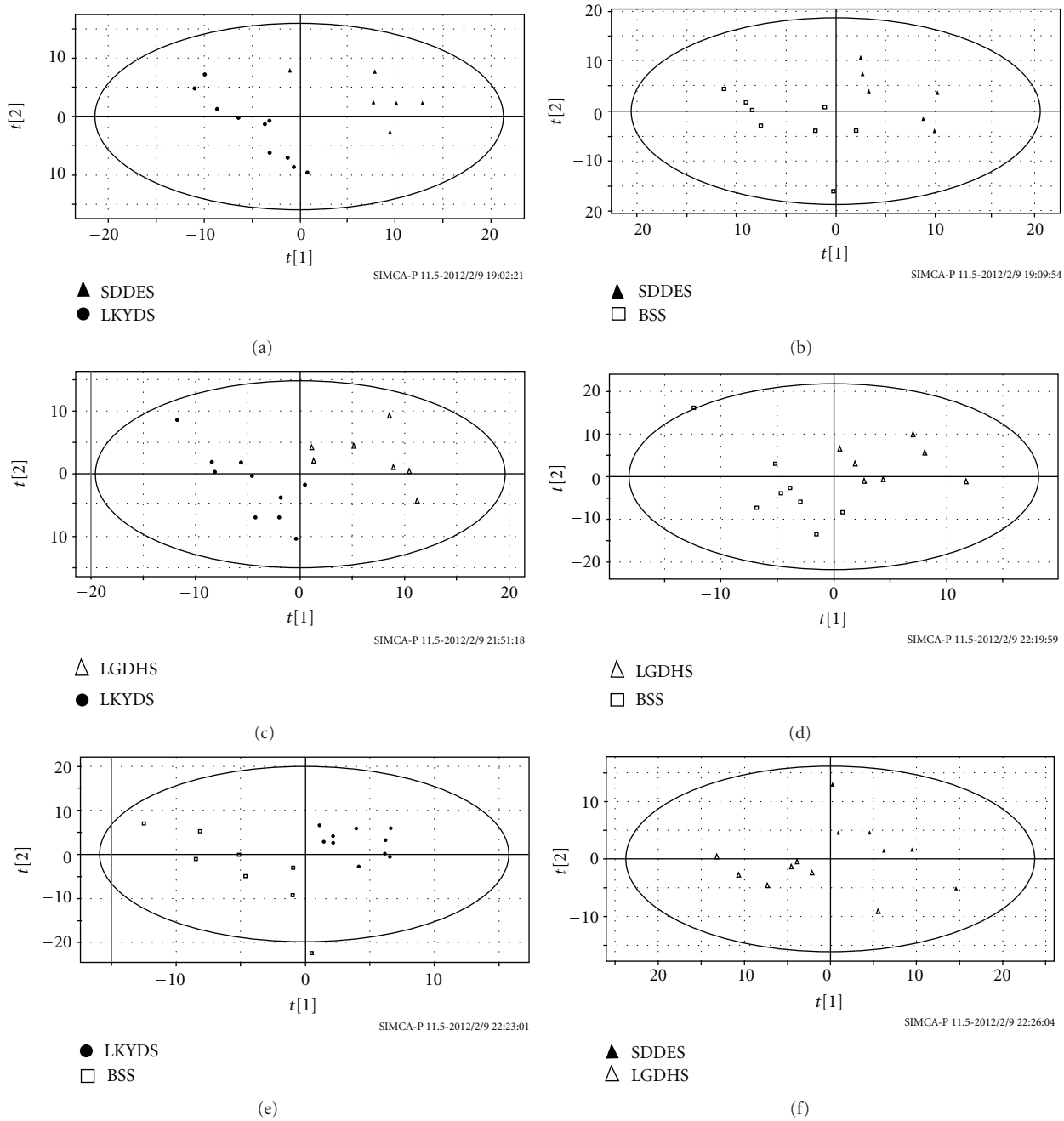


Figure 3: Score plot of PLS-DA for comparison among 4 ZHENG types. SDDES compared to LKYDS (a); SDDES compared to BSS (b); LGDHS compared to LKYDS (c); LGDHS compared to BSS (d); LKYDS compared to BSS (e); SDDES compared to LGDHS (f).

biomarkers ALT, AST, precollagen type III, and collagen type IV in this research, which suggests metabonomic technology or further studies could help diagnose HBC.

3.3. ZHENG Differentiation. Four ZHENG types were distinguished by PLS-DA analysis. The model information is shown in Table 2, and six maps of score plot are presented in Figure 3. The results prompt that ZHENG differentiation in TCM may be based on objective material, not only on practitioners' experience.

3.4. Efficiency of FZHY Tablet. The significantly changed metabolites of each group HBC patients from the healthy subjects have been selected. And the potential biomarkers of HBCG were previously listed in Table 1. The four TCM ZHEGNs' potential biomarkers are not summarized in tables but can be found on x-axis of Figure 4. The reversions of these metabolites were based on to evaluate the therapeutic effect of FZHY tablet. Consequently, we found that there were no significantly reversed potential biomarkers for all the subjects of HBC at both 12th week and 24th week. While for LSYDS at 12th week, most influential metabolites reversed,

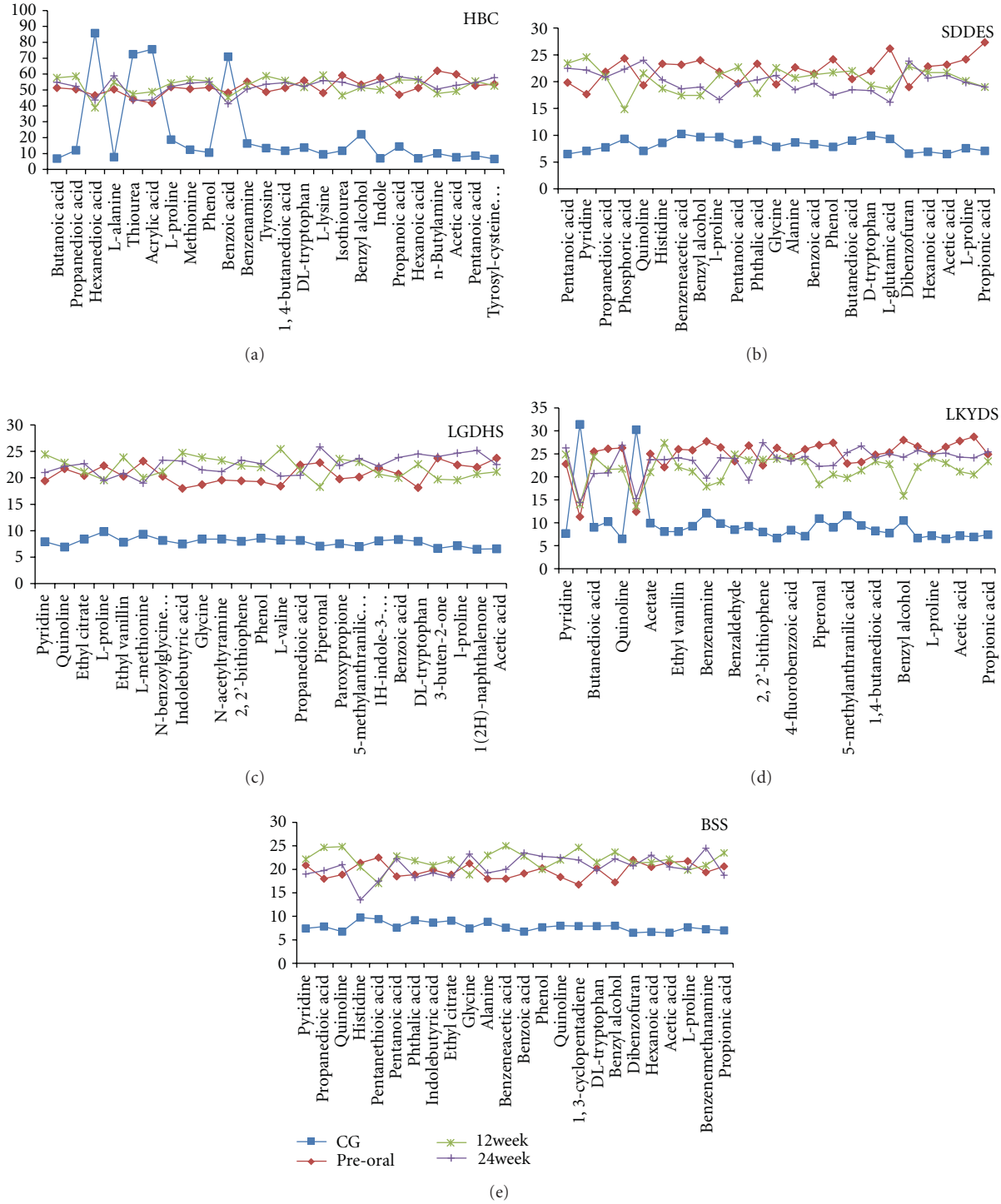


Figure 4: Five maps revealed the therapeutic effect of FZHY tablet for hepatitis-B-caused cirrhosis (HBC) and four TCM ZHENG types, respectively: spleen deficiency with dampness encumbrance syndrome (SDDES), liver-gallbladder dampness-heat syndrome (LGDHS), liver-kidney yin deficiency syndrome (LKYDS), and blood stasis syndrome (BSS), by the changing trend of significantly differential metabolites. CG is short for control group; preoral in each group stands for information before intervention of FZHY tablet; 12th week means effects after 12-week intervention by FZHY tablet; 24th week means effects after 24-week intervention. The x-axis represented the changed metabolites, and the y-axis was average rank in Mann-Whitney U-test, representing the contents of metabolites.

Table 2: Model information of PLS-DA for comparison of 4 ZHENGs with each other.

Model	Amount of components	R^2Y	Q^2Y
SDDES and LKYDS	3	0.969	0.462
SDDES and BSS	3	0.922	0.106
LGDHS and LKYDS	3	0.922	0.0934
LGDHS and BSS	6	0.999	0.682
LKYDS and BSS	4	0.988	0.44
SDDES and LGDHS	5	0.999	0.549

and at 24th week, the reversion of potential biomarkers showed good efficiency of FZHY for SDDES, as manifested in Figure 4 (LKYDS and SDDES). As we can see, most metabolites for LGDHS and BBS got further away from the healthy group than pre-oral of FZHY.

4. Discussion

The potential biomarkers that discriminate HBCG and CG were found by PLS-DA loading plot analysis. After retrieving literatures and the database KEGG, it was supposed that HBC might correlate with the disorder of immune metabolism, energy metabolism, ABC transporters, and protein digestion and absorption. The ZHENG differentiation of HBC demonstrated that a disease might be divided into more than one pattern. Different metabolic profiles or different phenotypes probably arise from disparate pathogenesis and etiological factors. Consequently, every ZHENG should be treated differently, which was in accordance with the theory of ZHENG differentiation and treatment [24].

The results showed that subjects with deficiency syndrome (SDDES and LKYDS) are more susceptible for FZHY tablet, which was in accordance with "boosting essence supplementing deficiency" in the principles of recipe composition. At 24th week, subjects with LKYDS did not appear the effects as good as that of 12th week, which may be interpreted by the dynamic and developmental characteristics of disease. The patients were diagnosed with LKYDS when enrolled, but after 12 weeks their ZHENG might have changed. So the treatment rules should be changed correspondingly, which precisely manifested the personalized medicine. In respect of that subjects with LGDHS and BSS still had no signs of recovery during the 24-week treatment, those two ZHENG types seemed not suitable for FZHY tablet. So the correspondence between ZEHNG and formula, called "fang zheng dui ying" in TCM, is very important [25]. And it is the main treatment principle after ZHENG differentiation.

To acquire results with high reliability and accuracy, large amount of samples should be collected. And after the multicenter and multiregional trial validation, biomarkers could only be transformed into clinical applications. In addition, if Metabonomics is validated by other "omics" or biochemical methods, it would be more convincing.

5. Conclusion

Subjects with HBC were distinguished from the healthy control with the method of Metabonomics based on GC/MS analysis and multivariate statistical techniques. The four ZHENGs in this study were also classified by PLS-DA. Without ZHENG differentiation, the efficiency of FZHY tablet for patients with HBC was not significant, through the holistic evaluating approach. However if the objects of treatment aim at subjects with spleen deficiency with dampness encumbrance syndrome or liver-kidney yin deficiency syndrome, the therapeutic effects would be increased remarkably. And at ones with liver-gallbladder dampness-heat syndrome, and blood stasis syndrome, within 24 weeks not any effects could be observed. As a result, the treatment effect of FZHY tablet indicated that accurately ZHENG differentiation could guide the appropriate TCM treatment in HBC. And this study indicated that Metabonomics technology can be utilized to evaluate the therapeutic effect of TCM recipes based on ZHENG differentiation and Treatment.

Acknowledgments

The study was financially supported by the National Science and Technology Major Project of China (no. 2012ZX10005001-004), National Key Technology Research and Development Program of China (no. 2006BAI08B02-6), and Leading Academic Discipline Project of Shanghai Municipal Education Commission (no. J50301).

References

[1] R.N. Anderson, "Deaths: leading causes for 2000.," *National Vital Statistics Reports*, vol. 50, no. 16, pp. 1–85, 2002.

[2] Agency for Healthcare Research and Quality, "HCUPnet: healthcare cost and utilization project (HCUP)," http://www.ahrq.gov/data/hcup/.

[3] W. F. Carman, M. R. Jacyna, S. Hadziyannis et al., "Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection," *Lancet*, vol. 2, no. 8663, pp. 588–591, 1989.

[4] G. Carosi, M. Rizzetto, A. Alberti et al., "Treatment of chronic hepatitis B: update of the recommendations from the 2007 Italian Workshop," *Digestive and Liver Disease*, vol. 43, no. 4, pp. 259–265, 2011.

[5] A. A. Hamza, "Ameliorative effects of Moringa oleifera Lam seed extract on liver fibrosis in rats," *Food and Chemical Toxicology*, vol. 48, no. 1, pp. 345–355, 2010.

[6] C. Liu, M. Sun, L. Wang et al., "Effects of Yinchenhao Tang and related decoctions on DMN-induced cirrhosis/fibrosis in rats," *Chinese Medicine*, vol. 3, article no. 1, 2008.

[7] J. L. Lou, M. N. Jiang, C. Li et al., "Herb medicine Gan-fu-kang attenuates liver injury in a rat fibrotic model," *Journal of Ethnopharmacology*, vol. 128, no. 1, pp. 131–138, 2010.

[8] J. L. Tang, B. Y. Liu, and K. W. Ma, "Traditional Chinese medicine," *The Lancet*, vol. 372, no. 9654, pp. 1938–1940, 2008.

[9] W. Long, P. Liu, J. Xiang, X. Pi, J. Zhang, and Z. Zou, "A combination system for prediction of Chinese Materia Medica properties," *Computer Methods and Programs in Biomedicine*, vol. 101, no. 3, pp. 253–264, 2011.

[10] C. Lu, C. Xiao, G. Chen et al., "Cold and heat pattern of rheumatoid arthritis in traditional Chinese medicine: distinct molecular signatures indentified by microarray expression profiles in CD4-positive T cell," *Rheumatology International*, vol. 32, no. 1, pp. 61–68, 2010.

[11] Y. Feng, Z. Wu, X. Zhou, Z. Zhou, and W. Fan, "Knowledge discovery in traditional Chinese medicine: state of the art and perspectives," *Artificial Intelligence in Medicine*, vol. 38, no. 3, pp. 219–236, 2006.

[12] S. Li, Z. Q. Zhang, L. J. Wu, X. G. Zhang, Y. D. Li, and Y. Y. Wang, "Understanding ZHENG in traditional Chinese medicine in the context of neuro-endocrine-immune network," *IET Systems Biology*, vol. 1, no. 1, pp. 51–60, 2007.

[13] P. Liu, Y. Y. Hu, C. Liu et al., "Multicenter clinical study on Fuzhenghuayu capsule against liver fibrosis due to chronic hepatitis B," *World Journal of Gastroenterology*, vol. 11, no. 19, pp. 2892–2899, 2005.

[14] J. K. Nicholson and J. C. Lindon, "Systems biology: metabonomics," *Nature*, vol. 455, no. 7216, pp. 1054–1056, 2008.

[15] Chinese Medical Association, "Chronic hepatitis B prevention and treatment guidelines," *Chinese Journal of Infectious Diseases*, vol. 19, no. 1, pp. 56–62, 2001.

[16] Y. X. Zhang and B. H. Wei, "Evaluation criteria of the clinical diagnosis, drug efficacy and TCM Syndrome differentiation for cirrhosis (pilot program)," *Chinese Journal of Integrative Medicine*, vol. 14, no. 4, pp. 237–238, 1994.

[17] Y. Qiu, M. Su, Y. Liu et al., "Application of ethyl chloroformate derivatization for gas chromatography-mass spectrometry based metabonomic profiling," *Analytica Chimica Acta*, vol. 583, no. 2, pp. 277–283, 2007.

[18] L. Eriksson, E. Johansson, and N. Kettaneh-Wold, *Multi- and Megavariate Data Analysis*, Part. 1: Basic Principles and Applications, Umetrics AB, Umeå, Sweden, 2nd edition, 2001.

[19] J. J. Chang and S. R. Lewin, "Immunopathogenesis of hepatitis B virus infection," *Immunology and Cell Biology*, vol. 85, no. 1, pp. 16–23, 2007.

[20] P. Borst and R. Oude Elferink, "Mammalian ABC transporters in health and disease," *Annual Review of Biochemistry*, vol. 71, pp. 537–592, 2002.

[21] E. Bugianesi, N. Leone, E. Vanni et al., "Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma," *Gastroenterology*, vol. 123, no. 1, pp. 134–140, 2002.

[22] D. M. Smal, "Role of ABC transporters in secretion of cholesterol from liver into bile," *Proceedings of the National Academy of Sciences*, vol. 100, no. 1, pp. 4–6, 2003.

[23] Z. H. Su, S. Q. Li, G. A. Zou et al., "Urinary metabonomics study of anti-depressive effect of Chaihu-Shu-Gan-San on an experimental model of depression induced by chronic variable stress in rats," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 55, no. 3, pp. 533–539, 2011.

[24] C. D. Gu, *The Inner Classic of the Yellow Emperor, Essential Questions (Huangdi Neijing, Suwen)*, People's Medical Publishing House, Beijing, China, 1956.

[25] T. X. Lin and Y. H. Li, "Discussion on the three levels of the prescription-composition principle of TCM," *Deutsche Zeitschrift fur Akupunktur*, vol. 52, no. 3, pp. 27–30, 2009.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 635702, 10 pages
doi:10.1155/2012/635702

Research Article

Tumor Microenvironment Varies under Different TCM *ZHENG* Models and Correlates with Treatment Response to Herbal Medicine

Zhen Chen,[1,2] Lian-Yu Chen,[1,2] Peng Wang,[1,2] Hai-Yan Dai,[1,2] Song Gao,[1,2] and Kun Wang[1,2]

[1] *Department of Integrative Oncology, Fudan University Shanghai Cancer Center, Shanghai 200032, China*
[2] *Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China*

Correspondence should be addressed to Peng Wang, wangp413@yahoo.com.cn

Received 11 January 2012; Accepted 1 April 2012

Academic Editor: Shi-Bing Su

In traditional Chinese medicine (TCM), diagnosis of pathology and choice of treatment prescriptions are based on a method of differentiation of signs and symptoms known as syndrome differentiation or *ZHENG*. The cornerstone of TCM, *ZHENG*, relies on the gathering of clinical information through inspection, auscultation and olfaction, inquiry, and palpation. However, the biomolecular basis of the *ZHENG* remains unclear. In this study, we established mouse xenograft pancreatic cancer models with *Shi-Re* (Dampness-Heat), *Pi-Xu* (Spleen-Deficiency), or *Xue-Yu* (Blood-Stasis) *ZHENG*, which are regarded as the three major *ZHENG*s in pancreatic cancer. We found that tumors of the different *ZHENG* models exhibited significantly altered cancer-associated fibroblast (CAF) proliferative activity and tumor-associated macrophage (TAM) infiltration, which led to altered levels of CAF- and TAM-derived secreted cytokines such as SDF-1 and CCL5. The *ZHENG* model type also significantly influenced tumor growth, and administration of herbal medicine to the *ZHENG* model modified the tumor microenvironment. Therefore, this study partially unveiled the molecular basis of TCM *ZHENG* in pancreatic cancer.

1. Introduction

Traditional Chinese medicine (TCM) has a history of over 3000 years. A holistic form of medicine, TCM, emphasizes bringing the patient's body, mind, and spirit into harmony. The theory and application of TCM are one of constant summarizing, inducing, and refining of the experiences accumulated in preventing and treating diseases in daily life and medical practice.

TCM rests squarely on *ZHENG* (syndrome) differentiation, a process of analyzing data collected through four combined diagnostic methods: inspection, auscultation and olfaction, inquiry, and palpation. All diagnostic and therapeutic methods in TCM are based on the differentiation of *ZHENG*. In modern times, TCM has become popular worldwide because of its reliable therapeutic efficacy [1]. However, diagnosis in TCM depends on the intuition and experience of the physician grounded in TCM theory, and this method seems to lack objectivity, accuracy, and reproducibility in the face of biomolecular science and Western-based medicine. Furthermore, the concept of *ZHENG* is often misinterpreted and unclear. For all these reasons, researchers from China and elsewhere have begun to investigate the *ZHENG* of TCM for a molecular foundation.

Tumors are now recognized as structures of multiple cell types, comparable to organs in complexity, which during tumorigenesis recruit the involvement of surrounding normal cells to construct and interact within a tumor microenvironment [2]. Continuous paracrine signaling with feedback within this microenvironment eventually leads to the end stages of cancer progression [3]. As cancer is no longer considered a discrete entity defined only by the traits of cancer cells within the tumor but may eventually involve the entire organism, TCM offers a holistic approach whose goal is regulating the integrity of all body functions as well as the interaction between the human and surrounding environment.

We have previously shown that the presence of *ZHENG* may influence tumor growth in pancreatic cancer. We also found that this effect might correlate with the CC chemokine

(β-chemokine) family [4]. This finding suggests an involvement between *ZHENG* and the tumor microenvironment and deserves further research. Accordingly, in the present study we evaluated the tumor microenvironment under different *ZHENG* conditions, specifically noting changes in the proliferative activity of cancer-associated fibroblasts (CAF) and the infiltration of tumor-associated macrophages (TAM). We confirm here that characteristics of the tumor environment correlated with the *ZHENG* of TCM, and herbal treatments modified the tumor microenvironment.

2. Materials and Methods

2.1. Cell Lines and Mice. Samples of the pancreatic cancer cell line Panc02 were obtained from the Cancer Research and Development Center and grown in complete growth medium as recommended by the manufacturer. The cultured cells were maintained in a humidified 5% CO_2 atmosphere at 37°C. All cells were regularly authenticated by observing cell morphology and tested for the absence of mycoplasma contamination using a MycoAlert Mycoplasma Detection kit (MycoAlert, Lonza, Rockland, ME, USA).

Male C57 mice, 4- to 6-week old, were obtained from the Shanghai Institute of Materia Medica at the Chinese Academy of Sciences (Shanghai, China) and housed in laminar flow cabinets under specific pathogen-free conditions with food and water supplied *ad libitum*. All experiments on mice were conducted in accordance with the guidelines of the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals. The Committee for the Use of Live Animals in Teaching and Research, Fudan University, Shanghai approved the study protocol.

2.2. Drugs and Reagents. Individual packets of herb powders for each herb were produced by Jiangyin Tianjiang Pharmaceutical. The final decoction of each prescription was prepared at the Department of Pharmacy, Fudan University Shanghai Cancer Center, Shanghai, China, by dissolving the herb powder into distilled water to the required concentration. The daily dosage of herb decoctions for the mice was calculated according to the following human-mouse transfer formula: $Db = Da \times (Rb/Ra) \times (Wb/Wa)\ 2/3$, where D, R, and W represent dosage, shape coefficient, and body weight, respectively, and a and b represent human mouse, respectively.

Honey supplied by Guan Sheng Yuan International Trade (Shanghai) was adjusted to a concentration of 20% in water. Wine (er guo tou) obtained from Hongxing (Beijing) was diluted to 55% in water. Pork fat was donated by Gu Jianzhong, Chinese Academy of Sciences (Shanghai). The following antibodies were used: anti-vimentin, anti-α-smooth muscle actin (SMA), anti-C-X-C chemokine receptor type 4 (CXCR4), and anti-C-C chemokine receptor type 5 (CCR5; all from Epitomics), and anticluster of differentiation 68 (CD68; Santa Cruz Biotechnology, Santa Cruz, CA).

2.3. Establishment of TCM ZHENG Model. We developed three types of TCM *ZHENG* models in respective mouse groups, namely, *Shi-Re* (Dampness-Heat), *Pi-Xu* (Spleen-Deficiency), and *Xue-Yu* (Blood-Stasis). The *Shi-Re* and *Pi-Xu ZHENG* models were established as we described previously [4]. Briefly, the *Shi-Re* condition was established by the wine and the pork fat combination (day 1 to day 7, 0.2 mL), and the food and honey-water were provided. *Pi-Xu* was developed by feeding the mice with a decoction of mirabilite and Chinese rhubarb, 0.2 mL for each mouse (day 1 to day 7). The *Xue-Yu ZHENG* was established by subcutaneous injection of 0.01% adrenaline (0.13 mg/kg) for each mouse (day 1 to day 7), as we described previously [5].

2.4. Subcutaneous Xenograft Tumor Model. Panc02 cells (2×10^6 cells in 200 μL) were injected subcutaneously into the right axilla of each C57 mouse. The length and width of tumors (in millimeters) were measured weekly with calipers. Tumor volume was calculated by the formula $(a \times b^2) \times 0.5$, where a and b were the long and short dimensions, respectively. Mice were euthanized under anesthesia when tumors reached 1.5 cm in diameter. The tumors were then resected and weighed. Each group had $\geq$10 mice.

2.5. Immunohistochemical Analysis. Specimens of tumor tissue were fixed in 10% formalin and embedded in paraffin wax. Unstained 3 μm sections were then cut from paraffin blocks for immunohistochemical (IHC) analysis. The sections were stained with rabbit anti-vimentin (1 : 100), rabbit anti-α-SMA (1 : 100), rabbit anti-CD68 (1 : 200), rabbit anti-CXCR4 (1 : 200), and rabbit anti-CCR5 at 4°C overnight. The secondary antibody and avidin-biotin peroxidase complex method was used according to the standard protocols provided by the manufacturer (Vector Laboratories, CA, USA). An immunoglobulin-negative control was used to rule out nonspecific binding. Two independent assessors and one pathologist performed all procedures, all of whom were blinded to the model/treatment type for this series of specimens.

To quantitatively evaluate the CAF proliferative activity and TAM infiltration in each group, we calculated the ratio of the area positive for vimentin or CD68 staining to the total area in histological sections from ten fields under light microscopy (200x). The procedure for evaluation of CXCR4 and CCR5 expression followed that of our previous report [4].

2.6. Enzyme-Linked Immunosorbent (ELISA) Assay for Cytokine Release. The concentrations of SDF-1 and CCL5 in the tumor samples were determined using a sandwich ELISA kit (DuoSet; R&D Systems, Minneapolis, MN) according to the protocol of the manufacturer. Briefly, frozen tumor tissue was homogenized in lysis buffer and thereafter centrifuged at 12,000 rpm for 15 minutes at 4°C; 50 μL of the supernatant was used for ELISA. Concentrations of immunoreactive SDF-1 and CCL5 were expressed as pg/mL.

2.7. Statistical Analyses. The data are expressed as the mean ± standard error (SE) of three or more independent experiments performed in triplicate. The statistical analyses

were performed using analysis of variance (ANOVA) models and Student's t-tests. A P value <0.05 was accepted as statistically significant.

3. Results

3.1. ZHENG Distribution in Pancreatic Cancer Patients. We firstly investigated the distribution of *ZHENG* conditions in populations of pancreatic cancer patients based on reports published from January 1, 1998 to December 31, 2008. Sixty-nine studies were identified by electronic and hand searches, among which 34 clinical articles were included for our study. Data on *ZHENG* distribution were extracted and analyzed. Twenty-seven *ZHENG*s were identified. The three *ZHENG*s in pancreatic cancer that were most reported were Dampness-Heat (in Chinese, *Shi-Re*; 33.9% of studies), Spleen-Deficiency (*Pi-Xu*; 29.10%), and Blood-Stasis (*Xue-Yu*; 19.8%; Figure 1).

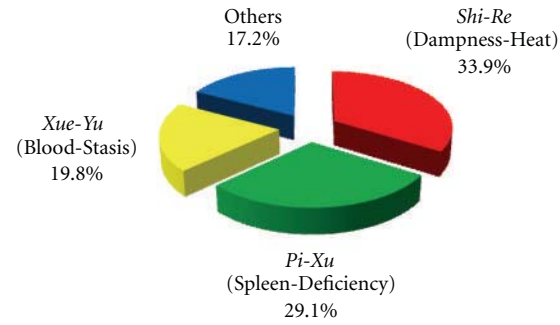


Figure 1: Percentages of pancreatic patients diagnosed with various TCM *ZHENG* conditions. Data on *ZHENG* distribution were extracted and analyzed from 34 clinical articles published between January 1, 1998 and December 31, 2008.

3.2. Alteration of Tumor Microenvironment under Different ZHENG Conditions. The tumor microenvironment plays an important role in the development and progression of cancer [6, 7]. Pancreatic carcinomas are surrounded by desmoplastic stroma consisting of fibroblasts, immune cells, endothelial cells, and pericytes [8]. We hypothesized that the tumor microenvironment would be altered under different *ZHENG* conditions. To verify this hypothesis, we first established 3 subcutaneous tumor models of pancreatic cancer in mice that exemplified the *ZHENG* conditions *Shi-Re*, *Pi-Xu*, and *Xue-Yu*, respectively. We sought to investigate the differences in the tumor microenvironment among these *ZHENG* models.

As it is recognized that in many tumors the stroma is characterized by an increase in fibroblast proliferation, we immunostained CAFs using the fibroblastic marker vimentin combined with the defined myofibroblast marker α-smooth muscle actin (α-SMA) to investigate the proliferative activity of CAF [9]. We found that the number of both vimentin- and α-SMA-positive cells was decreased in tumors from the *Shi-Re* and *Pi-Xu ZHENG* models of pancreatic cancer compared with the control tumor, while tumors from the *Xue-Yu* model exhibited no changes in CAF activity (Figures 2(a) and 2(b)). This observation suggested that CAF proliferative activity in tumors was altered differently on the basis of *ZHENG* conditions.

It is accepted that, in general, cancer- and host-cell-derived signals program TAMs to acquire an M2-like polarized and otherwise tumor-supportive phenotype [10]. In many cases increased numbers of TAMs are associated with a poorer prognosis [11, 12]. Therefore, we evaluated tumors in the different *ZHENG* models for TAM infiltration by staining for CD68 (also known as macrosialin in mice), a glycoprotein expressed on macrophages. We found that, compared with the control group, the number of macrophages was dramatically less in the *Shi-Re* group, followed by lesser degrees of decrease in the *Pi-Xu* groups (Figures 2(a) and 2(b)). This observation suggests relatedness between the inflammation characteristics of tumor microenvironments and the specific *ZHENG* conditions tested. Altogether, our study demonstrated a correlation between *ZHENG* conditions and the microenvironment of tumors in pancreatic cancer.

3.3. Correlation between Tumor Microenvironment and Growth under Different ZHENG Conditions. CAFs stimulate tumor cell proliferation and invasion through various growth factors, hormones, and cytokines [13]. SDF-1 is a CAF-derived chemokine that has been shown to directly boost the proliferation and invasion of pancreatic cancer cells [14]. Thus, we evaluated the levels of secreted SDF-1 in tumors under different *ZHENG* conditions, and the expression of CXCR4, the SDF-1 cognate receptor, in tumor cells. The results of ELISA assays showed decreased levels of SDF-1 released in tumors in the *Shi-Re* and *Pi-Xu* groups compared to the control mice. This was not observed in the *Xue-Yu* tumors (Figure 3(a)). This result was consistent with the observation that the *Shi-Re* and *Pi-Xu* tumors exhibited decreased CAF proliferative activity. However, there was no difference in CXCR4 expression among the *ZHENG* models and control tumor cells (Figures 3(b) and 3(c)).

Similarly, we wanted to verify whether the decreased TAM infiltration we observed above led to a reduction in the levels of the TAM-derived cytokine CCL5. We found that secreted CCL5 decreased dramatically in tumors under *Shi-Re* and *Pi-Xu ZHENG*s. This was also observed in the *Xue-Yu* tumors, although the difference was not significant ($P = 0.083$) (Figure 3(a)). We also found that tumor cells from the *Shi-Re*, *Pi-Xu,* and *Xue-Yu* models exhibited decreased CCR5 expression, especially for *Shi-Re* (Figures 3(b) and 3(c)).

After we confirmed that CAF-related SDF-1/CXCR4 and TAM-related CCL5/CCR5 expressions were changed under different *ZHENG* conditions, we next investigated an association between the altered tumor microenvironments and tumor growth. We found that altered tumor microenvironments were correlated with *in vivo* changes in tumor growth (Figure 3(d)). Taken together, these results suggest that tumors under different *ZHENG* conditions exhibited different tumor microenvironments, which may finally effect tumor growth.

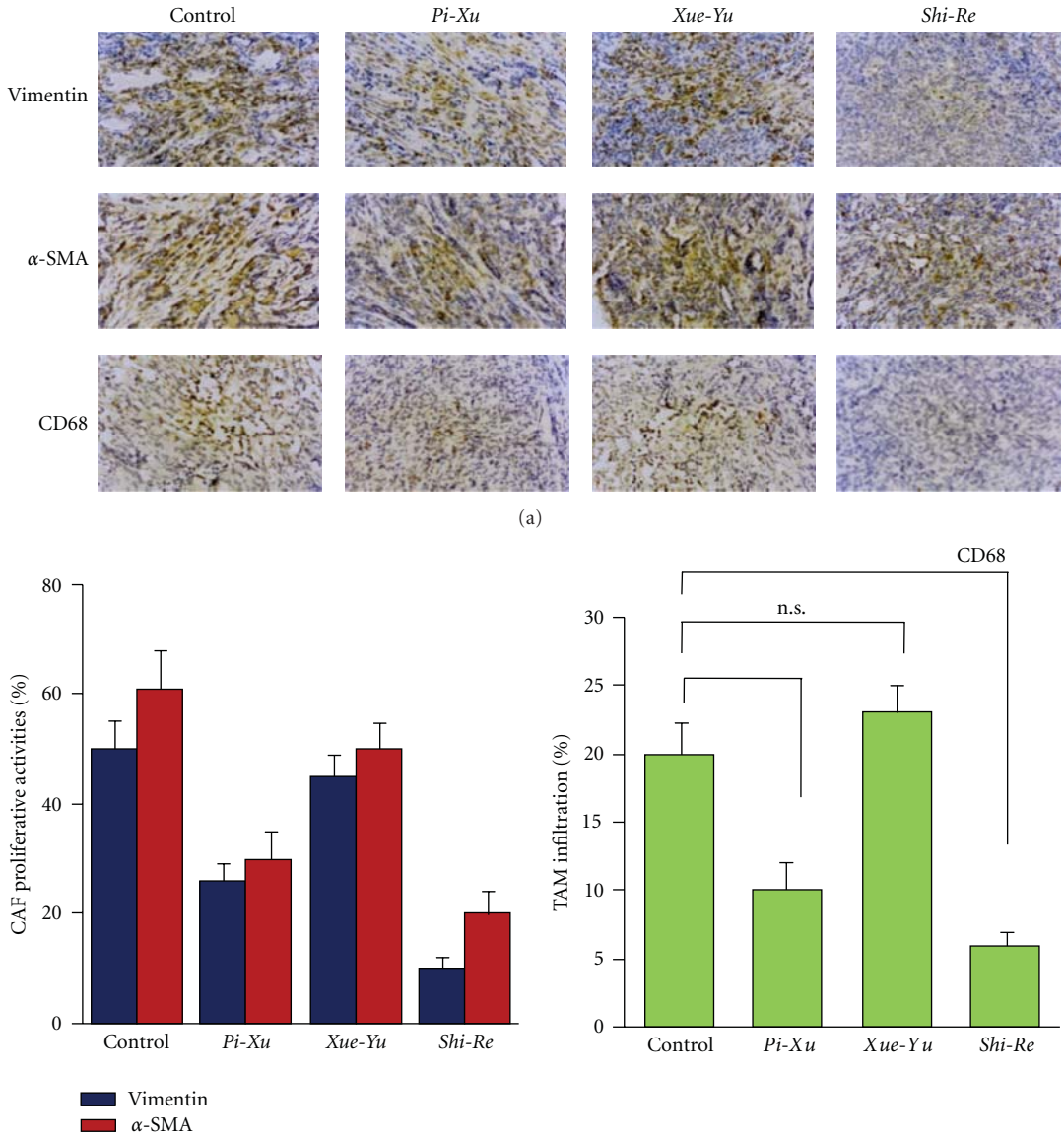


Figure 2: Alterations of tumor microenvironments under different *ZHENG* conditions. (a) Xenograft tumor models were established as described. On the next day, three types of *ZHENG*, namely, *Shi-Re*, *Pi-Xu*, and *Xue-Yu* were established. Tumors were obtained 4 wks after implantation. IHC staining for vimentin and α-SMA on sections of tumors was performed for evaluating CAF proliferative activities (top). IHC staining for CD68 was performed for evaluating TAM infiltration (low). Original magnification, 200x. (b) CAF proliferative activity (left) and TAM infiltration (right) were quantitatively evaluated by calculating the ratio of vimentin or CD68 antibody positive staining area to the total area in each field, and the mean value from ten fields under 200x microscopy was indicated. $^{*}P < 0.05$; $^{**}P < 0.01$; n.s.: not significant.

3.4. Tumor Response under Different ZHENG Conditions to Herbal Medicine Treatments. TCM usually means a comprehensive assessment of pathogenesis, location, and disease pathology, and the diagnosed *ZHENG* helps guide the application of Chinese herbal remedies. So, we used *Huang lian jie du* decoction (a traditional prescription used of treating *Shi-Re ZHENG*), *Si jun zi* decoction (a traditional prescription used of treating *Pi-Xu ZHENG*), and *Tao hong si wu* decoction (a traditional prescription used of treating *Xue-Yu ZHENG*) for *Shi-Re*, *Pi-Xu,* and *Xue-Yu* tumors, respectively. The herbal prescriptions used are shown in Table 1. We found that the herbal medicines had no or little effect on CAF proliferation or TAM infiltration (Figure 4(a)). We also evaluated the effects of the herbal medicines on SDF-1 secretion and CCL5 levels and found that the levels of which did not change after herbal medicine treatment (Figure 4(b)). Similarly, none of the herbal medicines had significant effects on CXCR4 or CCR5 expression in tumor cells (Figure 4(c)). These observations indicated that the herbal medicines had little effect on either the tumor or

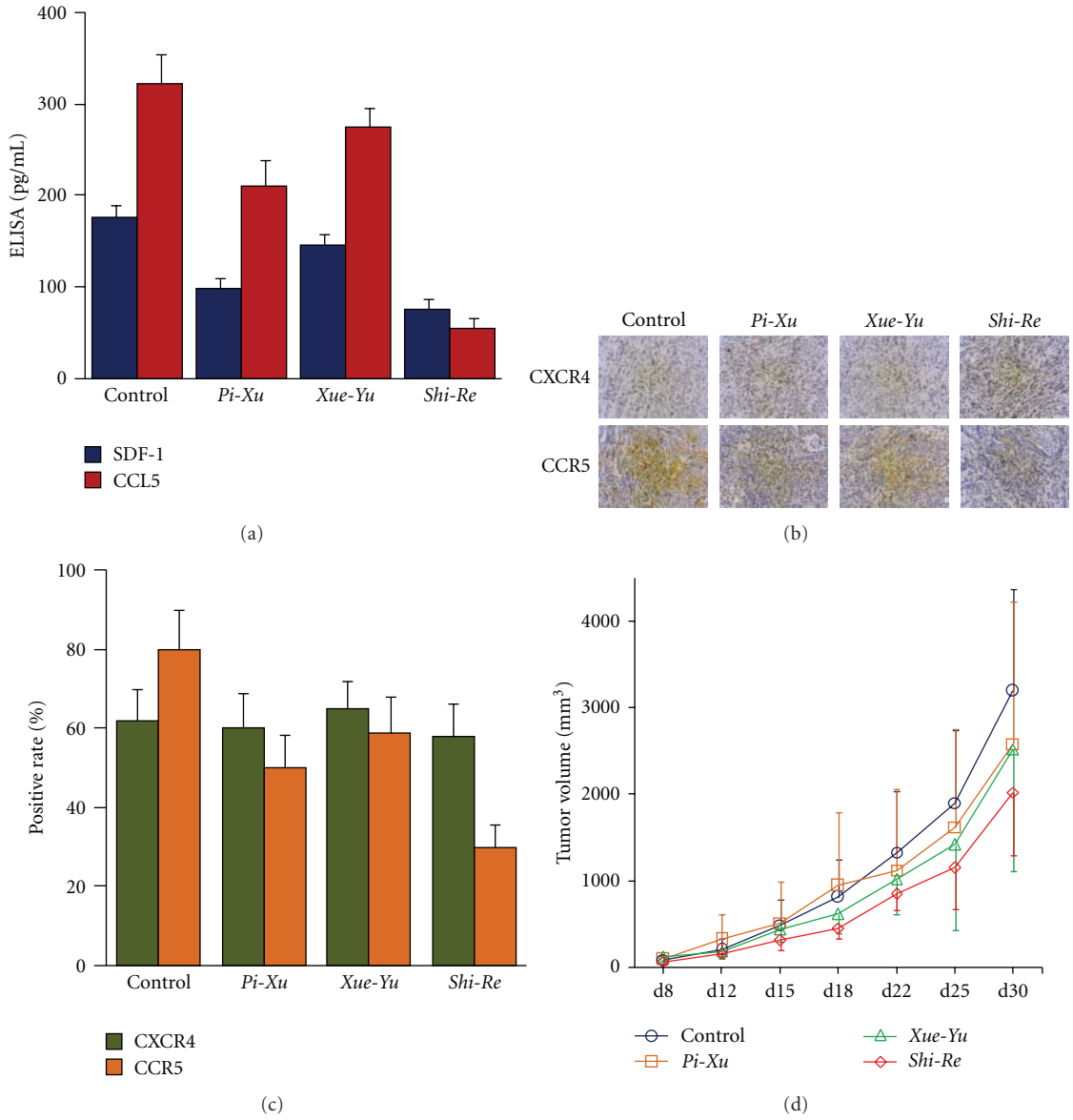


Figure 3: Correlation between microenvironment and tumor growth under different *ZHENG* conditions. (a) The levels of CAF- and TAM-derived secreted cytokines, SDF-1 and CCL5, in tumor from the *Shi-Re*, *Pi-Xu*, and *Xue-Yu ZHENG* models were evaluated with ELISA assay. Data are expressed as the mean ± SE. (b and c) IHC staining for CXCR4 and CCR5 on sections of tumors from *ZHENG* models was performed and representative photos are shown in (b). The positive rates of CXCR4 and CCR5 protein in tumors with indicated *ZHENG* are shown in (c). (d) Effect of *ZHENG* on tumor growth in a subcutaneously transplanted tumor model. Xenograft tumor model combined with *ZHENG* model was established as described in Figure 2(a). Mean volumes of tumors from each group were measured. Mean ± standard deviation was determined for 10 mice in each group.

microenvironment. Finally we evaluated the effects of the herbal medicines on tumor growth and could not find any difference when the tumors were treated with different types of herbal medicine (Figure 4(d)). Therefore, our results suggest that a prescription based solely on *ZHENG* does not always result in a satisfactory response.

3.5. Herbal-Medicine-Induced Alteration of Tumor Microenvironment Is Correlated with Treatment Response. Although tumors under different *ZHENG* conditions demonstrated differences in tumor microenvironment which may finally be reflected in tumor growth, the *ZHENG* conditions themselves seemed not to promote tumor growth. Thus, herbal medicines prescribed on the basis of the *ZHENG* condition alone did not affect tumor growth. To further understand the relatedness of tumor microenvironment, *ZHENG*, and the response to herbal treatment, we employed the *Qingyihuaji* formula (QYHJ), a prescription based on TCM theory whereby pancreatic cancer is considered to be of *Shi-Re* origin. QYHJ has been used to treat pancreatic cancer for

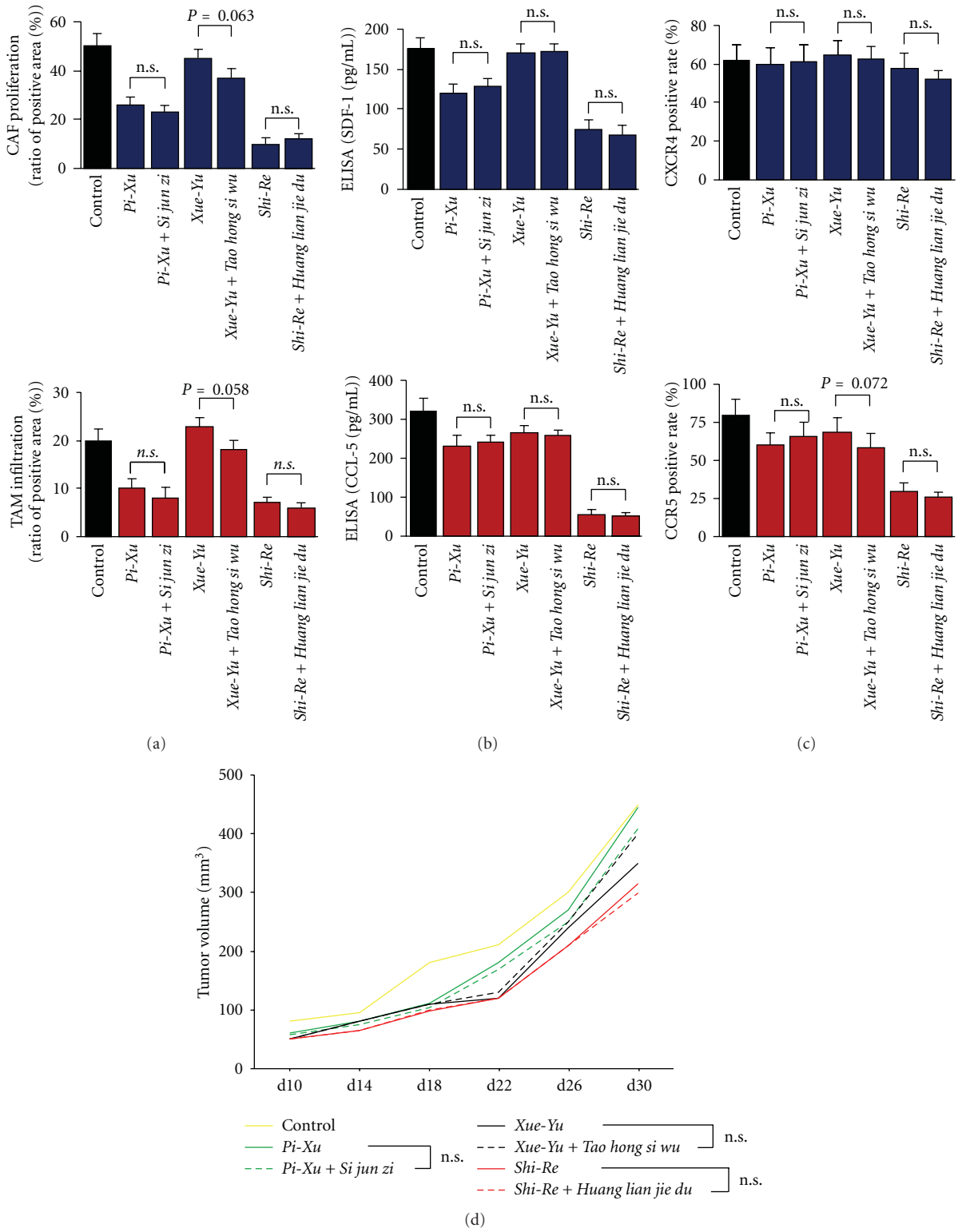


Figure 4: Response of tumors under different *ZHENG* conditions to herbal medicine treatments. (a) IHC staining for vimentin, *a*-SMA, and CD68 in tumors from the indicated group was performed for evaluating CAF proliferative activities and TAM infiltration, respectively. CAF proliferative activity and TAM infiltration were quantitatively evaluated as described in Figure 2(b). (b) The levels of SDF-1 and CCL5 in indicated tumors were evaluated with ELISA assay. Data are expressed as the mean ± SE. (c) IHC staining for CXCR4 and CCR5 on sections of indicated tumors. The positive rates of CXCR4 and CCR5 protein in tumors with indicated *ZHENG* was calculated. (d) Effect of herbal medicine on subcutaneously transplanted tumor with different *ZHENG*. The growth curves for each are shown. n.s.: not significant.

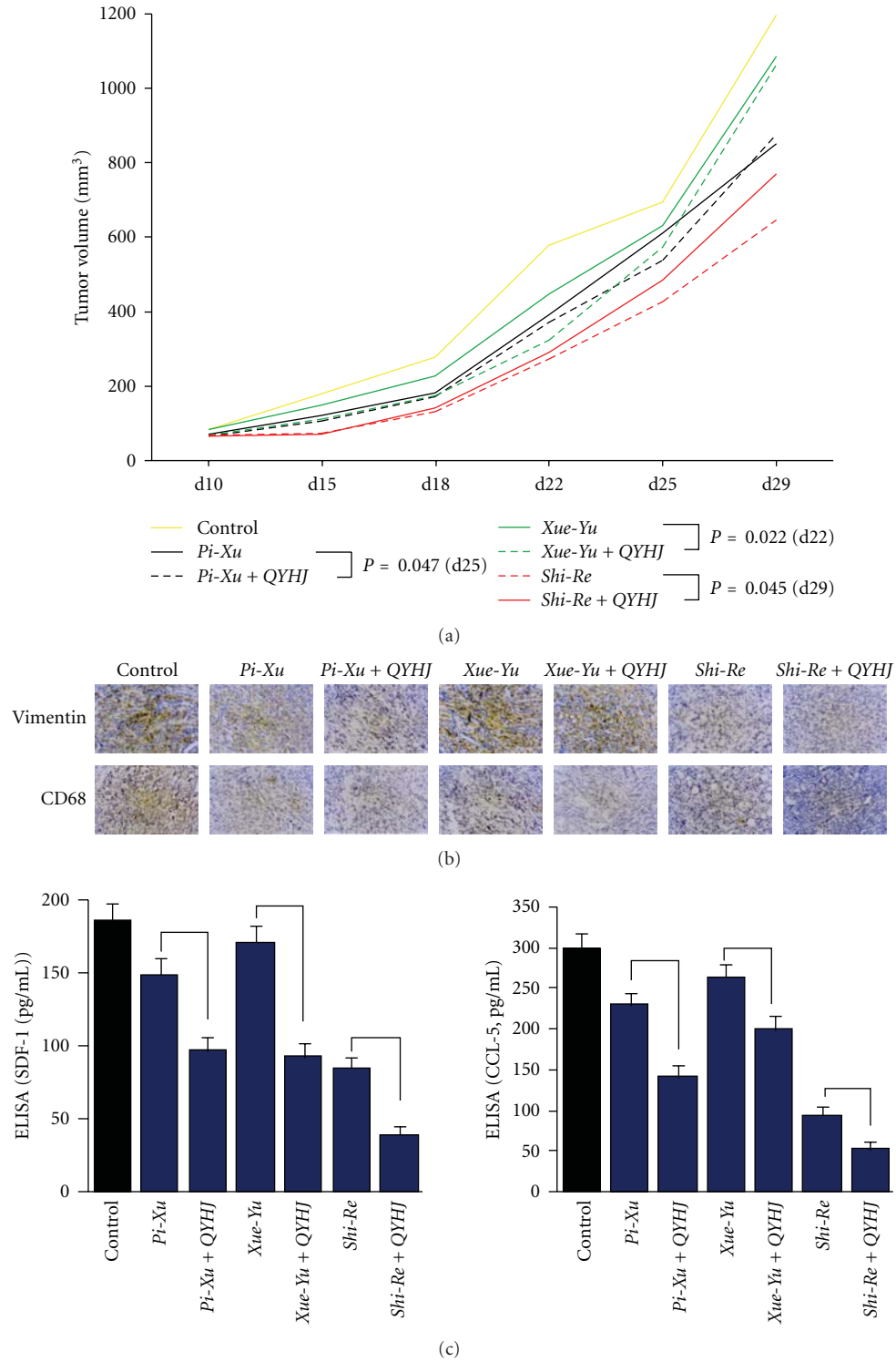


Figure 5: Treatment response to herbal medicine involved with modification of tumor microenvironment. (a) The antitumor effect of Qingyihuaji formula (QYHJ) on tumor with different *ZHENG*. (b) The effect of QYHJ on CAF proliferative activities and TAM infiltration were evaluated as described in Figure 2. (c) The effect of QYHJ on secreted SDF-1 and CCL5 levels were evaluated as described in Figure 3(a). $^{*}P < 0.05$; $^{**}P < 0.01$.

Table 1: Herbal prescriptions used in this study.

Prescription	Contents (g)
Huang lian jie du decoction	Coptis root (9), baical skullcap root (6), amur corktree bark (6), cape jasmine fruit (9)
Si jun zi decoction	Ginseng (9), largehead atractylodes rhizome (9), poria (9), liquorice root (6)
Tao hong si wu decoction	Peach seed (9), safflower (6), Chinese angelica root (9), rehmannia root (12), rhizome of szechwan lovage (6), radix paeoniae rubra (9)
Qingyihuaji formula	Herba scutellariae barbatae (15), hedyotidis herba (15), amorphophallus konjac (15), coix seed (15), fiveleaf gynostemma herb (15), java amomum fruit (10)

many years [15, 16]. We first established mouse tumor models with the accompanying *ZHENG* conditions *Shi-Re*, *Pi-Xu*, and *Xue-Yu*. The mice were then treated with QYHJ and the tumor microenvironment and tumor growth were evaluated. We found that tumors of the different *ZHENG* condition models exhibited altered tumor microenvironments (Figures 5(b) and 5(c)), which is consistent with our previous observations (Figure 2). The QYHJ treatments altered the tumor microenvironments in the *Shi-Re*, *Pi-Xu*, *Xue-Yu* models dramatically, as demonstrated by decreased CAF proliferation and TAM infiltration (Figure 5(b) and 5(c)). Then when we correlated tumor microenvironment alteration with tumor growth, we surprisingly found that QYHJ treatment led to growth inhibition of tumors under different *ZHENG* conditions, although the inhibition rate varied among the different *ZHENG* (Figure 5(a)). This suggests that disease identification is sometime a requisite for the treatment of cancer with TCM. Taken together, these results indicate that a combination of disease diagnosis and *ZHENG* identification is essential for clinical TCM practice in cancer treatment. They also showed a relatedness between the tumor environment and *ZHENG*, and treatment response to herbal medicine involved the modification of the tumor microenvironment.

4. Discussion

In this study, we established three different *ZHENG* mouse models according to TCM theory. We identified alterations in the tumor microenvironment under different *ZHENG* conditions. These tumor microenvironment modifications mediated a correlation between the *ZHENG* condition and response to herbal treatment. Therefore our study revealed a molecular basis for *ZHENG* in pancreatic cancer.

In TCM clinical practice, *ZHENG* helps guide the remedy prescription and therefore has an important position in the TCM system, that is, *ZHENG* is the key to recognizing diseases and the foundation to treat them. However, because of the complexity of the concepts (e.g., a single *ZHENG* involves multiple anatomical systems) and lack of nonprofessional descriptions, research of *ZHENG* is difficult to advance. The molecular basis underlying *ZHENG* in TCM remains unclear.

It has been confirmed that tumor cells do not act in isolation, but rather subsist in a rich microenvironment provided by resident fibroblasts, inflammatory cells, endothelial cells, pericytes, leukocytes, and extracellular matrix [17]. It is increasingly appreciated that, as the cancer progresses, the surrounding microenvironment is activated in support, coevolving through continuous paracrine communication and supporting carcinogenesis [3]. Pancreatic ductal adenocarcinoma is characterized by an extensive stromal response called desmoplasia. Within the tumor stroma, CAFs are the primary cell type; the importance of the role of CAFs in tumor progression is now well accepted. CAFs produce large amounts of secreted factors, including CXC, CC chemokines, and other inflammatory mediators that promote the proliferation, invasion, and metastasis of cancer cells [18]. It is also accepted that large numbers of tumor-associated leukocytes infiltrate solid tumors, and TAMs represent a major and important component of these leucocytes, which are driven toward functions that support cancer progression and poorer prognosis [12]. Therefore the stromal elements of tumors hold prognostic, as well as response-predictive, information. Abundant targeting opportunities within the tumor microenvironment are continually identified [3].

As TCM sustains systematic theories and is a holistic approach to health, and our previous study has indicated a correlation between *ZHENG* and levels of cytokines related to CAF and TAM [4], we hypothesized a correlation between the tumor microenvironment and the *ZHENG* syndromes of TCM. Thus we evaluated the tumor microenvironment by immunostaining for CAF and TAM and surprisingly found differences in microenvironment alterations under different *ZHENG* conditions. Furthermore, the alterations in CAF proliferative activity and TAM infiltration led to changed levels of CAF- and TAM-derived secreted cytokines which finally affected tumor growth.

Based on TCM theory and clinical experience, patients with pancreatic cancer usually exhibit *Shi-Re*, *Pi-Xu*, or *Xue-Yu*, and respective herbal decoctions for removing heat and dampness (*Huang lian jie du*), reinforcing Qi and strengthening the spleen (*Si jun zi)*, and promoting blood circulation and removing blood stasis (*Tao hong si wu*) are always prescribed. However the efficacy of these remedies is not always satisfactory. In the current study, the application of decoctions of these herbal medicines had little effect on tumor growth or the tumor microenvironment. This observation seems to contradict the TCM theories of treating the same disease with different methods and treating different diseases with the same methods. There are many reasons that may account for the lack of response to the TCM treatments in the present study. One is that each prescription

has its priority and focus, although they are within the same category for the same *ZHENG*. Another reason is that apart from the traditional relationship between disease and *ZHENG*, there may also exist analogous *ZHENG* in the same disease, which means that different patients who suffer from the same disease manifest the common basic *ZHENG* in spite of slight differences in their accompanying symptoms. Therefore, we can use a basic prescription with slight modifications to treat accompanying symptoms. In fact recent research emphasized the principle of analogous *ZHENG* existing in the same disease in TCM clinical practice [19], especially in cancer treatment. These two reasons may partially explain why the prescriptions based on *ZHENG* used in the present study had little effect on tumor growth.

The integration of disease diagnosis and identification of *ZHENG* have been widely used in cancer treatment [20]. Based on our previous studies, pancreatic cancer is characterized by dampness, heat, and toxicity and should be treated by removing heat and dampness, detoxification and resolving a mass [21]. According to this recognition, we recommend the QYHJ formula in the treatment of pancreatic cancer. The results of our clinical studies suggest that treatment with QYHJ resulted in prolonged survival time for patients with pancreatic cancer [15, 21]. Animal studies showed that QYHJ could inhibit the growth of subcutaneously transplanted pancreatic tumors in nude mice [16]. Just as we can see from this study, QYHJ had an effect on tumor growth and the tumor microenvironment, although the effect varied depending on the *ZHENG* type. Therefore, our study suggests an intrinsic disease-specific *ZHENG*, which should be considered during TCM practice. The study also indicated that the tumor microenvironment influences the tumor response to herbal medicine treatment.

In conclusion, our study showed alterations in the tumor microenvironment under different *ZHENG* conditions. We also confirmed a relatedness between the tumor environment and *ZHENG*, and herbal medicine treatments modified the tumor microenvironment. This study partially unveiled the molecular basis of TCM *ZHENG* in pancreatic cancer.

Acknowledgments

This study was supported by National Science Foundation of China (81001061); Shanghai Nature Science Fund, Shanghai, China (09ZR1406800); Doctoral Programs Foundation of Ministry of Education of China (20090071120076); Shanghai Science and Technology Committee Rising-Star Program (11QA1401300); Medical Talents Training Program of Health Bureau of Shanghai (XYQ2011008); Shanghai Municipal Health Bureau Grant (KY148).

References

[1] A. P. Lu, X. R. Ding, and K. J. Chen, "Current situation and progress in integrative medicine in China," *Chinese Journal of Integrative Medicine*, vol. 14, no. 3, pp. 234–240, 2008.

[2] D. Hanahan and R. A. Weinberg, "Hallmarks of cancer: the next generation," *Cell*, vol. 144, no. 5, pp. 646–674, 2011.

[3] K. Pietras and A. Östman, "Hallmarks of cancer: interactions with the tumor stroma," *Experimental Cell Research*, vol. 316, no. 8, pp. 1324–1331, 2010.

[4] H. Y. Dai, Peng Wang, L. Y. Feng et al., "The molecular mechanisms of traditional Chinese medicine ZHENG syndromes on pancreatic tumor growth," *Integrative Cancer Therapies*, vol. 9, no. 3, pp. 291–297, 2010.

[5] Z. Chen, L.-M. Liu, and Y.-B. Liu, "Characteristics of W256 tumor growth and liver metastasis in mice model with Blood stasis syndrome," *Zhong Yi Yao Xue Kan*, vol. 21, no. 6, pp. 866–867, 2003.

[6] J. A. Joyce and J. W. Pollard, "Microenvironmental regulation of metastasis," *Nature Reviews Cancer*, vol. 9, no. 4, pp. 239–252, 2009.

[7] K. Polyak, I. Haviv, and I. G. Campbell, "Co-evolution of tumor cells and their microenvironment," *Trends in Genetics*, vol. 25, no. 1, pp. 30–38, 2009.

[8] A. Neesse, P. Michl, K. K. Frese et al., "Stromal biology and therapy in pancreatic cancer," *Gut*, vol. 60, no. 6, pp. 861–868, 2011.

[9] L. A. Kunz-Schughart and R. Knuechel, "Tumor-associated fibroblasts (Part I): active stromal participants in tumor development and progression?" *Histology and Histopathology*, vol. 17, no. 2, pp. 599–621, 2002.

[10] A. Mantovani and A. Sica, "Macrophages, innate immunity and cancer: balance, tolerance, and diversity," *Current Opinion in Immunology*, vol. 22, no. 2, pp. 231–237, 2010.

[11] C. Steidl, T. Lee, S. P. Shah et al., "Tumor-associated macrophages and survival in classic Hodgkin's lymphoma," *The New England Journal of Medicine*, vol. 362, no. 10, pp. 875–885, 2010.

[12] A. Mantovani, T. Schioppa, C. Porta, P. Allavena, and A. Sica, "Role of tumor-associated macrophages in tumor progression and invasion," *Cancer and Metastasis Reviews*, vol. 25, no. 3, pp. 315–322, 2006.

[13] N. A. Bhowmick, E. G. Neilson, and H. L. Moses, "Stromal fibroblasts in cancer initiation and progression," *Nature*, vol. 432, no. 7015, pp. 332–337, 2004.

[14] F. Marchesi, P. Monti, B. E. Leone et al., "Increased survival, proliferation, and migration in metastatic human pancreatic tumor cells expressing functional CXCR4," *Cancer Research*, vol. 64, no. 22, pp. 8420–8427, 2004.

[15] H. Ouyang, P. Wang, Z. Meng et al., "Multimodality treatment of pancreatic cancer with liver metastases using chemotherapy, radiation therapy, and/or Chinese herbal medicine," *Pancreas*, vol. 40, no. 1, pp. 120–125, 2011.

[16] P. Wang, Z. Chen, Z. Q. Meng et al., "Ski acts as therapeutic target of Qingyihuaji formula in the treatment of SW1990 pancreatic cancer," *Integrative Cancer Therapies*, vol. 9, no. 1, pp. 50–58, 2010.

[17] R. R. Langley and I. J. Fidler, "The seed and soil hypothesis revisited-The role of tumor-stroma interactions in metastasis to different organs," *International Journal of Cancer*, vol. 128, no. 11, pp. 2527–2535, 2011.

[18] D. Mahadevan and D. D. Von Hoff, "Tumor-stroma interactions in pancreatic ductal adenocarcinoma," *Molecular Cancer Therapeutics*, vol. 6, no. 4, pp. 1186–1197, 2007.

[19] W. J. Wang, "Pay more attention to "Analogous syndrome complex in the same disease and treating the same disease with analogous methods"," *Zhong Xi Yi Jie He Xue Bao*, vol. 6, no. 5, pp. 441–445, 2008.

[20] L. M. Liu, "The prospective of integrative medical treatment of oncology in the next ten years," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 31, no. 7, pp. 870–872, 2011.

[21] Y.-H. Shen, L.-M. Liu, Z. Chen et al., "Combined with chemotherapy for treatment of 32 cases of advanced pancreatic cancer," *Journal of Traditional Chinese Medicine*, vol. 47, no. 2, pp. 115–117, 2006.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 298925, 6 pages
doi:10.1155/2012/298925

Research Article

Interleukin-10 Genotype Correlated to Deficiency Syndrome in Hepatitis B Cirrhosis

Qing-Ya Li,[1,2] Zhi-Zhong Guo,[1,2] Jian Liang,[3] Wei Zhang,[4] Lie-Ming Xu,[5] Yue-Qiu Gao,[5] Xiao-Su Wang,[6] Dong-Ying Xue,[7] and Shi-Bing Su[1]

[1] *Research Center for TCM Complexity System, Shanghai University of TCM, Shanghai 201203, China*
[2] *Henan University of TCM, Zhengzhou, Henan 450008, China*
[3] *Ruikang Hospital of Guangxi University of TCM, Nanning, Guangxi 530011, China*
[4] *Longhua Hospital, Shanghai 200126, China*
[5] *Shuguang Hospital, Shanghai 200021, China*
[6] *Yueyang Hospital, Shanghai 200437, China*
[7] *Putuo Hospital, Shanghai 200060, China*

Correspondence should be addressed to Shi-Bing Su, shibingsu07@163.com

Received 13 February 2012; Accepted 28 March 2012

Academic Editor: Shao Li

Traditional Chinese medicine (TCM) syndrome is an important basis for TCM diagnosis and treatment. As Child-Pugh classification as well as compensation and decompensation phase in liver cirrhosis, it is also an underlying clinical classification. In this paper, we investigated the correlation between single nucleotide polymorphisms (SNPs) of Interleukin-10 (IL-10) and TCM syndromes in patients with hepatitis B cirrhosis (HBC). Samples were obtained from 343 HBC patients in China. Three SNPs of IL-10 (−592A/C, −819C/T, and −1082A/G) were detected with polymerase chain-reaction-ligase detection reaction (PCR-LDR). The result showed the SNP-819C/T was significantly correlated with Deficiency syndrome ($P = 0.031$), but none of the 3 loci showed correlation either with Child-Pugh classification and phase in HBC patients. The logistic regression analysis showed that the Excess syndrome was associated with dizzy and spider nevus, and the Deficiency syndrome was associated with dry eyes, aversion to cold, IL-10-819C/T loci, and IL-10-1082A/G loci. The odds ratio (OR) value at IL-10-819C/T was 4.022. The research results suggested that IL-10-819C/T locus (TC plus CC genotype) is probably a risk factor in the occurrence of Deficiency syndrome in HBC patients.

1. Introduction

Hepatitis B virus (HBV) infection is a major health problem in China. It is one of the major causes of virus-related liver diseases such as liver cirrhosis (LC) and hepatocellular carcinoma (HCC) [1, 2]. There are 350 million HBV-infected people around the world, about 15–25% of who have the risk of dying of the HBV-caused LC and HCC [3]. During the course of HBV infection, liver is gradually damaged by this hepatotropic DNA virus, presenting a wide variety of clinical manifestations ranging from an asymptomatic carrier state to chronic hepatitis B (CHB), and to HBC, even to HCC [2, 4]. Five-year survival rate of patients with severe CHB and HBC is about 50% [5–7]. Whether chronic HBV infection would be developed to CHB or even LC, the process is affected by many factors such as hereditary susceptibility of patients.

DNA sequence and its variations are reflecting the human evolutionary process. SNP is a variation occurring when a single nucleotide in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in an individual [8]. It can help us to understand the occurrence and development of human diseases, and the response to drug therapy. Currently, it is known that at least 93% of human genes could present SNPs [9], and the SNPs have become the research focus of genetic susceptibility, and in the process of infectious diseases [10]. The previous studies have shown the correlation between the

hereditary susceptibility of gene and liver disease, such as SNPs of aldehyde dehydrogenase 2 (ALDH2) and HCC [11], interleukin-2 (IL-2), IFN-γ, IL-10 and the infection of HBV, HCV, or HBV/HCV coinfection [12]. However, the studies of the correlation between genetic susceptibility and HBC are quite few.

The classification of disease is an important basis for the diagnosis of disease. In the LC, the phenotypes have been used to the classification, such as Child-Pugh classification, compensation and decompensation phase, hepatocellular function, and traditional Chinese medicine (TCM) syndromes. The TCM syndrome, also called ZHENG or TCM pattern, is the basic unit and the key concept in TCM theory. All diagnostic and therapeutic methods in TCM are based on the differentiation of ZHENG [13]. ZHENG is an abstraction of TCM theory, which can be seen as a simply assemblage of disease symptoms.

In this study, we investigated the correlation between the IL-10 gene SNPs and phenotypes, which include TCM syndromes, Child-Pugh classification, and compensation or decompensation phase in HBC patients.

2. Materials and Methods

2.1. Patients and Samples. In this study, 343 patients were recruited from Longhua Hospital, Shuguang Hospital, Yueyang Hospital and Putuo Hospital in Shanghai, the First Affiliated Hospital of Henan University of TCM and Ruikang Hospital in Guangxi in China. The patients were selected based on their age, gender, disease classification and area distribution (Table 1). All patients were Chinese yellow race. Blood samples were obtained from all subjects with informed consent and approved by ethic committee in respective hospitals, based on the Declaration of Helsinki. Each subject donated 3 mL peripheral blood samples, which were collected and stored at −80°C before DNA extraction.

2.2. Child-Pugh, Phase, and TCM Syndrome. The patients were divided into class A, class B, and class C according to Child-Pugh-Turcotte (CPT) score, and the CPT score was calculated by rating the following five parameters including serum levels of bilirubin and albumin, prothrombin time, ascites, and encephalopathy [14, 15]. According to clinical symptoms of patients and development of the disease, liver cirrhosis was divided into compensation phase and decompensation phase.

The clinical information of HBC patients such as symptoms and signs was collected from the above 6 hospitals, and then TCM syndromes were classified into Excess, Deficiency, and Deficiency-Excess syndromes by 3 TCM senior physicians according to the define of diagnosis, and TCM syndrome differentiation of liver cirrhosis [16]. The Excess syndrome was including Liver-qi stagnation syndrome, Damp-heat syndrome, and Blood stasis syndrome. The Deficiency-Excess syndrome was Damp abundance and spleen asthenia syndrome. The Deficiency syndromes were including liver-kidney yin deficiency syndrome and Yang deficiency of spleen and kidney syndrome.

Table 1: Clinical data of 343 HBC patients.

Gender	No (%)
Male	242 (70.55)
Female (%)	101(29.45)
Mean age (yr)	49.57 ± 10.02
Child-Pugh-Turcotte score (%)	
A	240 (69.97)
B	75 (21.87)
C	28 (8.16)
Phase (%)	
Compensation phase	203 (59.18)
Decompensation phase	140 (40.82)
Area (%)	
Shanghai	226 (65.89)
Guangxi	69 (20.12)
Henan	48 (13.99)

2.3. DNA Extraction. Blood samples of all the patients were collected in K_2EDTA tubes. Genomic DNA was selected from 1 mL peripheral blood of each sample, using the TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China). Subsequently, the DNA was stored at −80°C for following genotype analysis.

2.4. SNP Genotyping. Firstly, the genomic DNA extracted from clinical samples was subjected to a multiplex PCR (Invitrogen, Carlsbad, CA). Briefly, 1 μL of genomic DNA was added to a 15 uL final volume containing 0.25 μL per primer (10 μmol/L) (prime sequence in Table 2), 0.2 μL Taq enzyme, 1.5 μL 10 × PCR buffer, 0.3 μL dNTPs (2.5 mmol/L), 1.5 uL Mgcl2 and 10 uL deionized water. The reaction mixture was followed by a denaturation step at 94°C for 2 min and 35 cycles of amplification (94°C for 20 s, 60°C for 20 s, and 72°C for 40 s) with a final extension step at 72°C for 3 min through ABI 9600 (Applied Biosystems).

Secondly, LDR assays were carried out using conditions similar to those described elsewhere with slight modifications [17]. Briefly, the final volume containing 2 uL PCR products, 1 uL 10 × Taq DNA ligase buffer, 0.125 uL Taq DNA ligase (40 U/uL, New England Biolabs, Beverly, MA) and 0.01 μL per probe (10 bp) and 6.845 μL deionized water. LDR probes were designed by the Generay Biotechnology Company (probes sequence in Table 3). LDR mixtures were thermally cycled for 20 cycles of 30 s at 94°C and 3 min at 64°C through ABI 9600 (Applied Biosystems).

Lastly, the mixture of 1 μL LDR product and 2 μL loading Dye was followed by a denaturation step at 95°C for 3 min and was immediately put into ice-water. Then, the products were detected by ABI 3730XL DNA sequencer (Applied Biosystems).

Additionally, about 5% of the samples were randomly selected and retested by direct DNA sequencing in Shanghai National Biochip Research Center Laboratory, and the results were concordant with PCR-LDR.

Table 2: The gene position, polymorphism, primer sequences, and gene frequencies of IL-10 SNPs.

Gene position	Rs number	Polymorphism	Primer sequence	Gene frequencies (%)
IL-10-592 A/C	rs1800872	A/C	F: AAGAGGTGGAAACATGTGCC R: TACCCAAGACTTCTCCTTGC	22.20
IL-10-819 C/T	rs1800871	C/T	F: ATGGTGTACAGTAGGGTGAG R: TTTCCACCTCTTCAGCTGTC	57.70
IL-10-1082 A/G	rs1800896	A/G	F: AGAAGTCCTGATGTCACTGC R: AAGTCAGGATTCCATGGAGG	15.10

Table 3: The LDR probes for IL-10 detection.

Gene position	Probes sequence
IL-10-592 A/C	A60-S7-TA: TTTTTTTTAACACATCCTGTGACCCCGCGTGTA
	A60-S7-TC: TTTTTTTTTTTAACACATCCTGTGACCCCGCGTGTC
	A60-S7-TR: -P-CTGTAGGAAGCCAGTCTCTGGAAAGTTTTTT-FAM-
IL-10-819 C/T	A60-S6-TC: TGTACCCTTGTACAGGTGAAGTAAC
	A60-S6-TT: TTTTGTACCCTTGTACAGGTGAAGTAAT
	A60-S6-TR: -P-ATCTCTGTGCCTCAGTTTGCTCACT-FAM-
IL-10-1082 A/G	A60-S5-TA: AACACTACTAAGGCTTCTTTCGGAA
	A60-S5-TG: TTTAACACTACTAAGGCTTCTTTCGGAG
	A60-S5-TR: -P-GGGGAAGTAGGGATAGGTAAGAGGA-FAM-

2.5. Statistical Analysis. The data determined by the frequency of genotype obeyed the Hardy-Weinberg equilibrium (HWE) between the observed and expected genotype values. The correlation between genotypes and phenotypes was compared by the X^2 test. $P < 0.05$ was considered statistically significant in all tests. A binary logistic regression analysis was used for the evaluation of the independent effect of IL-10 SNPs on the TCM symptoms of HBC. Odds ratio (OR) and 95% confidence interval (CI) were rated.

3. Results

3.1. Characteristics of the Study Population. The frequencies of 3 SNPs loci of IL-10 were assessed in 343 HBC patients in China. The Hardy-Weinberg equilibrium (HWE) test showed that the distribution of these tested genotypes was not significantly different from the expected distribution ($P > 0.05$) (Table 1). The ages of patients ranged from 18 to 65 years old (mean ± SD, 49.57 ± 10.02). There were no significant differences of age and sex in gene polymorphisms in research object ($P > 0.05$). Males were 242 (70.55%) and females were 101 (29.45%). In Child-Pugh classification, class A, class B, and class C were 240 (69.97%), 75 (21.87%), and 28 (8.16%), respectively. There were 203 (59.18%) in compensation phase and 140 (40.82%) in decompensation phase.

3.2. Correlation between IL-10 Genotypes and Child-Pugh Classification and Compensation or Decompensation Phase in HBC Patients. As shown in Table 4, there was no significant correlation between IL-10 genotypes and Child-Pugh classification in HBC patients. It showed that the P value was greater than 0.05 between IL-10 genotypes (−592A/C, −819C/T, and −1082A/G) and class A, class B and class C of Child-Pugh classification, respectively. Also, there was no significant correlation between IL-10 genotypes (−592A/C, −819C/T, and −1082A/G) and compensation or decompensation phase, respectively ($P > 0.05$).

3.3. Correlation between IL-10 Genotypes and TCM Syndromes in HBC Patients. The correlation was analyzed between IL-10 genotypes (−592A/C, −819C/T, and −1082A/G) and TCM syndromes in HBC patients. As shown in Table 5, TC plus CC genotype of IL-10-819C/T was significantly different with TT genotype ($P = 0.031$) between Deficiency syndrome and other TCM syndromes. However, there was no significant correlation between IL-10-592A/C and −1082A/G genotypes and TCM syndromes ($P > 0.05$). It indicated that the patients with TC plus CC genotype of IL-10-819C/T may be appearance of Deficiency syndrome.

3.4. Correlation between the TCM Syndromes and Clinical Data and IL-10 SNPs in HBC Patients. To further clarify the correlation between Excess syndrome or Deficiency syndrome and clinical data and IL-10 SNPs in HBC patients, the binary logistic regression analysis was carried out. The analytic parameters were including age, gender, IL-10 SNPs loci (−592A/C, −819C/T, and −1082A/G), clinical symptoms and signs (fatigue, poor appetite, abdominal distension, backache, limp aching knees, dry eyes, dizzy, pruritus, yellow urine, aversion to cold, loose stools, spider nevus, ascites) and hepatocellular function parameters (ALT, AST, bilirubin and albumin, prothrombin time). The results showed that the Excess syndrome was associated with dizzy and spider nevus (Table 6), and the Deficiency syndrome was associated with dry eyes, aversion to cold, IL-10-819C/T, and −1082A/G loci

Table 4: Correlation between IL-10 genotypes and Child-Pugh classification or compensation and decompensation phase in HBC patients.

Gene/genotype	Child-Pugh classification			P	Phase		P
	Class A (%) ($n = 240$)	Class B (%) ($n = 75$)	Class C (%) ($n = 28$)		Compensation ($n = 203$)	Decompensation ($n = 140$)	
IL-10-592 A/C							
AA	104 (43.7)	35 (46.7)	13 (46.4)		83 (40.9)	69 (50.0)	
AC	108 (45.4)	35 (46.7)	13 (46.4)	0.839	95 (46.8)	61 (44.2)	0.072
CC	26 (10.9)	5 (6.7)	2 (7.1)		25 (12.3)	8 (5.8)	
IL-10-819C/T							
TT	114 (47.5)	36 (48.0)	12 (42.9)		90 (44.3)	72 (51.4)	
CT	103 (42.9)	32 (42.7)	15 (53.6)	0.770	89 (43.8)	61 (43.6)	0.076
CC	23 (9.6)	7 (9.3)	1 (3.6)		24 (11.8)	7 (5.0)	
IL-10-1082A/G							
AA	212 (88.3)	65 (86.7)	22 (78.6)		182 (89.7)	177 (88.5)	
AG	27 (11.3)	10 (13.3)	6 (21.4)	0.340*	20 (9.9)	23 (11.5)	0.710*
GG	1 (0.4)	0 (0)	0 (0)		1 (0.5)	0 (0)	

* Between AA and AG + GG of IL-10-1082A/G.

Table 5: Correlation between IL-10 genotypes and TCM syndromes in HBC patients.

TCM syndrome type	IL-10-592		P	IL-10-819		P	IL-10-1082		P
	AA	AC + CC		TT	TC + CC		GG	AG + AA	
Excess syndrome	197	29	0.999	111	112	0.600	22	203	0.969
Deficiency-Excess syndrome	41	7	0.735	27	24	0.470	4	49	0.621
Deficiency syndrome	61	8	0.778	23	46	**0.031**	7	56	0.726
Total	299	44		163	180		33	308	

* X^2 test.

(Table 6). The odds ratio (OR) value at IL-10-819C/T was 4.022. It further indicated that IL-10-819C/T locus (TC plus CC genotype) is probably a very high risk in the occurrence of Deficiency syndrome in HBC patients.

4. Discussion

TCM syndrome classification, also defined as ZHENG differentiation, is the basic concept in the TCM theory. TCM syndrome, a profile of symptoms and signs as a series of clinical phenotypes, plays an important role in understanding the human homeostasis and guiding the applications of TCM treatment. All diagnostic and therapeutic methods in TCM are based on the differentiation of the TCM pattern, and this concept has been used for thousands of years in China [18]. The "Heat," "Cold," "Excess," and "Deficiency" are the four basic syndromes in TCM [19]. In TCM practice, an experiential diagnosis approach has been frequently used to classify Excess, Deficiency, and Deficiency-Excess syndrome in HBC patients. In order to replace the traditional experiential diagnosis, the scientific evidence for TCM syndrome classification is essential, and it would be beneficial to understand the classification and essence of the TCM syndrome.

IL-10 is an important immunoregulatory cytokine mainly produced by activated T cells, monocytes, B cells, and thymocytes. As an immune response modulator, IL-10 can both stimulate and suppress the immune response [20]. Several polymorphic sites of IL-10 gene promoter region have been described, including three biallelic polymorphisms at positions −1082A/G, −819C/T, and −592A/C from the transcription start site. The IL-10-819C/T C and T alleles were completely in linkage disequilibrium with the IL-10-592A/C A and C alleles, respectively. The −592A allele was exclusively associated with the −1082A allele. These result in three different haplotypes: GCC, ACC, and ATA [21]. It has been reported that IL-10 gene SNP was associated with several diseases such as breast cancer [22], cervical cancer [23], multiple myeloma [24], and gastric carcinoma [25]. Moreover, IL-10 promoter polymorphism was associated with the progression of HBV infection [26].

Previous studies have shown that TCM syndrome is associated with gene SNPs. For example, the people with 5-HTTLPR SS genotype polymorphism may be the susceptible population of Excess of liver Yang syndrome [27]. The K allele of ABCA1 gene may be protective factors of phlegm syndrome and blood stasis syndrome in coronary heart disease [28]. The kidney-Yang Deficiency syndrome (KDS) is closely related with special SNP linkage disequilibrium in the intragenic level, and genes within the flanks of these SNPs suggest some essential symptoms of KDS [29]. There was correlation between liver-qi stagnation syndrome and gene polymorphism of tryptophan hydroxylase (TPH) and G-proteinβ3 submit (GNB3) in HBC patients [30]. We have

Table 6: Correlation between Excess or Deficiency syndrome and clinical data and IL-10 gene SNPs in HBC patients.

Factors	B	SE	Wald	P	OR	95%CI	
						Lower	Upper
Excess syndrome							
Abdominal distension	0.277	0.148	3.509	0.061	1.319	0.987	1.763
Dizzy	0.658	0.203	10.458	0.001	1.931	1.296	2.876
Spider nevus	0.385	0.180	4.594	0.032	1.469	1.033	2.089
Constant	0.173	0.199	0.755	0.385	1.189		
Deficiency syndrome							
Dry eyes	0.448	0.191	5.518	0.019	1.566	1.077	2.276
Aversion to cold	0.605	0.203	8.868	0.003	1.830	1.230	2.725
IL-10-819C/T	1.392	0.442	9.921	0.002	4.022	1.692	9.563
IL-10-1082A/G	−0.903	0.430	4.415	0.036	0.406	0.175	0.941
Constant	−1.163	0.777	2.240	0.134	0.313		

been investigated some cytokine such as TNF-α, TGF-β_1, and IL-10 [31] and further found that IL-10 genotype may correlate with TCM syndrome in HBC patients [32].

In this study, therefore, to further investigate whether IL-10 genotypes correlated really to TCM syndromes, more samples from different area (Shanghai, Henan and Guangxi in China) were applied, compared to Child-Pugh classification and compensation or decompensation phase. The results showed that IL-10-819C/T locus was significantly correlated to Deficiency syndrome ($P = 0.031$), and IL-10 gene loci (−592A/C, −819C/T, and −1082A/G) were not correlated to either Child-Pugh classification or compensation and decompensation phase in HBC patients. The binary logistic regression analysis showed that the Deficiency syndrome was associated with dry eyes, aversion to cold, IL-10-819C/T and IL-10-1082A/G locus, and OR value at IL-10-819C/T was 4.022. The research results suggested that IL-10-819C/T locus (TC plus CC genotype) might correlate with the risk in the occurrence of Deficiency syndrome in HBC patients. The study provided a proof for TCM syndrome classification, which would be helpful to the TCM clinical diagnosis in HBC patients.

Though our results showed that IL-10 genotype might correlate with Deficiency syndrome in HBC patients, it is difficult to understand the relationship between IL-10 SNPs and TCM syndromes, while TCM syndrome changes following patient's condition and disease situation. In recent years, following the implementation of Human Genome Project and high throughput Genomic strategies, a large number of human complex diseases associated genetic variants have been identified through Genome-wide association studies (GWAS) [33]. To discover genetic base of TCM syndrome changes as well as other phenotypes of diseases, the GWAS method might provide important clues in future research.

5. Conclusion

In this study, we identified that IL-10-819C/T locus was significantly correlated to Deficiency syndrome, and the OR value was 4.022, and indicated that HBC patients with the CC genotype plus TC genotype at IL-10-819C/T might correlate with the risk in the occurrence of Deficiency syndrome.

Acknowledgments

This paper was supported by National Science and Technology Major Project of China (no. 2012ZX10005001-004), National Natural Science Funds (no. 81073134), Leading Academic Discipline Project of Shanghai Municipal Education Commission (no. J50301), and E-institutes of Shanghai Municipal Education Commission (no. E 03008).

References

[1] X. Zhang, H. Zhang, and L. Ye, "Effects of hepatitis B virus X protein on the development of liver cancer," *Journal of Laboratory and Clinical Medicine*, vol. 147, no. 2, pp. 58–66, 2006.

[2] B. J. McMahon, "Epidemiology and natural history of hepatitis B," *Seminars in Liver Disease*, vol. 25, supplement 1, pp. 3–8, 2005.

[3] H. S. Margolis, M. J. Alter, and S. C. Hadler, "Hepatitis B: evolving epidemiology and implications for control," *Seminars in Liver Disease*, vol. 11, no. 2, pp. 84–92, 1991.

[4] W. M. Lee, "Hepatitis B virus infection," *The New England Journal of Medicine*, vol. 337, no. 24, pp. 1733–1745, 1997.

[5] J. I. Weissberg, L. L. Andres, C. I. Smith, S. Weick, J. E. Nichols, and G. Garcia, "Survival in chronic hepatitis B. An analysis of 379 patients," *Annals of Internal Medicine*, vol. 101, no. 5, pp. 613–616, 1984.

[6] Y. F. Liaw, D. I. Tai, C. M. Chu, and T. J. Chen, "The development of cirrhosis in patients with chronic type B hepatitis: a prospective study," *Hepatology*, vol. 8, no. 3, pp. 493–496, 1988.

[7] F. E. de Jongh, H. L. A. Janssen, R. A. de Man, W. C. J. Hop, S. W. Schalm, and M. van Blankenstein, "Survival and prognostic indicators in hepatitis B surface antigen-positive cirrhosis of the liver," *Gastroenterology*, vol. 103, no. 5, pp. 1630–1635, 1992.

[8] http://en.wikipedia.org/wiki/Single nucleotide polymorphism.

[9] R. Sachidanandam, D. Weissman, S. C. Schmidt et al., "A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms," *Nature*, vol. 409, no. 6822, pp. 928–933, 2001.

[10] M. Helminen, N. Lahdenpohja, and M. Hurme, "Polymorphism of the interleukin-10 gene is associated with susceptibility to Epstein-Barr virus infection," *Journal of Infectious Diseases*, vol. 180, no. 2, pp. 496–499, 1999.

[11] M. Munaka, K. Kohshi, T. Kawamoto et al., "Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma," *Journal of Cancer Research and Clinical Oncology*, vol. 129, no. 6, pp. 355–360, 2003.

[12] Q. J. Gao, D. W. Liu, S. Y. Zhang et al., "Polymorphisms of some cytokines and chronic hepatitis B and C virus infection," *World Journal of Gastroenterology*, vol. 15, no. 44, pp. 5610–5619, 2009.

[13] S. Li, Z. Q. Zhang, L. J. Wu, X. G. Zhang, Y. D. Li, and Y. Y. Wang, "Understanding ZHENG in traditional Chinese medicine in the context of neuro-endocrine-immune network," *IET Systems Biology*, vol. 1, no. 1, pp. 51–60, 2007.

[14] E. Cholongitas, G. V. Papatheodoridis, M. Vangeli, N. Terreni, D. Patch, and A. K. Burroughs, "Systematic review: the model for end-stage liver disease—should it replace Child-Pugh's classification for assessing prognosis in cirrhosis?" *Alimentary Pharmacology and Therapeutics*, vol. 22, no. 11-12, pp. 1079–1089, 2005.

[15] E. Christensen, "Prognostic models including the Child-Pugh, MELD and Mayo risk scores—where are we and where should we go?" *Journal of Hepatology*, vol. 41, no. 2, pp. 344–350, 2004.

[16] Y. X. Zhang, "Dlagnosis, syndrome differentiation of TCM and evaluate the curative effect of liver cirrhosis (tentative scheme)," *Chinese Journal of Integrative Medicine*, vol. 14, pp. 237–238, 1994.

[17] Z. Xiao, J. Xiao, Y. Jiang et al., "A novel method based on ligase detection reaction for low abundant YIDD mutants detection in hepatitis B virus," *Hepatology Research*, vol. 34, no. 3, pp. 150–155, 2006.

[18] C. Keji and X. Hao, "The integration of traditional Chinese medicine and Western medicine," *European Review*, vol. 11, no. 2, pp. 225–235, 2003.

[19] W. Y. Jiang, "Therapeutic wisdom in traditional Chinese medicine: a perspective from modern science," *Trends in Pharmacological Sciences*, vol. 26, no. 11, pp. 558–563, 2005.

[20] S. Mocellin, F. M. Marincola, and H. A. Young, "Interleukin-10 and the immune response against cancer: a counterpoint," *Journal of Leukocyte Biology*, vol. 78, no. 5, pp. 1043–1051, 2005.

[21] J. Eskdale, V. Keijsers, T. Huizinga, and G. Gallagher, "Microsatellite alleles and single nucleotide polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus," *Genes and Immunity*, vol. 1, no. 2, pp. 151–155, 1999.

[22] F. Kong, J. Liu, Y. Liu, B. Song, H. Wang, and W. Liu, "Association of interleukin-10 gene polymorphisms with breast cancer in a Chinese population," *Journal of Experimental and Clinical Cancer Research*, vol. 29, no. 1, p. 72, 2010.

[23] G. A. Stanczuk, E. N. Sibanda, C. Perrey et al., "Cancer of the uterine cervix may be significantly associated with a gene polymorphism coding for increased IL-10 production," *International Journal of Cancer*, vol. 94, no. 6, pp. 792–794, 2001.

[24] C. Zheng, D. Huang, L. Liu et al., "Interleukin-10 gene promoter polymorphisms in multiple myeloma," *International Journal of Cancer*, vol. 95, no. 3, pp. 184–188, 2001.

[25] M. S. Wu, S. P. Huang, Y. T. Chang et al., "Tumor necrosis factor-α and interleukin-10 promoter polymorphisms in Epstein-Barr virus-associated gastric carcinoma," *Journal of Infectious Diseases*, vol. 185, no. 1, pp. 106–109, 2002.

[26] S. Miyazoe, K. Hamasaki, K. Nakata et al., "Influence of interleukin-10 gene promoter polymorphisms on disease progression in patients chronically infected with hepatitis B virus," *American Journal of Gastroenterology*, vol. 97, no. 8, pp. 2086–2092, 2002.

[27] J. S. You, S. Y. Hu, and H. G. Zhang, "Study on emotion mea surement of liver syndromes in TCM and its correlative study on polymorphism of serotonin (5 HT) transporter gene," *Zhongguo Yi Yao Xue Bao*, vol. 19, no. 11, pp. 669–671, 2004.

[28] Y. F. Wu, Y. C. Zhou, and X. S. Zhang, "Association between traditional Chinese medicine syndrome of coronary atherosclerotic heart disease and polymorphism of R219K of ABCAl gene in Chinese Han male patients," *Zhongguo Zu Zhi Gong Cheng Yan Jiu Yu Lin Chuang Kang Fu*, vol. 10, no. 7, pp. 7–9, 2006.

[29] W. J. Ding, Y. Z. Zeng, W. H. Li et al., "Identification of linkage disequilibrium SNPs from a Kidney-yang deficiency syndrome pedigree," *American Journal of Chinese Medicine*, vol. 37, no. 3, pp. 427–438, 2009.

[30] Y. B. Liu, W. Zhang, S. B. Su et al., "Association between liver cirrhosis of hepatitis B cases with Ganqi depression pattern and 5HTTLPRVNTRs,TPH A218C,GNB3 C825T polymorphism," *Chinese Journal of Integrated Traditional and Western Medicine on Liver Diseases*, vol. 20, no. 5, pp. 267–271, 2010.

[31] Z. L. Jiang, H. Zhang, S. B. Su, W. Zhang, and Y. B. Liu, "Relationship between gene polymorphisms of TNF-α, TGF-β1 and IL-10 and posthepatitis B-hepatitic cirrhosis," *World Chinese Journal of Digestology*, vol. 17, no. 31, pp. 3263–3268, 2009.

[32] Z. L. Jiang, W. Zhang, H. Zhang, Y. B. Liu, Q. Y. Li, and S. B. Su, "Relationship between gene polymorphisms of interleukin-10 and syndrome types of traditional Chinese medicine in post hepatitis B cirrhosis," *Zhong Xi Yi Jie He Xue Bao*, vol. 11, no. 7, pp. 1052–1056, 2009.

[33] J. Hardy and A. Singleton, "Genome-wide association studies and human disease," *The New England Journal of Medicine*, vol. 360, no. 17, pp. 1759–1768, 2009.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 697028, 11 pages
doi:10.1155/2012/697028

Research Article

Study on TCM Syndrome Identification Modes of Coronary Heart Disease Based on Data Mining

Qi Shi, Huihui Zhao, Jianxin Chen, Xueling Ma, Yi Yang, Chenglong Zheng, and Wei Wang

Beijing University of Chinese Medicine, 11 Bei San Huan Dong Lu, ChaoYang District, Beijing 100029, China

Correspondence should be addressed to Wei Wang, wangwei@bucm.edu.cn

Received 14 January 2012; Revised 5 March 2012; Accepted 16 March 2012

Academic Editor: Shao Li

Coronary heart disease (CHD) is one of the most important types of heart disease because of its high incidence and high mortality. TCM has played an important role in the treatment of CHD. Syndrome differentiation based on information from traditional four diagnostic methods has met challenges and questions with the rapid development and wide application of system biology. In this paper, methods of complex network and CHAID decision tree were applied to identify the TCM core syndromes of patients with CHD, and to establish TCM syndrome identification modes of CHD based on biological parameters. At the same time, external validation modes were also constructed to confirm the identification modes.

1. Introduction

Coronary heart disease (CHD) is one of the most important types of heart diseases because of its high incidence and high mortality. With the improvement of people's living standards, the prevalence tendency of CHD is rising and the population of youths suffering from CHD is growing. Coronary angiography has been considered as the "golden standard" in CHD diagnosis. CHD was called "thoracic obstruction" in TCM, with a variety of etiological factors; various clinical manifestations and complex syndromes [1]. Syndrome research has always been hot and difficult spots in TCM basic studies. Syndrome differentiation based on information from traditional four diagnostic methods has met challenges and questions with the rapid development and wide application of system biology. The "golden standard" of syndrome diagnosis has not been found yet. The large number and complexity, multilevel relationships of information from four diagnostic methods had constrained the accuracy of syndrome differentiation. Currently, the application of quantitative modes and data mining is developing rapidly [2, 3]. These technologies had provided approaches and methods for TCM syndrome differentiation. Our earlier study showed that the characteristics of information from four diagnostic methods above were in line with that of complex networks; not only in common with special nature on the basis of their own evolutionary mechanisms, but also closely contacted with nature and structural features. We also found that biological parameters could be considered as a reflection of the pathomechanism and physiological mechanism, which might be a reflection of syndrome in TCM too [4]. What is more, we have established a mode conducted by four biological parameters which could distinguish CHD patients with blood stasis syndrome from nonblood stasis syndrome patients by means of C5 Decision Tree [5]. This study indicated that core TCM syndromes could be identified by complex networks and biological parameters could be serviced as syndrome identification mode in CHD patients with the method of decision tree.

2. Material and Methods

411 cases of CHD in-patients, aged from 45 to 75, from Anzhen Hospital (Beijing), hospitals of Traditional Chinese Medicine in Zhengzhou, Wuhan and Hubei Province, China-Japan Friendship Hospital (Beijing) and Dongzhimen Hospital (Beijing) (March 1, 2010 to June 30, 2011). All selected patients were diagnosed and confirmed by coronary angiography. Diagnosis standards of CHD refer to "Treatment Guide of Stable Angina" (ACC/AHA/ACP-ASIM, 1999) and "Diagnosis and Treatment Recommendations of Unstable Angina" (Chinese Society of Cardiology, 2000) [6, 7].

Diagnosis standards of TCM syndrome refer to "Guiding principles for the clinical study of Chinese Medicines" (2002) and "Terminology for Traditional Chinese Medicine clinical practice-Part of the syndrome" (1997) [8, 9]. All hospitalized patients had signed informed consent voluntarily. Excluded cases were patients who suffered from acute myocardial infarction, myocarditis, pericardial disease, cardiac neurosis, intercostal neuralgia, menopausal syndrome, or severe spondylosis; angina caused by rheumatic fever, syphilis, congenital coronary artery abnormalities, hypertrophic cardiomyopathy, aortic stenosis, or regurgitation; stroke, lung infection, nephritis, renal failure, urinary tract infections, rheumatism, severe arrhythmia, heart failure, cancer, other primary, and serious diseases of liver, kidney, hematopoietic system. Pregnant or lactating women, patients with allergies or psychosis, were also excluded. Demographic details of CHD patients with or without qi deficiency syndrome and phlegm-blood stasis syndrome were showed in Supplemental Tables 1 and 2 (Supplementary material available online at doi: 10.1155/2012/697028).

2.1. TCM Syndrome Differentiation and Collections of Clinical Data. TCM syndrome was confirmed by three TCM deputy director physicians who had more than five years of clinical experiences. It should be performed within 24 hours since the patients were admitted to hospital. 90 clinical testing indicators from blood routine examination, urine routine examination, blood biochemical test, blood coagulation test, thyroid function, TNI, BNP, electrocardiogram (ECG), and echocardiography were collected within one week. 69 symptoms from four diagnostic methods were also collected, including chest pain, chest distress, short breath, cardiopalmus, cough, hypodynamia, spontaneous perspiration, night sweat, burning sensation of five centres, eyestrain, dry mouth, dizziness, amnesia, fainting feeling, tinnitus, insomnia, irritable tantrum, hypochondrium distending pain, sighing, depression, anorexia, abdominal distension, epigastric fullness, belching, nausea and vomiting, sore waist and knee, frequency of micturition at night, limb numbness, heel pain, pachylosis, obesity, white phlegm, yellow phlegm, frothy phlegm, tastelessness in the mouth, bitter taste in the mouth, sweet taste in the mouth, salty taste in the mouth, viscous and greasy taste in the mouth, yellow urine and oliguria, clear urine in large amounts, residual urine, cold abdomen and waist, heavy limbs, darkish complexion, red complexion, conjunctival congestion, dark color around eyes, dark red lip gingival, pale lips and finger nails, dark color in palatal mucosa, lower abdominal tenderness, faint low voice, emaciation, swollen tongue body, tooth-marked tongue, thick tongue coating, greasy tongue coating, thick and greasy tongue coating, yellow tongue coating, glossal petechia, lavender subglossal collateral vessels, blue purple subglossal collateral vessels, mauve subglossal collateral vessels, subglossal collateral vessels engorgement, deep pulse, thready pulse, uneven pulse, and weak pulse.

2.2. Data Processing of Four Diagnostic Information. We identified useful relationships among information from four diagnostic methods above by means of distance-based mutual information model (DMIM) [10]. Then, we established 120 association relationships among 69 symptoms from four diagnostic methods. The association data was consolidated into adjacency matrix and then converted into the format that Pajek software required.

2.3. Measurement of Network Properties and Complex Network Mapping. Pajek software 2.0 was used to analyze the node degrees and node core values of the four diagnostic information network. With the command of "Layout-Energy-Kamada-Kawai-Separate Components," we drew the K-core network figures according to different colors and different degrees, mediated positions of the nodes with manual operation. Nodes and edges of the network could not be deleted. Then, we exported the network figures in Bitmap format.

2.4. Construction of Identification Modes and Validation. Data standardization was used to analyze information of the cases from different hospitals. Next, we establish two identification modes of CHD core syndromes by chi-square automatic interaction detection (CHAID) decision tree. "Qi deficiency" and "phlegm-blood stasis" were considered as dependent variable and 90 biological parameters were independent variables. We set "Parent Node" 50 and "Child Node" 25, allowing the tree model to grow sufficiently. 10-fold cross-validation was used in this research to minimize the bias produced by random sampling of the training and test data samples.

2.5. Construction of External Validation Modes. 212 patients were selected from the 411 cases of CHD to establish new decision tree modes for external validation. Similarly, "qi deficiency" and "phlegm-blood stasis" were considered as dependent variable. 8 and 6 biological parameters got from research above were severed as independent variables. Due to the reduction in the number of independent variables, we set "Parent Node" 2 and "Child Node" 1 to allow the tree model growing much sufficiently. 10-fold cross validation was also used in this section for a validation.

3. Results

3.1. Results of Four Diagnostic Information Network Properties. Properties of four diagnostic information results showed that degree values of 69 nodes were from one to eleven. The degree values of subglossal collateral vessels engorgement, amnesia, faint low voice, white phlegm, heavy limbs, short breath, cough, anorexia, tastelessness in the mouth, as well as swollen tongue body were greater than six, and they indicated the core syndromes of CHD. The core deficiency syndrome was qi deficiency, and the core excessive syndrome was phlegm-blood stasis. From the results of network cores analysis, we found the core values of 31 four diagnostic information nodes were three. These nodes formed a 3-core network together (Table 1).

Table 1: Property values of four diagnostic information network.

Network nodes	Degree	Core value	Network nodes	Degree	Core value
chest pain	2	2	bitter taste in the mouth	3	2
chest distress	4	3	sweet taste in the mouth	3	2
short breath	7	3	salty taste in the mouth	3	2
cardiopalmus	3	3	viscous and greasy taste in the mouth	1	1
cough	7	2	yellow urine and oliguria	1	1
hypodynamia	5	3	clear urine in large amounts	2	1
spontaneous perspiration	4	3	residual urine	2	2
night sweat	2	1	cold abdomen and waist	1	1
burning sensation of five centres	1	1	heavy limbs	8	3
eyestrain	2	1	darkish complexion	2	2
dry mouth	1	1	red complexion	1	1
dizziness	3	3	conjunctival congestion	1	1
amnesia	10	3	dark color around eyes	5	3
fainting feeling	4	3	dark red lip gingiva	3	3
tinnitus	6	3	pale lips and finger nails	2	2
insomnia	2	2	dark color in palatal mucosa	2	1
irritable tantrum	8	3	lower abdominal tenderness”	2	1
hypochondrium distending pain	3	3	faint low voice	9	3
sighing	4	3	emaciation	1	1
depression	2	2	swollen tongue body	7	3
anorexia	7	3	tooth-marked tongue	6	3
abdominal distension	5	3	thick tongue coating	1	1
epigastric fullness	2	2	greasy tongue coating	1	1
belching	2	2	thick and greasy tongue coating	3	3
nausea and vomiting	4	3	yellow tongue coating	6	3
sore waist and knee	9	3	glossal petechia	3	3
frequency of micturition at night	2	2	lavender subglossal collateral vessels	2	2
limb numbness	3	3	blue purple subglossal collateral vessels	3	3
heel pain	1	1	mauve subglossal collateral vessels	1	1
pachylosis	1	1	subglossal collateral vessels engorgement	11	3
obesity	2	2	deep pulse	1	1
white phlegm	8	3	thready pulse	1	1
yellow phlegm	1	1	uneven pulse	2	2
frothy phlegm	1	1	weak pulse	5	3
tastelessness in the mouth	7	3			

3.2. Color Classification Results of Four Diagnostic Information K-Core Network. According to the core values of nodes, we drew the k-core network figure of information from four diagnostic methods (Figure 1). In the center of this network arrayed 31 nodes with the core value of 3, which were important for the network. Nodes with the same color indicated the same syndrome. 31 central nodes suggested 7 TCM syndromes: qi deficiency, qi stagnation, yin deficiency, yang deficiency, blood stasis, phlegm turbid, and heat syndrome. From the figure, qi deficiency, phlegm-blood stasis made up the basic syndromes of CHD patients. Dizziness, hypodynamia, spontaneous perspiration, short breath, faint low voice, cardiopalmus, chest distress, weak pulse, tooth-marked tongue, tastelessness in the mouth, anorexia formed qi deficiency syndrome; swollen tongue body, fainting feeling, white phlegm, as well as thick and greasy tongue coating were the performances of phlegm turbid syndrome. Blue purple subglossal collateral vessels, subglossal collateral vessels engorgement, glossal petechia, dark color around eyes, dark red lip gingival, as well as limb numbness were usually appeared in blood stasis patients.

3.3. Degree Classification Results of Four Diagnostic Information K-Core Network. Figure 2 showed another expression form of four diagnostic information k-core network. In the photo, sizes of the circle represented the degree values of the nodes. Degree value was a simple but most important property of complex network. The degree value of one node

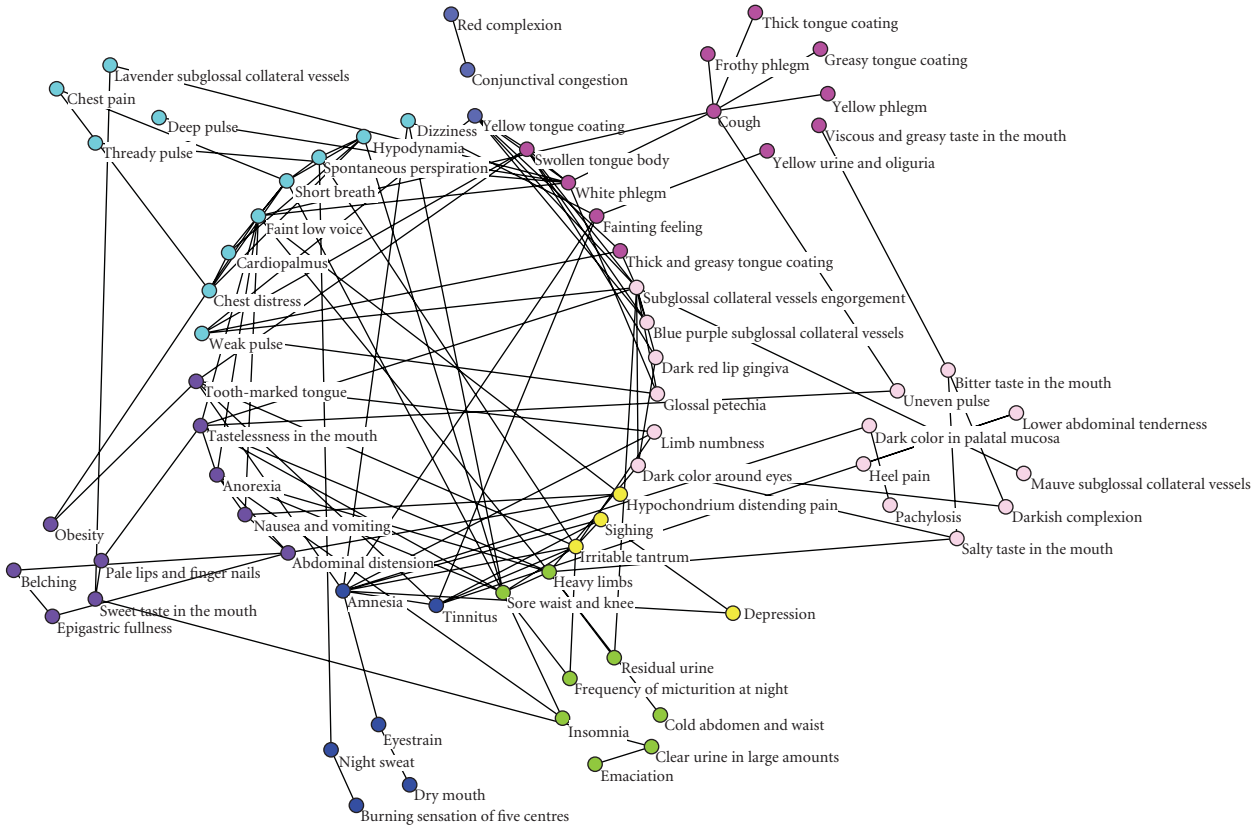


Figure 1: Color classification figure of information from four diagnostic methods K-Core network.

was defined as the number of other nodes that connected to it. In a complex network, the greater degree value that one node was, the more significant role it had played. In qi deficiency syndrome, short breath, hypodynamia, faint low voice, weak pulse, as well as tastelessness in the mouth had the maximum degree values. Swollen tongue body, white phlegm, and cough had played major roles in phlegm turbid syndrome. Subglossal collateral vessels engorgement was the key nodes in blood stasis syndrome, whose degree value was eleven. In this network, we can determine the importance of the nodes by combinations of degree values and core values.

3.4. Results of Identification Mode for Qi Deficiency Syndrome. Using the CHAID decision tree, an identification mode of qi deficiency was built with eight biological parameters. They were urine crystal (X TAL), erythrocyte distribution width-CV (RDW-CV), potassium ion (K), thyroid stimulating hormone (TSH), monocyte (MONO), high sensitive C-reactive protein (hs-CRP), low-density lipoprotein (LDL), and A peak in echocardiography. In this mode, there were 19 nodes and 11 terminal nodes and the tree depth was 3. From this mode we believed that the X TAL was the best predictive variable quantity of qi deficiency syndrome among the 8 parameters. However, we could not distinguish qi deficiency and non-qi deficiency completely only by X TAL. We could fall back on the second grade variable quantities: RDW-CV and MONO. The third grade variable quantities included K, TSH, hs-CRP, LDL, and A peak (Figure 3).

3.5. Results of Identification Mode for Phlegm-Blood Stasis Syndrome. Identification mode of phlegm-blood stasis syndrome was made up of six properties: high-sensitive C-reactive protein (hs-CRP), total bilirubin (TBIL), glutamyl-transpeptidase (GGT), platelet (PLT), fasting blood glucose (FBG), and P-R interval. The depth of this mode was 3. There were 14 nodes and 8 terminal nodes. The 6 parameters formed eight identification paths for phlegm-blood stasis syndrome. The best identification variable of the mode was hs-CRP. Hs-CRP was the only effective variable to identify phlegm-blood stasis syndrome if the value of hs-CRP was between 0.07143 and 0.10714. The second grade variable quantities were TBIL and FBG. The third grade variable quantities were GGT, PLT, and P-R interval (Figure 4).

3.6. Results of Validation for 411 Patients. The result of 10-fold cross-validation showed that in qi deficiency syndrome mode, 302 cases were predicted correctly, while the other 109 cases were wrong classified. The sensitivity and specificity of this mode were 70.2% and 77.4%. The percentage of correct prediction was 73.5%. In phlegm-blood stasis syndrome mode, 328 cases were predicted correctly, the other 83 cases were wrong classified. The sensitivity and specificity of this mode were 72.5% and 81.3%. The percentage of correct prediction was 79.8% (Table 2).

3.7. Results of External Validation Mode for Qi Deficiency Syndrome. With the same CHAID decision tree method,

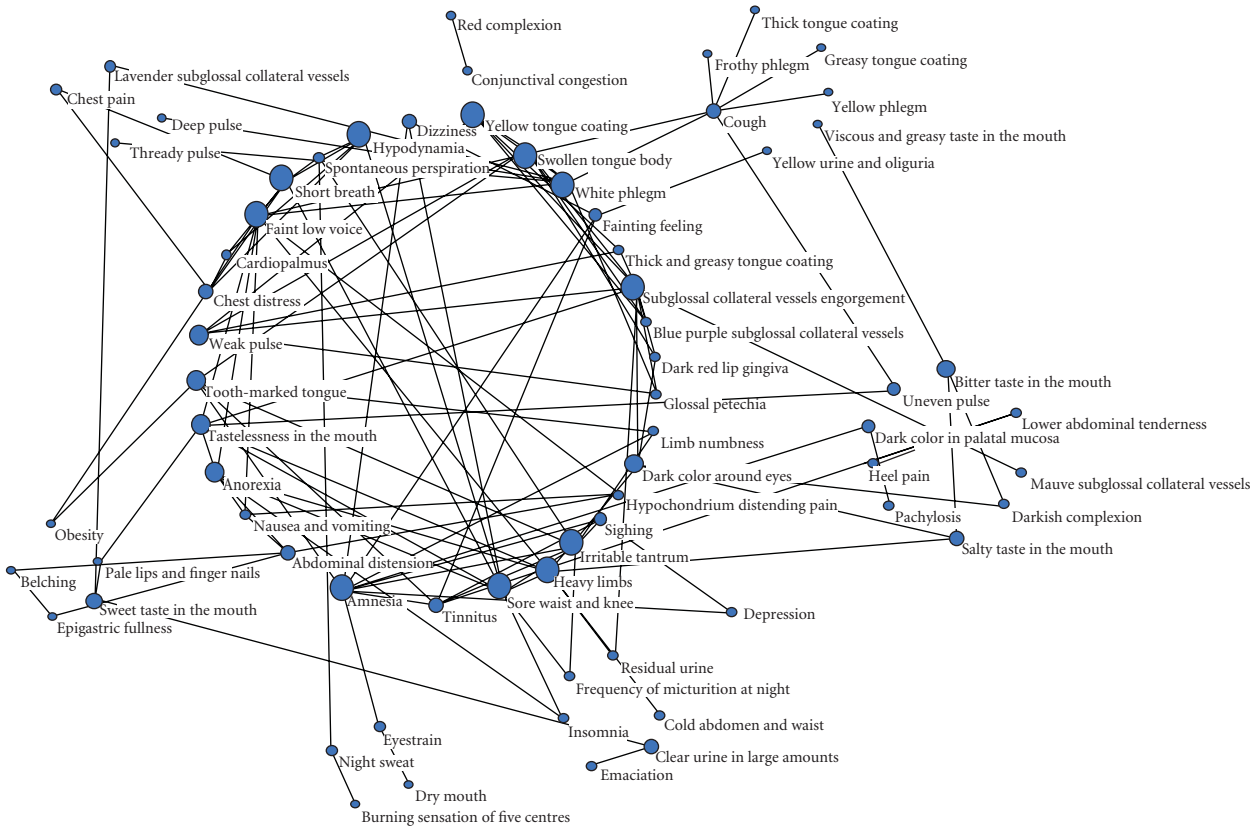


Figure 2: Degree Classification figure of information from four diagnostic methods K-Core network.

Table 2: 10-fold cross-validation results of classification for 411 cases.

CHAID	TN FN	FP TP	Sensitivity (%)	Specificity (%)	Accuracy (%)
Qi deficiency	144 67	42 158	70.2%	77.4%	73.5%
Phlegm-blood stasis	278 19	64 50	72.5%	81.3%	79.8%

Note: sensitivity = TP/(TP + FN); specificity = TN/(TN + FP); accuracy = (TP + TN)/(TP + FP + TN + FN).

an external validation mode of qi deficiency for 211 CHD patients was made up of six biological parameters. Unfortunately, this mode was lack of the parameters of hs-CRP and RDW-CV though we had made the tree model grow effectively as much as possible. The number of nodes in this mode was 18, and the number of terminal nodes was 10. MONO was the best predictive variable quantity of qi deficiency syndrome (Figure 5).

3.8. Results of External Validation Mode for Phlegm-Blood Stasis Syndrome. External validation mode of phlegm-blood stasis syndrome included the same six properties compared with the identification mode above. There were 23 nodes and 14 terminal nodes in this mode. The mode was much more complex, for these 6 parameters formed 12 identification paths for phlegm-blood stasis syndrome. The best identification variable of the mode was still hs-CRP. The second grade variable quantity was P-R interval, and the third ones were the remaining four quantities (Figure 6).

3.9. Results of Validation for 212 Patients. The result of 10-fold cross-validation showed that in qi deficiency syndrome, external validation mode, the sensitivity and specificity were 69.8% and 73.3%. The percentage of correct prediction was 71.7%. In phlegm-blood stasis syndrome external validation mode, the sensitivity and specificity were 86.8% and 75.9%. The percentage of correct prediction was 77.8% (Table 3).

4. Discussion

Data mining is a method of extracting the database which is still unknown while useful information is implied potentially. It establishes a computer program, automatically scrutinizes in the database and tries to find modes or rules

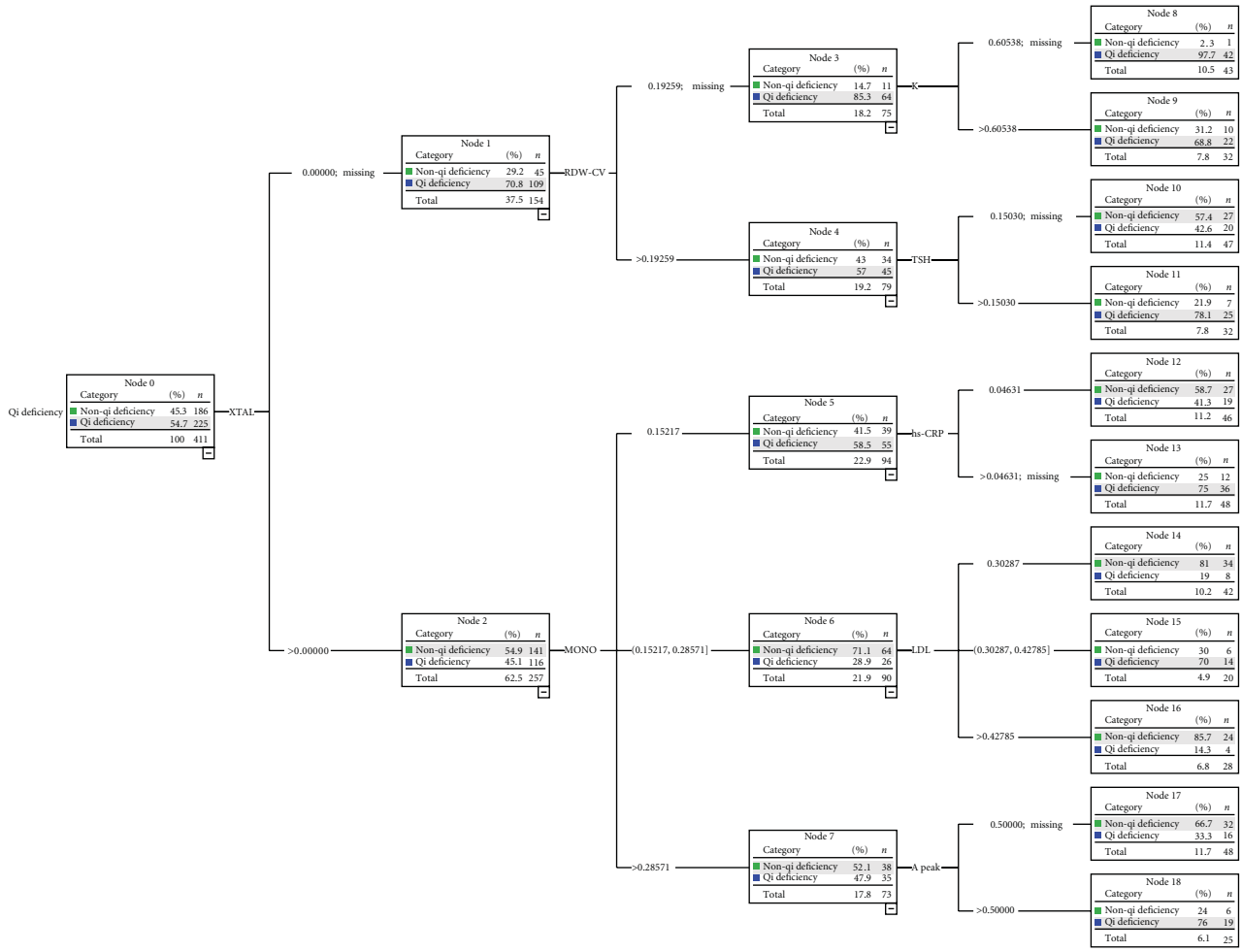


Figure 3: The 8 biological parameters made mode in identification Qi deficiency syndrome from 411 CHD patients.

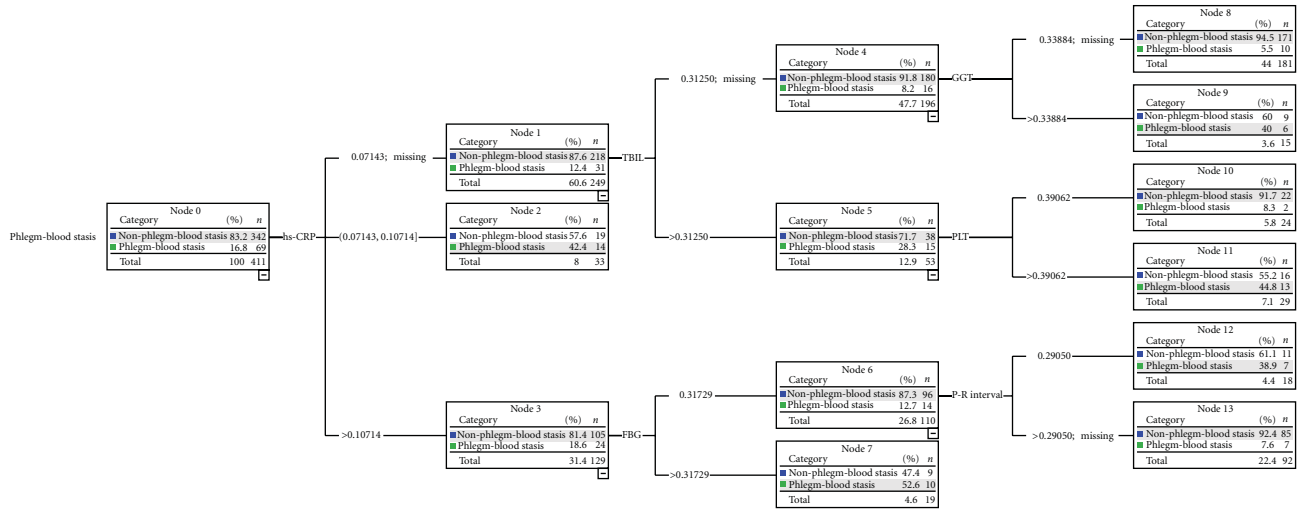


Figure 4: The 6 biological parameters made mode in identification phlegm-blood stasis syndrome from 411 CHD patients.

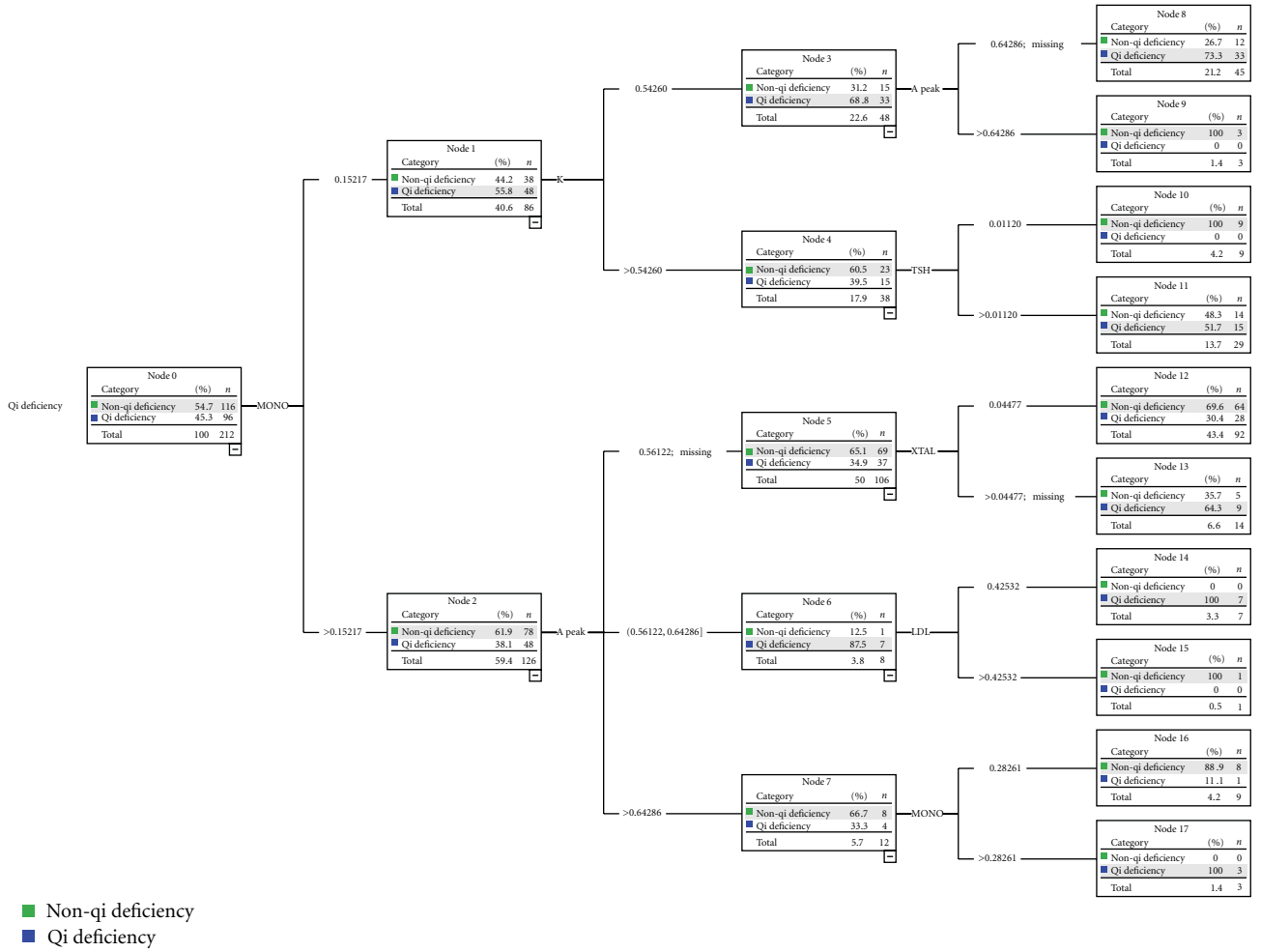


Figure 5: The 6 biological parameters made external validation mode in identification Qi deficiency syndrome from 211 CHD patients.

Table 3: 10-fold Cross-Validation results of classification for 212 cases.

CHAID	TN FN	FP TP	Sensitivity (%)	Specificity (%)	Accuracy (%)
Qi deficiency	85 29	31 67	69.8%	73.3%	71.7%
Phlegm-blood stasis	132 5	42 33	86.8%	75.9%	77.8%

Note: sensitivity = TP/(TP + FN); specificity = TN/(TN + FP); accuracy = (TP + TN)/(TP + FP + TN + FN).

[11]. Complex networks can be used to describe the social relations among persons, kinships, network connections among computers, semantic relations among words, relations of cooperation between scientists, and so forth [12–14]. With the suggestion of small world network concept by Watts and Strogatz in 1998 [15], and with the development of pioneering study on scaling in random networks byBarabási and Albert [16], more and more researchers had used complex networks in medical field. For example, researches on connection of the brain function [17], propagations of the diseases [18], studies of the drug efficacy and drug targets [19], gene regulatory networks [20], and interactions of protein [21].

The traditional approaches could not reveal the meaning of the four diagnostic information because the contents of them were numerous and the combination rules and relationships among the information were complex. TCM is a traditional medicine that capturing the variations of the disease based on the concept of wholism. Studies have shown that the diseases symptom networks had the characteristics of TCM syndromes classification [22]. In complex networks, the classification features, the demands of each role in the

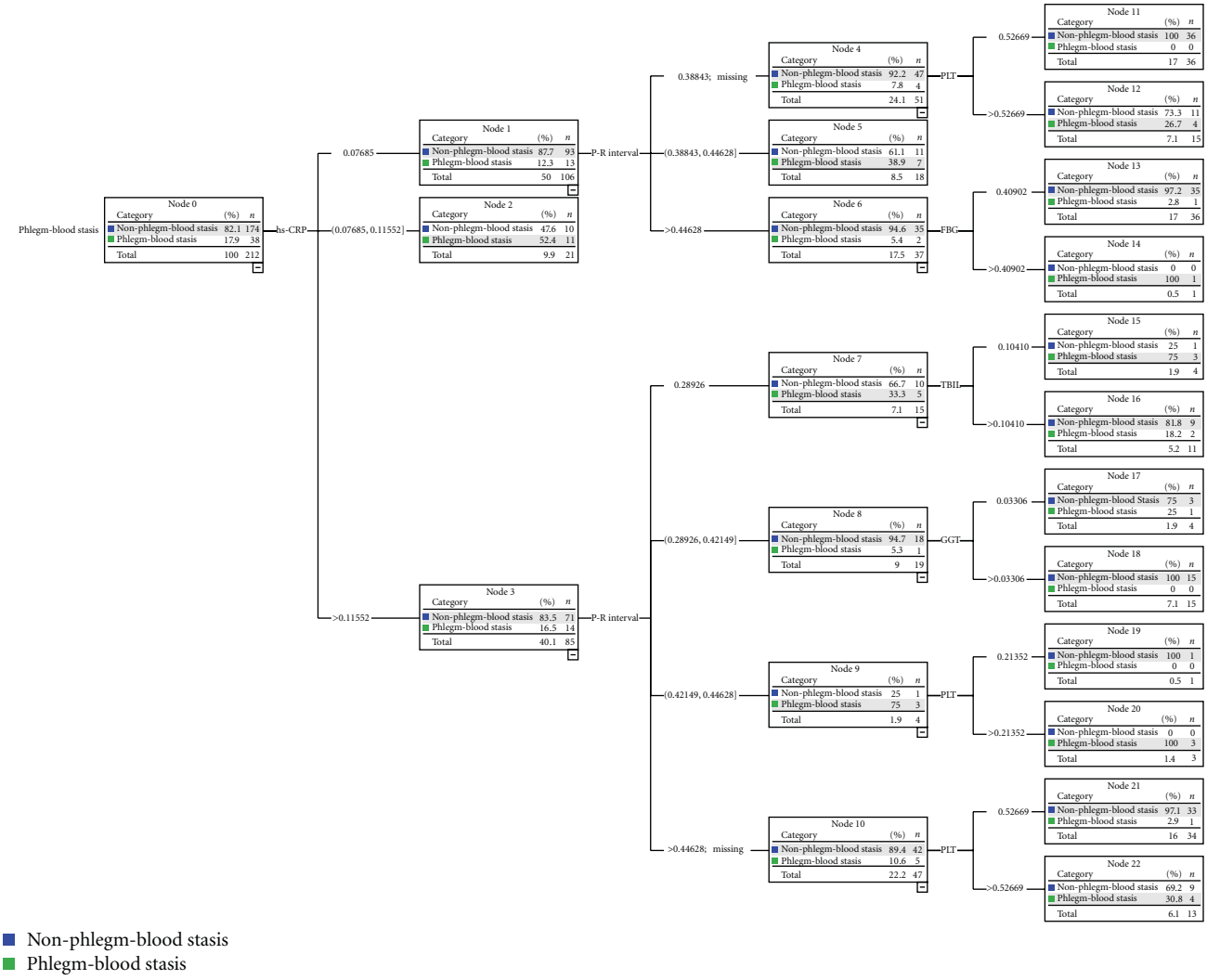


Figure 6: The 6 biological parameters made external validation mode in identification phlegm-blood stasis syndrome from 211 CHD patients.

network organization and the relations of the elements in the progress of organization constitute are the potential force of the network [23]. The process of clinical diagnosis and treatment in TCM are also very similar to complex system. In the analysis of relationships among syndrome, therapeutic and Chinese herbal medicine, the main syndromes and monarch drugs were similar to the hubs of the network, the therapeutic methods and therapeutic principles were abstract summarization of the complex relations [24]. As one of the data mining methods, complex networks provided new methods and ideas for the studies of TCM. It explained the integrity, nonlinearity, and dynamic character of TCM from another point of view.

Pajek is a software that can analyze the data very fast and effective and a kind of simulation for complex network. Unlike the common network analysis software, Pajek can deal with the large-scale networks that contain millions of nodes and have broken the bottleneck that numbers of network analysis software can only process the small-scale data. It usually extracts small-scale networks from the large-scale ones in order to achieve a more detailed study by the classical algorithm and display the analysis results through powerful visualization capability [25, 26]. In many complex networks, there is a phenomenon that although the node number of the network is very large, but the "core" node number is still very small for the entire complex network. Intuitively, the "core" refers to the nodes that play important roles in the complex network. In a network, if any of the nodes has k neighbors that were also in this network, then the network is called "k-core network." Researching the core of the complex network is to identify the entire "k-core network" in the complex network.

In our study, complex network was employed to identify the TCM core syndromes of CHD patients. The core syndromes included qi deficiency subjected to the deficiency syndromes, phlegm-blood stasis syndrome belonged to excessive syndromes. There are two reasons for the conclusions. Firstly, in this study, we gained a 3-core network,

in the center of which arranged 31 nodes. These nodes played a major role. Among these nodes, there were 13 nodes reflected the qi deficiency syndrome, and 10 nodes reflected the phlegm-blood stasis syndrome. The 8 remaining nodes represented yin deficiency, yang deficiency, qi stagnation, and heat syndromes. Secondly, the degree value is a simple but most important property of complex network. The degree value of one node is defined as the number of other nodes that connected to it. In a complex network, the greater degree value that one node is, the more significant role it plays. In the network of four diagnostic information, the degree values of subglossal collateral vessels engorgement, amnesia, faint low voice, white phlegm, heavy limbs, short breath, cough, anorexia, tastelessness in the mouth, and swollen tongue body were higher than 6, most of which reflected the core syndromes we mentioned. The identifications of these core syndromes accurately laid the foundation for the constructions of syndrome identification modes by biological parameters.

Decision Tree is a decision support tool that uses a tree-like graph or model of decisions and their possible consequences, including chance event outcomes, resource costs, and utility. It is a way to display an algorithm. Decision trees are usually applied to cost-benefit studies, especially in decision-making analysis, to help identify a strategy most likely to reach a goal [27]. In many fields of clinical medicine, decision trees have been used successfully to solve complex and chaotic problems without mathematical models or a precise understanding of the mechanisms involved, such as genetic and molecular sequence analysis [28], hospital information system mining [29], and health care [30].

Chi-squared automatic interaction detector (CHAID) decision tree is a method of chi-square automatic interaction detection put forward by Kass in 1980 for the analysis of classification data [31]. It has the functions of target selection, variable selection, and clustering. Its core idea is to split the cases optimally according to the response variables and screened explanatory variables and to determine the grouping automatically of multiple contingency tables on the basis of significance results from chi-square test. The classification process of CHAID algorithm is described as follows. First, select the response variable of category, cross-classification goes into explanatory variables and response variables, then results in a series of two-dimensional classifications. Calculate the $\chi 2$ value of the two-dimensional classification, compare the P value. The best initial two-dimensional classification table with the minimum P value comes into being. Explanatory variables will continually be used to classify the response variables based on the best two-dimensional classification table. Repeat the process until the P value is greater than α value, then the classification stops and mode is formed [32]. Our previous results showed that CHAID decision tree can analyze the large and dormant data from clinical information due to the nonlinear relationship and the interactions between blood stasis syndrome and biological parameters.

The methods of syndrome studies cannot be completed without modern medicine. Due to the complexity itself, it is hard to find the "golden index" for syndrome identification. However, the combinations of different biological parameters may demonstrate the characteristics of different syndromes. Data mining methods have solved those problems mentioned above, which make it possible that macroinformation and microinformation could be combined effectively. Using the CHAID decision tree, an identification mode for qi deficiency syndrome was established with eight biological parameters, and another identification mode for phlegm-blood stasis syndrome was constructed with six biological parameters in our research. We could diagnose patients with or without qi deficiency by 11 paths and diagnose phlegm-blood stasis syndrome by 8 paths.

Studies showed that hs-CRP was significantly increased in CHD patients and had a moderate predictive value for CHD. It had a correlation with phlegm-blood stasis syndrome and provided objective basis for phlegm-blood syndrome differentiation [33]. Meanwhile, significant positive correlation was observed between hs-CRP and qi deficiency syndrome [34]. TBIL is a harmful metabolite in the body under the traditional view. In recent years, domestic and foreign researches showed that TBIL, as a kind of physiological oxidant, had played a role in antiarteriosclerosis. Low express of serum TBIL is an independent risk factor of CHD [35]. Serum GGT value may be the index of oxidative stress *in vivo*. The elevation of GGT can predict the myocardial infarction and stroke, and reflect the cell damage caused by oxygen free radical [36]. When the activated platelet adheres to the vessel wall, the platelet dusts (endothelial granules) are released. This process is closely related to the occurrence of CHD [37]. Study on the relationship between CHD and FBG proved CHD patients were more easily combined with abnormalities of FBG [38]. A correlation study on TCM syndromes and ventricular diastolic functions showed E peak decreased and A peak increased significantly in qi deficiency patients. It prompted the dysfunctions of heart early filling [39]. Some scholars believed that the elevation of RDW suggested the underlying inflammation of the body. Inflammation is one of the most important mechanisms of atherosclerosis. Increase of RDW may be a predictor of the CHD severity [40, 41]. The physiological functions of CHD patients with qi deficiency syndrome were weakened. When the promotion effect of qi was weakened, growth and development of the body would be hurt, physiological functions of the meridian and viscera declined for the earlier failure. Study showed in CHD patients with qi deficiency syndrome, the thyroxine (TH) decreased the ability to feedback regulate the pituitary. Correlations were found between TSH and CHD with qi deficiency syndrome [42]. Compared with healthy people, mononuclear cell count of CHD patients often increased. MONO may be the pathogenesis of CHD. Increases of MONO may indicate earlier happens of CHD especially in middle-aged people [43]. LDL is a reflection of the severity of coronary artery lesions. Its level increased with the aggravation and the severity of coronary lesions. Considering the prevention and treatment of CHD and the physiological need level of LDL, some scholars put forward the proper LDL level for 1.3–1.8 mmol/L [44].

In summary, it showed that application of CHAID decision tree may provide more biological indicator basis for

TCM syndromes differentiation, which may also pave a way for further research on TCM syndrome.

5. Conclusion

Complex networks contributed a lot in the identification of the core TCM syndromes of CHD patients. We found that qi deficiency syndrome and phlegm-blood stasis syndrome were the basic syndromes of CHD patients in our study. Moreover, we established syndrome identification modes for CHD patients with or without core syndromes by CHAID decision tree. Qi deficiency identification mode included eight biological parameters: X TAL, RDW-CV, K, TSH, MONO, hs-CRP, LDL, and A peak. The accuracy of this mode was 73.5%, the sensitivity was 70.2% and specificity was 77.4%. The identification mode of phlegm-blood stasis syndrome included 6 biological parameters: hs-CRP, TBIL, GGT, PLT, FBG, and P-R interval, and the accuracy of this mode was 79.8%, the sensitivity was 72.5%, and the specificity was 81.3%. Constructions of the two external validation modes improved further reliabilities of the identification modes.

Authors' Contribution

Qi Shi, Huihui Zhao and Jianxin Chen contributed equally to the work.

Acknowledgment

This study was supported by the National Basic Research Program of China (no. 2003CB517105 and 2011CB505106), the Creation for Significant New Drugs of China (no. 2009ZX09502-018), the National Science Foundation of China (no. 30902020, 81173463, and 81102730), and self-topics of Beijing University of Chinese Medicine (no. 2009JYBZZ-XS014).

References

[1] M. Qu, M. X. Zhang, L. Zhang, Y. P. Chang, D. N. Wu, and H. H. Chen, "Concerning "heart-qi deficiency" in the role of coronary heart disease (CHD)'s outbreak," *Chinese Archives of Traditional Chinese Medicine*, vol. 28, pp. 282–286, 2010.

[2] M. Pirson, M. Dramaix, P. Leclercq, and T. Jackson, "Analysis of cost outliers within APR-DRGs in a Belgian general hospital: two complementary approaches," *Health Policy*, vol. 76, no. 1, pp. 13–25, 2006.

[3] R. P. Peters, J. W. Twisk, M. A. van Agtmael, and A. B. Groeneveld, "The role of procalcitonin in a decision tree for prediction of bloodstream infection in febrile patients," *Clinical Microbiology and Infection*, vol. 12, no. 12, pp. 1207–1213, 2006.

[4] C. H. Shi, H. H. Zhao, N. Hou et al., "Identifying metabolite and protein biomarkers in unstable angina in-patients by feature selection based on data mining method," *Chemical Research in Chinese Universities*, vol. 27, pp. 87–93, 2011.

[5] H. H. Zhao, S. W. Guo, J. X. Chen et al., "Characteristic pattern study of coronary heart disease with blood stasis syndrome based on decision tree," in *Proceedings of the 4th International Conference on Bioinformatics and Biomedical Engineering (iCBBE '10)*, pp. 1–3, June 2010.

[6] R. J. Gibbons, K. Chatterjee, J. Daley et al., "ACC/AHA/ACP-ASIM guidelines for the management of patients with chronic stable angina," *Journal of the American College of Cardiology*, vol. 33, no. 7, pp. 2092–2197, 1999.

[7] Society of Cardiology, Chinese Medical Association, Editorial Committee of Chinese Journal of Cardiology, "Diagnosis and treatment recommendation of unstable angina cordis," *Chinese Journal of Cardiology*, vol. 28, pp. 409–412, 2000.

[8] X. Y. Zheng, *Guiding Principle of Clinical Research on New Drugs of Traditional Chinese Medicine (Trial)*, China Medico-Pharmaceutical Sciences and Technology Publishing House, Beijing, China, 2002.

[9] W. F. Zhu and Y. Y. Wang, *Terminology for Traditional Chinese Medicine Clinical Practice-Part of the Syndrome*, China Standard Publishing House, Beijing, China, 1997.

[10] S. Li, B. Zhang, D. Jiang, Y. Y. Wei, and N. B. Zhang, "Herb network construction and co-module analysis for uncovering the combination rule of traditional Chinese herbal formulae," *BMC Bioinformatics*, vol. 11, supplement 11, article S6, 2010.

[11] I. H. Witten and E. Frank, *Data Mining Practical Machine Learning Tools and Techniques*, China Machine, Beijing, China, 2005.

[12] B. Phillip, "Exploratory social network analysis with Pajek," *Sociological Methods Research*, vol. 36, pp. 563–564, 2008.

[13] B. Vladimir and M. Andrej, "Analysis of kinship relations with Pajek," *Social Science Computer Review*, vol. 26, no. 2, pp. 224–246, 2008.

[14] M. S. Vitevitch, "What can graph theory tell us about word learning and lexical retrieval?" *Journal of Speech, Language, and Hearing Research*, vol. 51, no. 2, pp. 408–422, 2008.

[15] D. J. Watts and S. H. Strogatz, "Collective dynamics of "small-world" networks," *Nature*, vol. 393, no. 6684, pp. 440–442, 1998.

[16] A. L. Barabási and R. Albert, "Emergence of scaling in random networks," *Science*, vol. 286, no. 5439, pp. 509–512, 1999.

[17] F. F. Zhang, C. H. Chen, and L. Jiang, "Brain functional connection research based on complex network," *Complex Systems and Complexity Science*, vol. 8, pp. 18–23, 2011.

[18] X. H. Qin and Y. J. Guan, "Viruses spread of influenza A H1N1 based on complex networks," *Statistics and Information Forum*, vol. 25, pp. 86–90, 2010.

[19] M. A. Yildirim, K. I. Goh, M. E. Cusick, A. L. Barabási, and M. Vidal, "Drug-target network," *Nature Biotechnology*, vol. 25, no. 10, pp. 1119–1126, 2007.

[20] H. J. Zhou, "Application of complex network theory in gene regulatory networks," *Journal of Chongqing University of Science and Technology*, vol. 11, pp. 141–144, 2009.

[21] Z. Fang, Y. Z. Li, J. M. Xiao, G. B. Li, Z. N. Wen, and M. L. Li, "Complex network-based random forest algorithm for predicting the impact of amino acid mutation on protein stability," *Chemical Research and Application*, vol. 23, pp. 554–558, 2011.

[22] J. J. Sun, J. Jiang, G. L. Yan et al., "Complex networks theory and its application in study of traditional Chinese medicine," *Complex Systems and Complexity Science*, vol. 5, pp. 55–61, 2008.

[23] X. Z. Zhou, B. Y. Liu, Y. H. Wang, R. S. Zhang, N. L. Yao, and M. Cui, "Study on complex networks theory of compound drug compatibility," *Chinese Journal of Information on Traditional Chinese Medicine*, vol. 25, pp. 98–100, 2008.

[24] Z. Y. Gao, J. C. Zhang, H. Xu et al., "Analysis of relationships among syndrome, therapeutic treatment, and Chinese herbal

medicine in patients with coronary artery disease based on complex networks," *Journal of Chinese Integrative Medicine*, vol. 8, no. 3, pp. 238–243, 2010.

[25] B. Wand, W. Wu, and C. Q. Xun, "A survey on visualization of complex network," *Computer Science*, vol. 16, pp. 2841–2844, 2004.

[26] C. Amelia, "The application of social network theory to animal behaviour," *Bioscience Horizons*, vol. 2, pp. 32–43, 2009.

[27] R. P. Peters, J. W. Twisk, M. A. van Agtmael, and A. B. Groeneveld, "The role of procalcitonin in a decision tree for prediction of blood stream infection in febrile patients," *Clinical Microbiology and Infection*, vol. 12, pp. 1207–1213, 2006.

[28] F. Xie, "The classification of tumor gene expression profiles based on genetic algorithm and decision tree," *Computer Knowledge and Technology*, vol. 6, pp. 2493–2495, 2010.

[29] L. Y. Luo and J. X. Chen, "Data mining and investigation of surgical operation information based on decision tree," *Medicine Information*, vol. 21, pp. 1936–1939, 2008.

[30] L. Huang, J. M. Yuan, A. H. Ou, Y. R. Lao, X. B. Yang, and Z. M. Yang, "Pattern of sub-health state factors based on decision tree," *The Journal of Practical Medicine*, vol. 27, pp. 121–124, 2011.

[31] G. V Kass, "An exploratory technique for investigating large quantities of categorical data," *Applied Statistics*, vol. 29, pp. 119–127, 1980.

[32] L. Shi and Y. Wang, "Studies of risk factors in infant mortality-theory and application of CHAID method," *Chinese Journal of Health Statistics*, vol. 19, pp. 283–285, 2002.

[33] Y. D. Hong and H. X. Yang, "Multiple-factor analysis of phlegm blood-stasis syndrome of coronary heart disease," *Journal of Guangzhou University of Traditional Chinese Medicine*, vol. 27, pp. 184–188, 2010.

[34] G. Qiqi, Y. Y. Li, J. X. Chen, S. Z. Guo, L. F. Wang, and W. Wang, "Related research on qi deficiency sydrome of CHD unstable angina pectoris and inflammation," *Chinese Journal of Integrative Medicine on Cardio/Cerebrovascular Disease*, vol. 8, pp. 1418–1420, 2010.

[35] G. Q. Feng, J. Yang, X. B. Wang, C. Meng, L. D. Lin, and H. L. Wu, "The relationship between serum bilirubin levels and coronary artery disease in the elderly," *Chinese Journal of Arteriosclerosis*, vol. 15, pp. 311–312, 2007.

[36] D. H. Lee, R. Blomhoff, and D. R. Jacobs Jr., "Is serum gamma glutamyltransferase a marker of oxidative stress?" *Free Radical Research*, vol. 38, no. 6, pp. 535–539, 2004.

[37] J. Y. Xia and Q. F. Hua, "Endothelial microparticles and coronary heart disease," *Chinese Journal of Arteriosclerosis*, vol. 19, pp. 160–164, 2011.

[38] L. H. Xu and X. H. Wu, "A study on the relationship between coronary heart disease and fasting glucose," *Jilin Medicine*, vol. 31, pp. 3638–3639, 2010.

[39] S. F. Chen, H. Huang, H. J. Zhao., and L. L. Zhang, "Related research on TCM syndrome of CHD and ventricular diastolic function," *Journal of Fujian College of Traditional Chinese Medicine*, vol. 16, pp. 15–16, 2006.

[40] G. M. Felker, L. A. Allen, S. J. Pocock et al., "Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM program and the Duke Databank," *Journal of the American College of Cardiology*, vol. 50, no. 1, pp. 40–47, 2007.

[41] M. Tonelli, F. Sacks, M. Arnold, L. Moye, B. Davis, and M. Pfeffer, "Relation between red blood cell distribution width and cardiovascular event rate in people with coronary disease," *Circulation*, vol. 117, no. 2, pp. 163–168, 2008.

[42] M. Z. Xie, Y. Xie, H. H. Tian, N. Mo, H. Y. Huang, and H. T. Wu, "Study on the correlation of TSH and the heart-qi deficiency syndrome in coronary heart disease," *Chinese Journal of Information on Traditional Chinese Medicine*, vol. 13, pp. 22–23, 2006.

[43] R. Olivares, P. Ducimetière, and J. R. Claude, "Monocyte count: a risk factor for coronary heart disease?" *American Journal of Epidemiology*, vol. 137, no. 1, pp. 49–53, 1993.

[44] J. H. O'Keefe, L. Cordain, W. H. Harris, R. M. Moe, and R. Vogel, "Optimal low-density lipoprotein is 50 to 70 mg/dl: lower is better and physiologically normal," *Journal of the American College of Cardiology*, vol. 43, no. 11, pp. 2142–2146, 2004.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 965947, 5 pages
doi:10.1155/2012/965947

Research Article

Preparation of Blood-Deficient Model and Research of Angelica Polysaccharide on Enriching Blood in Chickens

Haifeng Hou,[1,2] Yongzhan Bao,[1] Qian Li,[3] and Wanyu Shi[1]

[1] *College of Veterinary Medicine, Agricultural University of Hebei, Baoding 071001, China*
[2] *Department of Animal Husbandry and Veterinary Medicine, Baoding Vocational and Technical College, Baoding 071051, China*
[3] *Egg-Type Chicken Laboratory, Animal Husbandry and Veterinary Institute of Hebei, Baoding 071000, China*

Correspondence should be addressed to Wanyu Shi, shwybyzh@vip.sina.com

Received 16 February 2012; Revised 23 March 2012; Accepted 24 March 2012

Academic Editor: Shi-Bing Su

In this study cyclophosphamide was used to prepare the blood-deficient model. The red blood cell count and hemoglobin content were measured. The experimental chickens presented the symptoms of blood-deficient syndrome, dullness, shrinkinginto oneself, broken winded, loose feather, waxy eyelid, and pale tongue. At the same time, red blood cell count and hemoglobin content decreased significantly. Angelica polysaccharide as the effective component of Angelica Sinensis could significantly increase the red blood cell count and the hemoglobin content of blood-deficient chickens. The results indicated that cyclophosphamide could significantly reduce the red blood count and hemoglobin content, and make the ideal blood-deficient model successfully. Angelica polysaccharide had the function of enriching blood in different ways. On the one hand Angelica polysaccharide enriched he blood directly, increased the number of RBC and hemoglobin; on the other hand it regulated the hematopoietic factors, enriched the blood indirectly.

1. Introduction

Based on the traditional Chinese veterinary medicine, syndrome of blood deficiency is a morbid condition of insufficient blood supply to the visceral organs and channels for their nutrition due to improper feeding, malnutrition, profuse bleeding, chronic hemorrhage, or diminished production of blood on account of hypofunction of the internal organs. The main manifestations are pale tongue, dullness, sparse hair, thready-weak pulse, and so forth [1]. Animal experimental study is an indispensable way to illustrate the etiology and pathogenesis of blood-deficient syndrome. Likewise, animal experiment is a method to study the mechanism of hematinics on treating deficiency of blood. An animal model of blood-deficient syndrome is an important step in the research on the modernization of traditional Chinese veterinary medicine [2]. Angelica Sinensis, known as Danggui in China, is one of the most popular traditional Chinese medicines as blood-enriching drugs. The medicinal part is composed of its dried roots with sweet-acrid taste, warm property, and attribution to the heart, liver and spleen channels. It is commonly used to enrich or nourish blood and activate blood circulation. Angelica Sinensis is contained by more than 80 composite formulae. Modern researches indicate that phthalides, organic acids, and their esters, polysaccharides are the main chemical components related to the bioactivities and pharmacological properties of Danggui [3–5]. Angelica polysaccharide is water-soluble organic compound extracted from the root of Angelica Sinensis. Angelica polysaccharide, as the effective component of Angelica Sinensis in enriching blood, has the efficacy of purifying blood quality, emmenagogue, acesodyne, lenitive, improving circulation and immunity, antiviral, antitumor, antioxidation, and so forth [6–9]. It is used frequently in clinics and also frequently appears as the main ingredient in prescriptions for bone injures. Cyclophosphamide is a cytostatic agent that produces systemic toxicity especially on cells with high proliferative capacity, while polysaccharides from Angelica polysaccharide have been shown to increase the turnover of hemopoietic stem cells [10]. In the modern society Angelica polysaccharide has a better potential for drug development [11]. Previous research showed Angelica polysaccharide had effects on enriching blood in mice/human with blood deficiency [10, 12]. However, there's

less reports on its hematopoiesis of Angelica polysaccharide about poultry. In this study, Cyclophosphamide was used to investigate whether the blood-deficient model was successfully made in chickens, at the same time to discuss the mechanisms on enriching and nourishing blood of Angelica polysaccharide [12–16].

2. Materials and Methods

2.1. Animals. One-day-old Hyline Brown chickens (male) were purchased from Hebei laboratory animal center, housed in cages and lighted for 24 h at the beginning of pretrial period. The chicks were given free access to feedstuff and water.

2.2. Reagent. Angelica polysaccharide was purchased from Beijing Biochem Co., Ltd. China. Cyclophosphamide was purchased from Jiangsu Hengrui Medicine Co., Ltd (no. 06121021).

2.3. Grouping and Treatment. Eighty 14-day-old normal chickens were randomly divided into eight groups with the same number and similar body weight. Angelica polysaccharide (1%) diluted with distilled water was drenched to the chickens by oral administration in a sterile syringe without a needle. The healthy chickens in group I as control, the healthy chickens in group II, III, and IV were given three gradient dosages (50 mg/Kg, 100 mg/Kg, 150 mg/Kg) of Angelica polysaccharide, respectively, for 7 days. Chickens in group V, VI, VII, and VIII were given Cyclophosphamide by intraperitoneal injection, 6 days later the blood-deficient chicken model was made. After the blood-deficient chicken model was set up, chickens in group VI, VII, and VIII were given three gradient dosages (50 mg/Kg, 100 mg/Kg, 150 mg/Kg) of Angelica polysaccharide, respectively, for 7 days. All the experimental animals were treated in accordance with the guidelines of the Chinese Council for Animal Care.

2.4. Blood Specimen Collection and Examination. Until the last Angelica polysaccharide administration, the blood specimens were collected from heart for red blood cells (test tube method) and hemoglobin (turbidimetry) tests. The steps were followed with [17].

2.5. Data Statistics. All data had a normal distribution presented as mean ± standard deviation (SD) and analysed by SPSS11.0 statistical software. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by student's t-test. A probability of less than 0.01 was considered to be statistically significant.

3. Results

3.1. Symptoms. The blood-deficient model was made by intraperitoneal injection of Cyclophosphamide for 6 days (80 mg/Kg·d). 6 days later, the chickens behaved as follows: dullness, shrinking into oneself, broken winded, loose-feather, waxy eyelid, and pale tongue. It can be concluded from the clinical manifestations that chicken for blood-deficient syndrome model has been successfully set up. Healthy chickens given Angelica polysaccharide behaved as follows, physical agility, pink tongue and bright feather. The blood-deficient chickens returned to the normal symptoms after given Angelica polysaccharide. Blood-deficient chickens given Angelica polysaccharide had no notable changes than the control group.

3.2. Effects of Angelica Polysaccharide on Blood Physiological Index

3.2.1. Effects of Angelica Polysaccharide on the Red Blood Cell Count. It showed in Table 1 that the red blood cell count in group (II) had no more change than the control group ($P > 0.05$). Angelica polysaccharide in group (III) and (IV) had significant effects on the red blood cell count than group I ($P < 0.01$). When the healthy chickens were given Cyclophosphamide, there was a significant decrease in the number of erythrocyte in group V ($P < 0.01$). However, after the blood-deficient chickens were given Angelica polysaccharide, the number of erythrocyte gradually increased. In added, the red blood cell count in group VIII was notably higher than the control ($P < 0.05$). The number of erythrocyte in group VII and VIII were significantly higher than group V ($P < 0.01$). In a word, Cyclophosphamide can lead to the red blood cell count reduction and Angelica polysaccharide as hematinic can increase it.

3.2.2. Effects of Angelica Polysaccharide on Hemoglobin Content. It showed in Table 2 that hemoglobin content in group II had no more change than the control group ($P > 0.05$). Angelica polysaccharide in group III and IV had significant effects on hemoglobin content than group I ($P < 0.01$). When the healthy chickens were given Cyclophosphamide, there was a significant decrease in the hemoglobin content in group V ($P < 0.01$). However, after the blood-deficient chickens were given Angelica polysaccharide, the hemoglobin content gradually increased. The hemoglobin content in group VII and VIII were significantly higher than group V ($P < 0.01$). In a word, Cyclophosphamide can lead to the hemoglobin content reduction and Angelica polysaccharide as hematinic can increase it.

4. Discussion

At present many methods which have different characteristics could set up blood-deficient animal model, such as radiation damage method, chemical method, and immune induce method [18]. Bleeding method which does not use special equipment is simple, with definite index. Bleeding method brings about the decrease of the peripheral blood cells immediately; however, the bloodletting quantity is hard to control and has little effect on the hematopoietic system of organism [19]. Radiation damage method which uses 60Co-γ to irradiate animals could damage bone marrow and affect the hematopoietic function. Radioactive ray has direct damage to stem cells and bone marrow microenvironment.

Table 1: Effects of Angelica polysaccharide on red blood cell count in chickens.

Group	N	Dosage (mg/kg)	Count of RBC ($\times 10^{12}$/L)
(I) Control of healthy chicken	10	0	$276.453 \pm 23.412^{\Delta\Delta}$
(II) Low dosage in healthy chicken	10	50	279.579 ± 25.890
(III) Middle dosage in healthy chicken	10	100	$312.132 \pm 12.900^{**}$
(IV) High dosage in healthy chicken	10	150	$313.256 \pm 17.203^{**}$
(V) CY	10	0	$241.557 \pm 14.498^{**}$
(VI) CY with low dosage	10	50	262.624 ± 15.560
(VII) CY with middle dosage	10	100	$283.233 \pm 13.412^{\Delta\Delta}$
(VIII) CY with high dosage	10	150	$298.684 \pm 21.287^{*\Delta\Delta}$

**Superscript differs significantly ($P < 0.01$) compared with group I, *superscript differs notably ($P < 0.05$) compared with group I. $^{\Delta\Delta}$Superscript differs significantly ($P < 0.01$) compared with group V, $^{\Delta}$superscript differs notably ($P < 0.05$) compared with group V. CY stands for Cyclophosphamide. RBC stands for red blood cell.

Table 2: Effects of Angelica polysaccharide on hemoglobin content in chickens.

Group	N	Dosage (mg/kg)	Hemoglobin content (g/L)
(I) Control of healthy chicken	10	0	$71.203 \pm 9.898^{\Delta\Delta}$
(II) Low dosage in healthy chicken	10	50	77.316 ± 2.493
(III) Middle dosage in healthy chicken	10	100	$84.611 \pm 3.752^{**}$
(IV) High dosage in healthy chicken	10	150	$86.684 \pm 5.610^{**}$
(V) CY	10	0	$63.797 \pm 3.337^{**}$
(VI) CY with low dosage	10	50	72.541 ± 5.630
(VII) CY with middle dosage	10	100	$78.255 \pm 7.266^{\Delta\Delta}$
(VIII) CY with high dosage	10	150	$78.256 \pm 3.538^{\Delta\Delta}$

**Superscript differs significantly ($P < 0.01$) compared with group I, *superscript differs notably ($P < 0.05$) compared with group I. $^{\Delta\Delta}$Superscript differs significantly ($P < 0.01$) compared with group V, $^{\Delta}$superscript differs notably ($P < 0.05$) compared with group V. CY stands for Cyclophosphamide.

Radioactive ray brings down the bone marrow and affects CFU-E, BFU-E, and CFU-GM at certain dosage. Radiation damage method needs to use special equipment, at the same time the radiation dosage is difficult to control. When the radiation dosage is low it cannot meet the damage requirement. Likewise, if the radiation dosage is high it may lead to death [20].

As the chemical method, in this experiment Cyclophosphamide was used to make the blood-deficient model [21]. The dosage, route and times of Cyclophosphamide administration were all easily to control by intraperitoneal injection. Cyclophosphamide is a kind of nitrogen mustards alkylating agent produced synthetically. Cyclophosphamide which is a broad spectrum anti-tumor medicine is widely used to treat acute/chronic lymphocytic leukemia, malignant lymphoma, myelomatosis multiplex, and so forth. When Cyclophosphamide got into animal body, it broke down into chloroethyl phosphopeptamine with alkylating function induced by hepatomicrosome P450 enzyme system. Then chloroethyl phosphopeptamine led to single-strand and double-strand DNA broken by cross-linking DNA strands. As an antitumor drug, Cyclophphosphamide destroyed the structure of DNA directly, interfered the transcription of DNA, inhibited the synthesis of RNA and protein, thus Cyclophosphamide prevented the proliferation of cell and reduced the blood supply. So Cyclophosphamide caused widespread destruction of hemopoietic system and immune system [22, 23]. In clinic, the effects of Cyclophosphamide are performed mainly in immunological function repression, marrow inhibition and peripheral blood cell decrease [24, 25]. Experiments indicated Cyclophosphamide had obvious inhibitive effects on red cell immune function [26]. Studies showed that Cyclophosphamide could also damage the bone marrow microenvironment and the proliferation and differentiation of hematopoietic cell, in order to inhibit the hematopoietic function [23, 27]. The two inhibitive effects made the deficiency of blood.

In order to research the pathophysiological changes of blood-deficient syndrome, experimental index interrelated with Ying blood function was chosen to examine [28]. Erythrocyte and hemoglobin could reflect the quantitative and functional changes of blood-deficient syndrome. The red blood cell count and hemoglobin content are both important standards to measure. Clinically, blood loss and anemia often present with the reduction of erythrocyte and hemoglobin content [29–31]. Angelica Sinensis as the traditional Chinese medicine is one of the most popular medicine to treat blood-deficient syndrome, and Angelica polysaccharide is the major component of Angelica Sinensis in enriching blood. Recent studies demonstrated that Angelica polysaccharide could effect the hemopoietic system of animal and had obvious promoter action on proliferation and differentiation of myelogenous hemopoietic progenitor cell of human and rats. It was also reported that indicated Angelica polysaccharide could increase C3b receptor rates, leucocyte and thrombocyte in peripheral blood of mice with radiation-injury remarkably. Likewise, Angelica polysaccharide could improve the hemopoietic function of radiation injured mice [32]. Studies showed that supernatant induced by Angelica polysaccharide of bone marrow macrophage could increase the colony-forming efficiency of myelogenous hemopoietic progenitor cell. Induced by Angelica polysaccharide, protein levels of EPO, GM-CSF, IL-3, and IL-6 expressed by bone marrow macrophage were improved differently, at the same time the expression levels and strength of mRNA of EPO and GM-CSF were increased significantly [33]. At the gene

level and protein level, Angelica polysaccharide promoted the synthesis and excretion hemopoiesis regulatory factors and then promoted the proliferation and differentiation of hemopoietic progenitor cell. It can be seen that Angelica polysaccharide not only supports the normal hemopoiesis but also inhibits the proliferation of tumor cell such as leukemia. It can be used for a natural revulsant. The hematopoietic function of Angelica polysaccharide works in different ways. On the one hand Angelica polysaccharide enriches he blood directly, increases the number of RBC and hemoglobin; on the other hand it regulates the hematopoietic factors, enriches the blood indirectly.

In this experiment the results indicated that Angelica polysaccharide could improve the symptoms of blood-deficient syndrome made by Cyclophosphamide, at the same time, significantly increase the red blood cell count and the hemoglobin content of blood-deficient chickens. With the development of scientific and technological and traditional Chinese medicine theory, mechanism of Angelica polysaccharide on enriching blood will be deepened and systematized gradually.

5. Conclusion

This study suggests that Cyclophphosphamide can make the ideal blood-deficient model successfully by intraperitoneal injection for 6 days (80 mg/Kg·d) when the chickens were 14-day-old. The experimental chickens presented the symptoms of blood-deficient syndrome, dullness, shrinking into oneself, broken winded, loose feather, waxy eyelid, and pale tongue. At the same time, red blood cell count and hemoglobin content decreased significantly. This blood-deficient model remains the advantage of high survival rate as well as long duration of blood-deficient symptoms. The results also show that Angelica polysaccharide can significantly increase the red blood cell count and hemoglobin content of blood-deficient chickens.

In conclusion, the blood-deficient model made by intraperitoneal injection of Cyclophphosphamide is more suitable for the clinical manifestations. As the main component of Angelica Sinensis, Angelica polysaccharide has the function of enriching blood. The finding provides a better basis for the clinic use of hematopoietic.

Conflict of Interests

The authors have declared that they have no conflict of interests.

Acknowledgment

This study was financially supported by the Ministry of Science and Technology of China (no. 2011BAD34B02).

References

[1] Y. Liang, X. H. Lu, and R. Q. Chen, "Research progress of blood-deficient syndrome in traditional Chinese Medicine," *Chinese Journal of Information on TCM*, vol. 6, no. 1, pp. 16–18, 1999.

[2] R. M. Jin, L. Ning, and C. X. Chen, "Prepartion of blood-deficient model and effects of Tangkuei blood-supplementing on hemopoiesis," *Chinese Traditional Patent Medicine*, vol. 23, no. 4, pp. 268–271, 2001.

[3] L. Yi, Y. Liang, H. Wu, and D. Yuan, "The analysis of Radix Angelicae Sinensis (Danggui)," *Journal of Chromatography A*, vol. 1216, no. 11, pp. 1991–2001, 2009.

[4] J. A. Wei, Z. S. Huang, and L. Wu, "Research on enriching blood function and material base of Dangguibuxuetang," *Pharmacology and Clinics of Chinese Materia Medica*, vol. 19, no. 2, pp. 8–10, 2003.

[5] Z. S. Huang and J. A. Wei, "Research on enriching blood function and material base of Dangguibuxuetang," *Pharmacology and Clinics of Chinese Materia Medica*, vol. 19, no. 5, pp. 11–12, 2003.

[6] F. Varga, M. Rumpler, R. Zoehrer et al., "T3 affects expression of collagen I and collagen cross-linking in bone cell cultures," *Biochemical and Biophysical Research Communications*, vol. 402, no. 2, pp. 180–185, 2010.

[7] H. Wang, W. Li, J. Li et al., "The aqueous extract of a popular herbal nutrient supplement, Angelica sinensis, protects mice against lethal endotoxemia and sepsis," *Journal of Nutrition*, vol. 136, no. 2, pp. 360–365, 2006.

[8] P. J. Liu, W. T. Hsieh, S. H. Huang, H. F. Liao, and B. H. Chiang, "Hematopoietic effect of water-soluble polysaccharides from Angelica sinensis on mice with acute blood loss," *Experimental Hematology*, vol. 38, no. 6, pp. 437–445, 2010.

[9] P. P. Wang, Y. Zhang, L. Q. Dai, and K. P. Wang, "Effect of Angelica sinensis polysaccharide-iron complex on iron deficiency anemia in rats," *Chinese Journal of Integrative Medicine*, vol. 13, no. 4, pp. 297–300, 2007.

[10] M. K. C. Hui, W. K. K. Wu, V. Y. Shin, W. H. L. So, and C. H. Cho, "Polysaccharides from the root of Angelica sinensis protect bone marrow and gastrointestinal tissues against the cytotoxicity of cyclophosphamide in mice," *International Journal of Medical Sciences*, vol. 3, no. 1, pp. 1–6, 2006.

[11] S. D. Sarker and L. Nahar, "Natural medicine: the genus Angelica," *Current Medicinal Chemistry*, vol. 11, no. 11, pp. 1479–1500, 2004.

[12] M. Zheng and Y. P. Wang, "Study on mechanism and effect of Angelica polysaccharide on human bone marrow hematopoietic progenitor proliferation and differentiation," *Journal of Anatomy*, vol. 25, no. 2, pp. 105–108, 2002.

[13] Z. S. Huang and J. A. Wei, "Experimant study of pharmacology of Tangkuei blood-supplementing," *Chinese Journal of Traditional Medical Science and Technology*, vol. 8, no. 3, pp. 205–206, 2002.

[14] L. R. Yang and L. Yang, "Experiment summarization of Tangkuei blood-supplementing," *Journal of Practical Traditional Chinese Medicine*, vol. 21, no. 2, pp. 123–124, 2005.

[15] Y. C. Chen and Y. Q. Gao, "Research on the mechanism of blood-tonifying effect of danggui buxue decoction," *China Journal of Chinese Materia Medica*, vol. 19, no. 1, pp. 43–63, 1994.

[16] H. F. Li, Q. L. Liu, J. H. Song et al., "Research on pharmacodynamic function of Tangkuei blood-supplementing," *China Pharmaceuticals*, no. 10, p. 22, 1997.

[17] North-East Agricultural College, *Basis of Clinical Diagnosis and Treatment*, Agriculture Press, Beijing, China, 1991.

[18] L. Yang, B. D. Zhu, and C. Peng, "Study on standard animal models with blood deficiency sign," *Sichuan Journal of Zoology*, vol. 25, no. 1, pp. 160–164, 2006.

[19] Q. Chen, *Methodology in the Study of Herbal Pharmacology*, People's Medical Publishing House, Beijing, China, 1993.
[20] W. J. Gong and X. D. Wo, "Review of modern study on blood deficiency," *Progress in Modern Biomedicine*, vol. 7, no. 6, pp. 934–937, 2007.
[21] M. S. Mao, L. P. Zhang, X. Y. Fang et al., "Studies of Dangguibuxuetang polysaccharide of the blood-deficient model mice," *China Journal of Basic Medicine in Traditional Chinese Medicine*, vol. 8, no. 12, pp. 926–927, 2002.
[22] S. M. Chen and X. T. Nie, "The effect of the alcohol extract of pilose antler on the red blood cells' immunologic function in the mice treated by Cyclophosphamide," *Journal of Economic Animal*, vol. 4, no. 1, pp. 23–25, 2000.
[23] H. Q. Ji, B. Y. Wang, and Q. Y. Jiang, "The resistance of bursin to decreasing of RBC immune function induced by Cyclophosphamide in Chicken," *China Poultry*, vol. 8, no. 1, pp. 38–40, 2004.
[24] M. S. Mao, L. P. Zhang, and X. Y. Fang, "Thinking about copy of blood-deficient animal model," *Guangming Journal of Chinese Medicine*, vol. 18, no. 3, pp. 8–9, 2003.
[25] Y. Feng, H. T. Zhao, J. B. Liu et al., "The effect of different dosage Cyclophosphamide on immunological suppression in broiler chicken," *Feed Industry*, vol. 26, no. 17, pp. 33–35, 2005.
[26] Y. Zhang, J. F. Cheng, J. R. He et al., "The protection of Angelica polysaccharide on bone marrow inhibition and toxicity of mice induced by Cyclophosphamide," *Journal of The Fourth Military Medical University*, vol. 24, no. 5, pp. 447–448, 2003.
[27] J. G. Lee, W. T. Hsieh, S. U. Chen et al., "Hematopoietic and myeloprotective activities of an acidic Angelica sinensis polysaccharide on human $CD34^+$ stem cells," *Journal of Ethnopharmacology*, vol. 139, no. 3, pp. 739–745, 2012.
[28] S. L. Wu, *Modern Hematonosis Diagnostic Manual*, Beijing Medical University and Peking Union Medical College Press, Beijing, China, 1998.
[29] R. Hatano, F. Takano, S. Fushiya et al., "Water-soluble extracts from Angelica acutiloba Kitagawa enhance hematopoiesis by activating immature erythroid cells in mice with 5-fluorouracil-induced anemia," *Experimental Hematology*, vol. 32, no. 10, pp. 918–924, 2004.
[30] J. L. Teng, "Qi supply mechanism of Tangkuei blood-supplementing," *Pharmacology and Clinics of Chinese Materia Medica*, vol. 5, no. 10, p. 42, 1994.
[31] Y. Q. Tong and H. M. Hou, "Study of anemia mechanism of Tangkuei blood-supplementing," *Jiangxi Journal of Traditional Chinese Medicine*, vol. 37, no. 2, pp. 62–63, 2006.
[32] Y. Hong, "The effect of angelica polysaccharide on the cell immune function of radiated mice," *Medical Journal of Wuhan University*, vol. 22, no. 1, pp. 27–28, 2001.
[33] J. Li and Y. P. Wang, "Effect of angelica polysaccharide on bone marrow macrophage and its relationship to hematopoietic regulation," *Chinese Traditional and Hebal Drugs*, vol. 1, pp. 69–71, 2005.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 298014, 8 pages
doi:10.1155/2012/298014

Review Article

Applications of New Technologies and New Methods in ZHENG Differentiation

Jianye Dai,[1] Shujun Sun,[1] Huijuan Cao,[1] Ningning Zheng,[1] Wenyu Wang,[1] Xiaojun Gou,[2] Shibing Su,[3] and Yongyu Zhang[1]

[1] *Research Center for Traditional Chinese Medicine and Systems Biology, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Pudong, Shanghai 201203, China*
[2] *Key Laboratory of Liver and Kidney Diseases of Ministry of Education, Shuguang Hospital, Institute of Liver Diseases, Shanghai University of Traditional Chinese Medicine, 528 Zhanghen Road, Pudong, Shanghai 201203, China*
[3] *Research Center for Traditional Chinese Medicine Complexity System, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Pudong, Shanghai 201203, China*

Correspondence should be addressed to Shibing Su, shibingsu07@163.com and Yongyu Zhang, dryyz@sina.com

Received 12 February 2012; Accepted 21 March 2012

Academic Editor: Aiping Lu

With the hope to provide an effective approach for personalized diagnosis and treatment clinically, Traditional Chinese Medicine (TCM) is being paid increasing attention as a complementary and alternative medicine. It performs treatment based on ZHENG (TCM syndrome) differentiation, which could be identified as clinical special phenotypes by symptoms and signs of patients. However, it caused skepticism and criticism because ZHENG classification only depends on observation, knowledge, and clinical experience of TCM practitioners, which is lack of objectivity and repeatability. Scientists have done fruitful researches for its objectivity and standardization. Compared with traditional four diagnostic methods (looking, listening and smelling, asking, and touching), in this paper, the applications of new technologies and new methods on the ZHENG differentiation were systemically reviewed, including acquisition, analysis, and integration of clinical data or information. Furthermore, the characteristics and application range of these technologies and methods were summarized. It will provide reference for further researches.

1. Introduction

Personalized medicine is looming all over the world, especially following the accomplishment of the Human Genome Project (HGP). Major investments in basic science have created an opportunity for significant progress in clinical medicine. Scientists are developing and using diagnostic tests based on genetics or other molecular mechanisms to better predict patients' responses to targeted therapies [1]. Yet, myriad obstacles must be overcome to achieve these goals.

As a holistic approach attempting to bring the body, mind, and spirit into harmony, TCM may bring personalized medicine to the light in an efficient way. As the essential part of its theory, ZHENG, also called Traditional Chinese Medicine (TCM) syndrome or pattern, is a characteristic profile of all clinical manifestations identified by TCM practitioners and consists of not only the body condition, but also mind and spirit. With the general knowledge of ZHENG and its evolution, TCM emphasizes on early diagnosis and prognosis of diseases, especially preventing its recurrence. In TCM clinical practice, "Treatment based on ZHENG Differentiation" (Bian Zheng Lun Zhi) often gets better effect. For example, He et al. [2] reveal that the effective rate of a combination therapy of two Chinese patent medicines (Glucosidorum Tripterygll Totorum Tablet and Yishenjuanbi Tablet) on rheumatoid arthritis was 53.3%, without ZHENG differentiation. Nevertheless, the effective rate was up to more than 80%, with ZHENG differentiation. Besides, Lu et al. [3] found that the effective rate of biomedical combination therapy (diclofenac, methotrexate, and sulfasalazine) in cold syndrome was much higher than in heat syndrome ($P < 0.01$). After 12-week treatment, the effective rates in patients with cold syndrome and heat syndrome were 51.67% and 29.09%, respectively, but for 24-week treatment, it changed to 88.52%

and 57.40%. These researches may suggest that treatment based on ZHENG differentiation could improve the specificity and efficiency in both TCM and Western Medicine.

Although TCM has been practiced effectively more than 3000 years, ZHENG differentiation is still argued, because it depended on clinical observation and TCM practitioner's experience, which would be subjective and unrepeatable. Since the success of personalized medicine relies on having accurate diagnostic tests that identify patients who can benefit from targeted therapies [1], a great breakthrough in TCM diagnosis with objectivity and repeatability is needed. For this goal, TCM researchers have currently done fruitful works with beneficial technologies and methods, such as literature mining and system biological analysis. Here, the new technologies and the new methods of applied in ZHENG differentiation were reviewed, at the aspects of acquisition, analysis, and integration of clinical data or information, respectively.

2. Data Acquisition

As the saying goes, "one cannot make bricks without straw," a qualitative or a quantitative data is required before ZHENG differentiation. The acquisition of the applicable data using the appropriate technologies or methods is first step.

2.1. Qualitative Data Acquisition. The qualitative data is usually got from literatures, epidemiological questionnaire, and parameters by the traditional four diagnostic methods (looking, listening and smelling, asking, and touching). It could be used to describe the characteristics, distribution, and evolution of ZHENG, further to classification.

2.1.1. Literature Retrieval. Like as that we could have a further view standing on the shoulders of predecessors, literature retrieval is undoubtedly a feasible method for researching ZHENG differentiation. A research [4] was performed to probe into the characteristics of ZHENGs and their elements distributions in polycystic ovary syndrome. Literatures from 1994 to 2009 on ZHENGs were retrieved with keyword search and classified, then especially a database was set up by Excel for further analysis based on the collected data. With the help of those, the frequencies of 36 syndromes and their elements have been analyzed.

It is worthy to say that quality control should be taken seriously in the process of literature collection. And we cannot simply copy the Western standards, for example, Cochrane Statement [5]. An evaluation system for ZHENG differentiation should be established, which is suitable for TCM; otherwise, it will restrict even hold back its development.

2.1.2. Epidemiological Design. Clinical epidemiological study is widely used to acquire data, with the methods of retrospective, cross-sectional, and longitudinal study. With retrospective analysis, diagnostic information of 438 patients with chronic severe hepatitis B (CSHB) was investigated by Peng et al. [6]. The principle signs of TCM syndromes were analyzed by frequency and variable cluster analysis for ZHENG differentiation on three clinical stages. Especially, the research on evolution of "dampness-heat," "spleen deficiency," and "blood stasis" may provide assistance for dynamical ZHENG differentiation.

2.1.3. Improvement of Four Diagnostic Methods. As the most important traditional methods, four diagnostic methods (looking, listening and smelling, asking, and touching) have to be developed. As depended on TCM practitioners' observation and clinical experience, the shortcoming of these methods is absence of objective criteria and repeatability. Yue and Liu [7] and Pang et al. [8] have digitalized tongue images using computer technology, to bring tongue observation to semiquantitative measure. And TCM pulse detector was utilized to improve the accuracy and repeatability of pulse diagnosis and provide the data but just feeling [9].

2.2. Quantitative Data Acquisition. As above data is all acquired by observation and clinical experience, it is not only qualitative and unrepeatable, but also hard to conduct statistical analysis, pattern recognition, and integration with absence of totally digitalization. Therefore, the acquisition of quantitative data is calling for further progress.

2.2.1. "Omics" Technologies. "Omics" consists of genomics, transcriptomics, proteomics, and metabonomics, with the rapid growth of large-scale detection technologies [10]. It directly focuses on biochemistry networks, pathways, metabolites, and molecule targets of whole bodies, at the top-to-down views. With the features of nondestructiveness, integrity, multitarget, high-throughput, and digitalization, "Omics" technologies may provide feasibility to investigate ZHENG, which would be characterized by multifactor, multiphenotype, and dynamic state.

Genomics/transcriptomics, also known as global gene expression profiling, is a tool for evaluating gene expression levels of thousands of genes in parallel. Technologies such as gene chip, gene sequencing, and differential display are usually applied. Wu et al. [11] performed genomics to assess the correlation between genetic variations of metabolic genes including PPARD, PPARG, and APM1 and the constitutions. The result suggested that SNP and haplotypes of PPARD, PPARG, and APM1 may underlie the genetic basis of the ZHENG classification. Moreover, gene chip technology was used by Lu et al. [12] to reveal gene expression profiles in $CD4^+$ T cells to classify cold and heat syndromes.

Proteomics can be defined as the science and technologies associated with mapping, visualizing, and/or quantitating the expression of all or a majority of the proteins in living systems [13]. Technologies used in proteomics have been around two-dimensional polyacrylamide gels combined with mass spectrometer (MS) or liquid chromatography (LC). With the method of two-dimensional electrophoresis (2DE) combined with matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF-MS), Liu et al. [14] evaluated the levels of plasma proteins in health donors and patients with the different ZHENGs of chronic

hepatitis B. Objective data was provided for ZHENG differentiation and further to suggest the diagnostic standards and guide the clinical treatment. Wu et al. [15] analyzed the plasma from healthy subjects and patients of coronary heart disease. The result found 3 decreased proteins and 6 increased proteins in blood stasis syndrome, compared with normal group. It suggested that fibrinogen and granzyme might be potential diagnostic biomarkers of blood stasis syndrome in coronary heart disease.

Metabonomics is the study of global metabolite profiles in a biological system (isolated cells, tissue, urine, saliva, blood plasma, etc.) under a given set of conditions [16]. Gas chromatography-mass spectrometer (GC-MS), liquid chromatography-mass spectrometer (LC-MS), and nuclear magnetic resonance (NMR) are widely applied in this area. With the technology of GC/MS, Van Wietmarschen et al. [17] have analyzed the plasma metabolism profiles in patients with cold and heat syndromes of rheumatoid arthritis. They classified the two ZHENGs and got seven differential metabolites. Moreover, using UPLC-QTOF-MS, Sun et al. [18] have analyzed urine samples from liver-Qi invasion patients with premenstrual syndrome. The potential biomarkers and metabolic pathways were found from the metabolic profiles. Furthermore, Liu et al. [19] have detected plasma samples using NMR to explore the dynamic evolution and phase characteristics of phlegm and blood stasis syndromes from the biological features of lipid metabolism.

2.2.2. Physiology and Pathology Detection Technology. Signs, symptoms, and biochemical parameters of patients were collected by Yuan et al. [20] from self-designed questionnaires regarding the four diagnostic methods of TCM. The result suggested that different syndromes have different pathological features. Taking an example, dampness-heat syndrome was characterized by obvious hepatic inflammation, poor synthesis function, and more ascites.

2.2.3. Molecular Biology Detection Technology. The correlation between biochemical indicator and ZHENGs was evaluated by Zhao [21]. Seventy female RA patients with cold or heat syndrome were enrolled in this trial. However, as for the expression of cytokine (TNF-α, IL-10, IL-8), clinical inflammatory indexes (ESR), and immune indexes (IgA, IgG, IgM, RF, C3), subjects with heat and cold syndrome showed no significant difference, except CRP.

3. Data Mining

For the complexity of biomedicine, it is circumscribed for researches only based on experimental data. Therefore, objective and accurate description of phenomenon and regularity in TCM is getting out from statistical analysis and data mining, drawing assistance from computer technologies. As a multidiscipline fused artificial intelligence, statistics, pattern recognition, and so on, data mining in database is equal to knowledge discovery [22, 23], which is initially utilized for genome designator in biomedicine [24].

3.1. Association Rule Mining. Association rule mining is one of the major approaches of data mining and perhaps the most common method of knowledge discovery in unsupervised learning systems [25]. It is used to describe significant associations or correlation relationships among a large set of data items. Especially, Wu et al. [26] associated the gene function from the MEDLNE with TCM literatures. And then they established the relationship between diseases and ZHENGs, combined with validating the relationship between ZHENGs and genes.

3.2. Rough Sets Theory. As a new math tool to deal with ambiguous and uncertain information, rough sets theory introduced by Pawlak [27] is applied to get some decisions and classification. By deleting unrelated or unimportant information, it is able to simplify information on the premise of keeping classification ability unchanged. The information of symptoms and signs from 287 posthepatitic cirrhosis patients were collected by Zhang et al. to explore the application of rough sets theory in TCM ZHENG diagnosis. The result showed that this model was meaningful for the diagnosis, with 83% coincidence to main six ZHENGs in TCM [28].

3.3. Cluster Analysis. Cluster analysis, an exploring way of classification, could describe a set of multivariate methods and techniques. It is often used to classify data into groups, types, profiles, and so on [29]. With multicenter and large-sample survey, two-step cluster analysis was utilized to study the ZHENG distribution rule of essential hypertension by Gu et al. [30]. Compared with the current ZHENG differentiation criteria, this method could add two more ZHENGs which may be used to reflected etiological factor.

3.4. Bayesian Networks. Bayesian network is a kind of probability network which is based on probabilistic reasoning, with the foundation of Bayes formula. Especially through their ability to coordinate bidirectional probabilistic inferences, Bayesian networks are now considered to be a general representation scheme for uncertain knowledge [31, 32]. Qu et al. [33] used Bayesian network to classify ZHENGs in 611 depression patients. The ZHENGs of depression were differentiated by various principle or peripheral ZHENGs and their combinations. The ZHENGs described in their study were in line with clinical TCM and might provide a good guidance for treatment.

3.5. Decision Trees. Decision trees are characterized by a logic function which is constant over some box-shaped regions of the X range. These regions are usually represented by a binary decision tree consisting of nodes and binary splits [34]. It can be applied in the development of ZHENG classification. Zhong et al. [35] developed a method of decision trees combined with association rules to study Qi stagnation syndrome in gastritis, getting satisfactory prediction.

3.6. Artificial Neural Network. With ability to fitting function at any precision, artificial neural network is powerful to use a

structure similar with cerebrum neural synapse to deal with information. It has been demonstrated successfully in many classification tasks [36]. Neural network model trained by conjugate gradient algorithm was built by Sun et al. [37] to classify ZHENGs of coronary heart disease, with 89.2% accuracy. The research got satisfactory results and overcame the shortcomings of traditional BP algorithm effectively.

3.7. Principal Component Analysis. Beginning with the interrelation of the variables, principal component analysis based on the dimension reduction is a statistical method that could translate many variables to fewer unrelated integrated variances [38]. Metabonomics based on UPLC/MS had been performed by Lu et al. [39] to study Kidney-Yang deficiency syndrome and therapeutic effect of *Rhizoma Drynariae*. With PCA, a clear separation of model group and predose group was achieved. The time-dependent regression tendency in *Rhizoma Drynariae* treatment group from 1 to 15 days was obtained, which provided a visual, overall, and dynamic progress.

3.8. Partial Least Squares Method. Partial least squares (PLSs) method was proposed by Wold, which extracts characteristics based on the principle of maximizing covariance of independent and dependent variable [40]. It makes the characteristics to have much associativity with the dependent variable, improving the precision of the ZHENG classification followed. As clinical samples have more individual variations than animal samples, the supervised methods like PLS are better at concerning the main intergroup difference of clinical samples than unsupervised methods like PCA. Van Wietmarschen et al. [17] used partial least squares-differentiation analysis (PLS-DA) to distinguish cold and heat syndromes of RA patients which were not distinguished by PCA, getting satisfactory result of 3-oxo-propionic acid and other differential metabolites.

3.9. Factor Analysis. Factor analysis is used to find the least number of factors to account for the common variance of a large set of statistical expert system variables, excluding variable-specific (unique) variance [41]. It could be applied in analyzing the correlativity of many primitive markers, and then finding out the limited and unobserved potential variance which dominates and explains the correlativity. Multicenter prospective research on TCM ZHENG in 815 cases of unstable angina was conducted by using factors analysis with the nonlinear dimension reduction. Wang et al. [42] suggested that this method could help to classify ZHENG and establish the preliminary diagnostic criteria.

3.10. Structural Equation Modeling. Structural equation modeling is based on statistical methodology to study and deal with complex and multivariable data. This technique allowed for the computation of individual measurement errors associated with the observed variables [43]. What is more, it allows testing of a priori hypotheses about the complex causality between the latent variables of diseases and ZHENG**s**. Here, the ZHENGs and domain changes of menopause syndrome on samples of 236 women from literature retrieval were identified by exploratory factor analysis. After finding principle ZHENG of Kidney-Yang and Kidney-Yin deficiency by latent tree, structural equation modeling was applied to confirm the former result [44].

In addition, set pair analysis [45], logistic regression [46], entropy cluster algorithm [47], and support vector machines [48] were applied in ZHENG differentiation with satisfactory results.

4. Integration of Data or Bioinformation

An example is shown about how to integrate information. Systems biology approach with the combination of computational analysis and animal experiment was used to investigate this complex issue, ZHENG, in the context of the neuroendocrine immune (NEI) system. By using the methods of literature mining, network analysis, and topological comparison, it was revealed that hormones and immune factors were predominant in the cold and heat syndromes networks, respectively, which were connected by neurotransmitters. In addition, genes related to heat-related diseases are mainly present in the cytokine-cytokine receptor interaction pathway; whereas genes related to cold-related diseases are linked to the neuroactive ligand-receptor interaction pathway. Also, it was in a position to interpret the scientific basis of both ZHENG and associated herbal treatments [49].

The "interaction-network-function" strategy of integration reflecting from "Entity Ontology" to "Relation Ontology" was according to the holism of TCM in methodology.

5. Summary and Prospect

With the features of high throughput and multilevel, "Omics," and bioinformatics technologies are appropriate tools to investigate the holistic characteristics of ZHENG differentiation. In order to easily understand technologies and methods, application range, advantages and disadvantages of "Omics," and bioinformatics, it was resumptively summarized in Table 1.

To find the characteristics and pathogenesis of ZHENGs through high throughput and multilevel, qualitative, and qualitative data, the data mining methods were applied. The advantage and the disadvantage of these methods were resumptively summarized in Table 2.

Given the limitation of single method and single subject, the multidisciplinary such as mathematics, physics, biology, and statistics would be combined underlying the direction of system theory, which may bring ZHENG researches to an objective and quantized way. For example, cold and heat syndrome has been studied with multiple technologies and methods such as "Omics," bioinformatics and laboratory index [50]. And Bayesian network, rough set, and generalize connected coefficient were combined to classify ZHENG in liver cirrhosis [28]. And we advocate that systematically combined the appropriate technologies or methods to establish a characteristic "net-marker" of ZHENG differentiation

Table 1: Brief introduction of "Omics" and bioinformatics.

Omics	Objects	Technologies and methods	Advantages	Disadvantages	Literatures
Genomics (transcriptomics)	DNA, mRNA	Gene sequence, differential display, subtractive hybridization, EST, SAGE, chip technology	Gene polymorphism Susceptibility for prognosis and treatment Completed database High throughput	Nonassociation to regulation of life activities Nonconsistent strictly with mRNA expression	Wu et al. [11] Lu et al. [12]
Proteomics	Amino acids, protein	Cleaving isotope-coded affinity tag, 2D-MS, 2D-HCLP	Performer of life function	Instability Variability	Liu et al. [14] Wu et al. [15]
Metabonomics	Metabolites	NMR, GC-MS, LC-MS	Amplified action Simplicity to detect Less numbers Similarities in different species	Lack of beneficial supports Interferences by physiological factors	Van Wietmarschen et al. [17] Sun et al. [18] Liu et al. [19]
Bioinformatics	Data, bioinformation	Data mining, network analysis, topological comparison, and so on	Totally holism Exploration of the potential of information Focusing on function relation	Needing of self-development	Li [49]

Table 2: Brief introduction of data mining methods.

Methods	Advantages	Disadvantages	Literatures
Logistic regression	Multifunction	Needing of sample size	Luo et al. [46]
Bayesian networks	Utilization of incomplete and inaccurate data	Needing of preceding researches as guidance	Qu et al. [33]
Rough sets theory	Without priori information; simplicity; handling ambiguous and uncertain information	Needing of self-development	Zhang et al. [28]
Association rules mining	Supporting indirect data mining	Nonselectivity; subjectivity	Wu et al. [26]
Set pair analysis	Suitability for changing systems	Handicap in handle relatively precise problems	Li et al. [45]
Structural equation modeling	Analyzing the causality between the latent variables	Needs of 200 samples at least	Chen et al. [44]
Cluster analysis	Minimization errors caused by subjective judgment	Too much calculation; handicap in clustering data with multidimensions and multilevel	Gu et al. [30]
Decision trees	Handling in nonnumeric data; Simplicity	Maybe misleading	Zhong et al. [35]
Principal component analysis	Dimension reduction; holism	Less specificity	Lu et al. [39]
Partial least squares method	Specificity	Handicap in deciding principal component	Van Wietmarschen et al. [17]
Artificial neural network	Simplicity; nonlinear	Handicap in obtaining the hidden information	Sun et al. [37]
Entropy cluster algorithm	Little demand on variances' types; analysis on any statistical dependence of the variances	Needing of self-development	Wang et al. [47]
Factor analysis	Correction capability; views to latent variables	Absence of domination and relationship between primary and secondary	Wang et al. [42]
Support vector machine	Classification without representing the feature space explicitly	Expressing the more complex prior information; analyzing limited samples	Yang et al. [48]

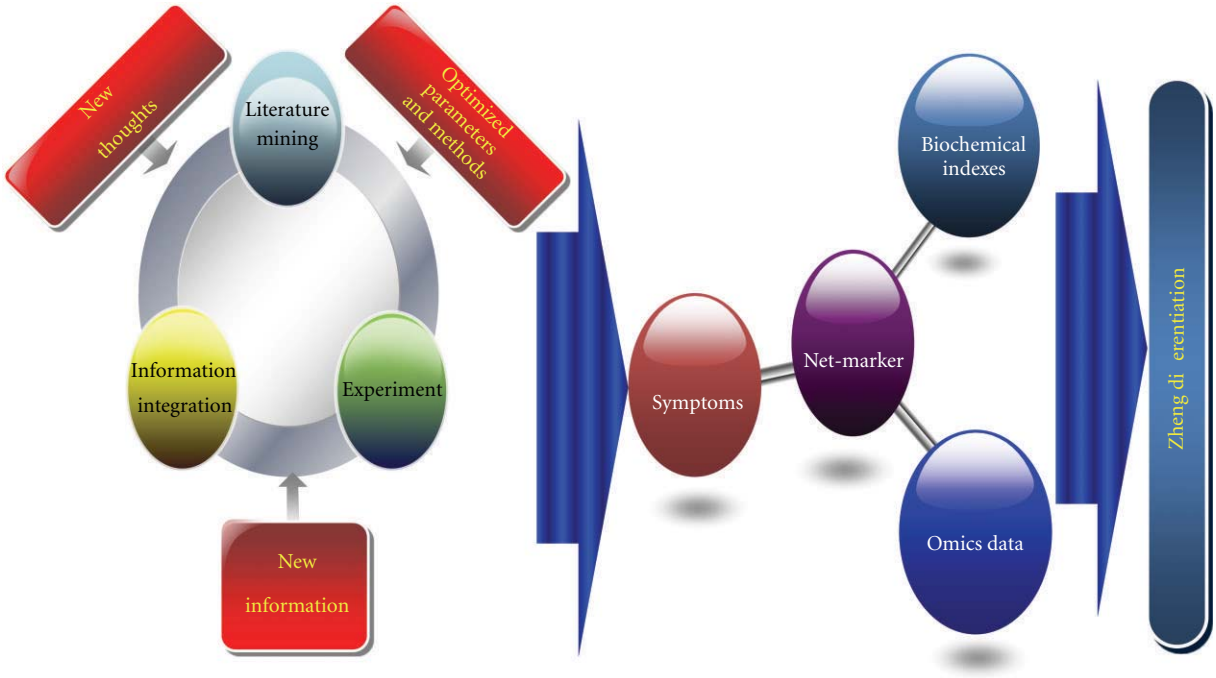


Figure 1: Schematic diagram of research approach for ZHENG differentiation.

using clinical signs, syndromes, biochemical indicators, and "Omics" data.

Furthermore, we proposed a ZHENG differentiation research approach bases on a computer-aided "information-experiment-information" model (Figure 1). By literature mining, researchers firstly could get necessary information to provide ideas, which include clinical syndromes and signs, laboratorial samples using the suitable methods. The ideas could guide new information which comes from experiments and supply validation. Then, analysis and integration of new data will produce further information for ZHENG differentiation.

In this progress, assistances are drawn from computer technologies. Data mining could provide the comprehensive and efficient way to deal with the massive data. Suitable methods with broader vision and optimized parameters could be explored by the objective data, but experience and subjective decision. And then, feedback will be got timely from the experiments by powerful statistical analysis, to guide next ones. Furthermore, the "net-marker" acquired from integration of former results may provide an overall and novel understanding of ZHENG for differentiation. The approach shows many differences to traditional thoughts on feasibility and directivity, reducing blindness and consumption (Figure 1).

In addition, for the clinical transformation in ZHENG differentiation, a further research of dynamic changes of ZHENG is needed. Following the development of high-throughout and noninvasive methods, especially the system biological technologies, may give the support to the dynamically differentiating ZHENG. Furthermore, the TCM information and bioinformation would be combined to make TCM syndrome network with the dynamic characteristic by bioinformatics and computer technologies.

Following the development of new technologies and new methods, the upgrade of TCM researchers' ability, and the expansion of views on the research of ZHENG differentiation, we all believe that objective and accurate approach would be beneficial to TCM diagnosis and treatment. As a result, TCM may play a more important role in personalized medicine.

Acknowledgments

This study was supported by National Science and Technology Major Project of China (no. 2012ZX10005001-004) and Leading Academic Discipline Project of Shanghai Municipal Education Commission (no. J50301).

References

[1] M. A. Hamburg and F. S. Collins, "The path to personalized medicine," *The New England Journal of Medicine*, vol. 363, no. 4, pp. 301–304, 2010.

[2] Y. He, A. Lu, Y. Zha, and I. Tsang, "Differential effect on symptoms treated with traditional Chinese medicine and western combination therapy in RA patients," *Complementary Therapies in Medicine*, vol. 16, no. 4, pp. 206–211, 2008.

[3] C. Lu, Q. Zha, A. Chang, Y. He, and A. Lu, "Pattern differentiation in traditional chinese medicine can help define specific indications for biomedical therapy in the treatment of rheumatoid arthritis," *Journal of Alternative and Complementary Medicine*, vol. 15, no. 9, pp. 1021–1025, 2009.

[4] X. M. Liu and Q. Hua, "Investigation on distributed feature of TCM syndrome and syndrome elements about polycystic

ovary syndrome from modern literature," *Chinese Journal of Information on TCM*, vol. 18, no. 2, pp. 34–36, 2011.

[5] J. H. Li, J. L. Wang, and C. Meng, "Present researches and analysis of quality valuation on literatures on clinical traditional Chinese medicine," *Chinese Journal of Information on TCM*, vol. 15, no. 6, pp. 95–98, 2008 (Chinese).

[6] J. Peng, B. Chen, K. W. Sun et al., "Retrospective analysis of distribution and evolution characteristics of syndrome dampness heat-blood stasis-spleen deficiency of chronic severe hepatitis B," *Chinese Journal of Integrated Traditional and Western Medicine on Liver Diseases*, vol. 21, no. 3, pp. 135–138, 2011.

[7] X. Q. Yue and Q. Liu, "Analysis of studies on pattern recognition of tongue image in traditional Chinese medicine by computer technology," *Journal of Chinese Integrative Medicine*, vol. 2, no. 5, pp. 326–329, 2004.

[8] B. Pang, D. Zhang, N. Li, and K. Wang, "Computerized tongue diagnosis based on Bayesian networks," *IEEE Transactions on Biomedical Engineering*, vol. 51, no. 10, pp. 1803–1810, 2004.

[9] J. Q. Hu, T. Zhao, G. C. Xu et al., "Assessment on the accuracy of four types of pulse by SM-1A TCM pulse detector," *World Science and Technology/Modernization of Traditional Chinese Medicine and Materia Medics*, vol. 13, no. 1, pp. 74–77, 2011.

[10] J. B. German, D. E. Bauman, D. G. Burrin et al., "Metabolomics in the opening decade of the 21st century: building the roads to individualized health," *Journal of Nutrition*, vol. 134, no. 10, pp. 2729–2732, 2004.

[11] Y. Wu, Y. Cun, J. Dong et al., "Polymorphisms in PPARD, PPARG and APM1 associated with four types of Traditional Chinese Medicine constitutions," *Journal of Genetics and Genomics*, vol. 37, no. 6, pp. 371–379, 2010.

[12] C. Lu, C. Xiao, G. Chen et al., "Cold and heat pattern of rheumatoid arthritis in traditional Chinese medicine: distinct molecular signatures indentified by microarray expression profiles in CD4-positive T cell," *Rheumatology International*, vol. 32, no. 1, pp. 61–68, 2010.

[13] N. G. Anderson and N. L. Anderson, "Twenty years of two-dimensional electrophoresis: past, present and future," *Electrophoresis*, vol. 17, no. 3, pp. 443–453, 1996.

[14] Y. P. Liu, P. Liu, R. Y. Dai et al., "Analysis of plasma proteome from cases of the different traditional Chinese medicine syndromes in patients with chronic hepatitis B," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 59, pp. 173–178, 2012.

[15] J. H. Wu, Z. C. Ma, Y. Gao, and S. Q. Wang, "Proteomics application in Research related proteins of blood stasis syndrome in coronary heart disease by Proteomics," *Chinese Journal of Integrative Medicine on Cardio-/Cerebrovascular Disease*, vol. 3, no. 3, pp. 189–191, 2005 (Chinese).

[16] S. Rochfort, "Metabonomics reviewed: a new "omics" platform technology for systems biology and implications for natural products research," *Journal of Cerebral Blood Flow & Metabolism*, vol. 68, no. 12, pp. 1813–1820, 2005.

[17] H. Van Wietmarschen, K. Yuan, C. Lu et al., "Systems biology guided by Chinese medicine reveals new markers for sub-typing rheumatoid arthritis patients," *Journal of Clinical Rheumatology*, vol. 15, no. 7, pp. 330–337, 2009.

[18] P. Sun, G. A. Luo, M. Q. Qiao et al., "Studies on the metabonomics of premenstrual syndrome liver-Qi invasion patient interfered by jingqianping granules," *World Science and Technology/Modernization of Traditional Chinese Medicine and Materia Medics*, vol. 12, no. 2, pp. 195–201, 2010.

[19] W. H. Liu, X. Z. Yan, L. Zhang, Q. Zhang, L. H. Wang, and F. H. Yu, "Metabonomics study on phlegm and blood stasis evolution of hyperlipidemia and atherosclerosis," *Liaoning Journal of Traditional Chinese Medicine*, vol. 49, no. 8, pp. 738–741, 2008.

[20] J. L. Yuan, H. Zhang, L. Wang et al., "Biochemical characteristics of traditional Chinese medicine syndromes and their elements in patients with hepatitis B cirrhosis," *Journal of Chinese Integrative Medicine*, vol. 9, no. 4, pp. 374–381, 2011.

[21] L. H. Zhao, C. Xiao, X. P. Yan et al., "Correlation between heat or cold syndrome and cytokine, and laboratory index in women with early rheumatoid arthritis," *Acta Universitatis Traditionis Medicalis Sinensis Pharmacologiaeque Shanghai*, vol. 20, no. 1, pp. 21–24, 2006.

[22] Y. C. Shen, X. Y. Wang, and Y. M. Cai, "Application and expectation of data mining in traditional Chinese medical research of syndrome and treatment," *Chinese journal of Integrated Traditional and Western Medicine*, vol. 28, no. 9, pp. 847–850, 2008.

[23] L. Zhu, B. Wu, and C. Cao, "Introduction to medical data mining," *Journal of Biomedical Engineering*, vol. 20, no. 3, pp. 559–562, 2003.

[24] W. Ding, Y. G. Jiang, Y. P. Song, M. X. Wu, and K. Li, "Data mining application in traditional Chinese medicine," *Journal of Mathematical Medicine*, vol. 20, no. 3, pp. 403–404, 2007 (Chinese).

[25] M. S. Chen, J. Han, and P. S. Yu, "Data mining: an overview from a database perspective," *IEEE Transactions on Knowledge and Data Engineering*, vol. 8, no. 6, pp. 866–883, 1996.

[26] Z. H. Wu, X. Z. Zhou, B. Y. Liu, and J. L. Chen, "Text mining for finding functional community of related genes using TCM knowledge," *Knowledge Discovery in Database*, vol. 3202, pp. 459–470, 2004.

[27] Z. Gong, B. Sun, and D. Chen, "Rough set theory for the interval-valued fuzzy information systems," *Information Sciences*, vol. 178, no. 8, pp. 1968–1985, 2008.

[28] Y. H. Zhang, P. Liu, Q. Zhang et al., "Bayesian model to traditional Chinese medicine syndrome classification based on the rough set theory and generalized-relating-degree coefficient," *Statistics and Decision*, vol. 264, pp. 159–161, 2008 (Chinese).

[29] S. T. Leonard and M. Droege, "The uses and benefits of cluster analysis in pharmacy research," *Research in Social and Administrative Pharmacy*, vol. 4, no. 1, pp. 1–11, 2008.

[30] W. L. Gu, Z. X. Shi, Y. X. Yu, Y. W. Wu, B. W. Lu, and K. K. Hui, "Distribution characteristics of syndrome types in essential hypertension," *Journal of Chinese Integrative Medicine*, vol. 8, no. 9, pp. 842–847, 2010.

[31] K. B. Korb and A. E. Nicholson, *Bayesian Artificial Intelligence, Chapman and Hall/CRC Computer Science and Data Analysis Series*, Taylor & Francis, 2010.

[32] A. Biedermann and F. Taroni, "Bayesian networks for evaluating forensic DNA profiling evidence: a review and guide to literature," *Forensic Science International*, vol. 6, no. 2, pp. 147–157, 2012.

[33] M. Qu, Q. S. Tang, Z. X. Bao, Y. Wang, and L. Ma, "Bayesian network application in traditional Chinese medicine syndrome," *Chinese archives of Traditional Chinese Medicine*, vol. 26, no. 7, pp. 1497–1498, 2008.

[34] A. Neumann, J. Holstein, J. R. Le Gall, and E. Lepage, "Measuring performance in health care: case-mix adjustment by boosted decision trees," *Artificial Intelligence in Medicine*, vol. 32, no. 2, pp. 97–113, 2004.

[35] Y. Zhong, X. L. Hu, and J. F. Lu, "Diagnosis in traditional Chinese medicine on gastritis based on relation rules and

decision trees," *Chinese Journal of Information on Traditional Chinese Medicine*, vol. 15, no. 8, pp. 97–99, 2008 (Chinese).

[36] P. K. Simpson, *Artificial Neural Systems*, Pergamon Press, Oxford, UK, 1989.

[37] G. X. Sun, C. Y. Liao, Z. K. Yuan, Z. X. Hu, and T. Ren, "Neural Network Modelsto Traditional Chinese Medicine Syndrome in coronary heart disease based on conjugate-gradientalgorithm," *Chinese Journal of Integrative Medicine on Cardio-/Cerebrovascular Disease*, vol. 6, no. 3, pp. 339–340, 2008 (Chinese).

[38] K. J. Friston, C. D. Frith, P. F. Liddle, and R. S. J. Frackowiak, "Functional connectivity: the principal-component analysis of large (PET) data sets," *Journal of Cerebral Blood Flow and Metabolism*, vol. 13, no. 1, pp. 5–14, 1993.

[39] X. Lu, Z. Xiong, J. Li, S. Zheng, T. Huo, and F. Li, "Metabonomic study on 'Kidney-Yang Deficiency syndrome' and intervention effects of Rhizoma Drynariae extracts in rats using ultra performance liquid chromatography coupled with mass spectrometry," *Talanta*, vol. 83, no. 3, pp. 700–708, 2011.

[40] K. Tang and T. Li, "Comparison of different partial least-squares methods in quantitative structure-activity relationships," *Analytica Chimica Acta*, vol. 476, no. 1, pp. 85–92, 2003.

[41] R. L. Gorsuch, *Factor Analysis*, Lawrence Erlbaum Associates, Hillsdale,, NJ, USA, 1983.

[42] J. Wang, Q. Y. He, H. X. Li, and Y. T. Fang, "Factor analysis of traditional Chinese medicine syndromes in 815 patients with unstable angina," *Journal of Chinese Integrative Medicine*, vol. 6, no. 8, pp. 788–792, 2008.

[43] D. Kaplan, *Structural Equation Modeling: Foundations and Extensions*, Sage, 2006.

[44] R. Q. Chen, C. M. Wong, K. J. Cao, and T. H. Lam, "An evidence-based validation of traditional Chinese medicine syndromes," *Complementary Therapies in Medicine*, vol. 18, no. 5, pp. 199–205, 2010.

[45] B. Li, R. Xu, F. L. Li, K. Q. Zhao, M. Zhang, and Z. C. Xu, "Study of syndrome differentiation factor of gouty arthritis with blood stasis syndrome based on connection number," *Journal of Chinese Integrative Medicine*, vol. 7, no. 8, pp. 724–728, 2009.

[46] H. G. Luo, Z. Han, J. X. Li et al., "Study on regularity of Traditional Chinese Medicine Syndrome in precancerous lesion of stomach cancer," *China Journal of Traditional Chinese Medicine and Pharmacy*, vol. 22, no. 6, pp. 399–401, 2007 (Chinese).

[47] J. Wang, H. X. Li, Z. Q. Sun et al., "Study on syndrome manifestations of Chinese medicine based on complicated algorithm," *Journal of Beijing Unveristy of Traditional Chinese Medicine*, vol. 29, no. 9, pp. 581–585, 2006 (Chinese).

[48] X. B. Yang, Z. H. Liang, and Y. J. Luo, "P-SVM applications in TCM syndrome classifications," *World Science and Technology/Modernization of Traditional Chinese Medicine and Materia Medics*, vol. 9, no. 1, pp. 28–31, 2007.

[49] S. Li, "Computational systems biology-based TCM research: a case study of cold/Hot zheng and associated formula," *World Science and Technology/Modernization of Traditional Chinese Medicine and Materia Medica*, vol. 9, no. 1, pp. 105–111, 2007.

[50] C. Lu, Y. Y. Liu, C. Xiao, M. Jiang, Q. L. Zha, and A. P. Lu, "Biological basis of cold and heat pattern of rheumatoid arthritis in traditional Chinese medicine," *World Science and Technology/Modernization of Traditional Chinese Medicine and Materia Medica*, vol. 12, no. 5, pp. 814–817, 2010.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 986427, 8 pages
doi:10.1155/2012/986427

Research Article

Combining ZHENG Theory and High-Throughput Expression Data to Predict New Effects of Chinese Herbal Formulae

Shuhao Yu,[1] Zhizhong Guo,[2] Yan Guan,[2] Yi-Yu Lu,[2] Pei Hao,[3] Yixue Li,[1,3] and Shi-Bing Su[2]

[1] *College of Life Science and Biotechnology, Shanghai Jiaotong University, 800 Dongchuan Road, Shanghai 200240, China*
[2] *Research Center for Complex System of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China*
[3] *Key Lab of Systems Biology/Key Laboratory of Synthetic Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, China*

Correspondence should be addressed to Shi-Bing Su, shibingsu07@163.com

Received 10 January 2012; Accepted 9 March 2012

Academic Editor: Shao Li

ZHENG is the key theory in traditional Chinese medicine (TCM) and it is very important to find the molecular pharmacology of traditional Chinese herbal formulae. One ZHENG is related to many diseases and the herbal formulae are aiming to ZHENG. Therefore, many herbal formulae whose effects on a certain disease have been confirmed might also treat other diseases with the same ZHENG. In this study, the microarrays collected from patients with QiXuXueYu ZHENG (Qi-deficiency and Blood-stasis syndrome) before treatment and after being treated with Fuzheng Huayu Capsule were analyzed by a high-throughput gene microarrays-based drug similarity comparison method, which could find the small molecules which had similar effects with Fuzheng Huayu Capsule. Besides getting the results of anti-inflammatory and anti-fibrosis drugs which embody the known effect of Fuzheng Huayu Capsule, many other small molecules were screened out and could reflect other types of effects of this formula in treating QiXuXueYu ZHENG, including anti-hyperglycemic, anti-hyperlipidemic, hyposenstive effect. Then we integrated this information to display the effect of Fuzheng Huayu Capsule and its potential multiple-target molecular pharmacology. Moreover, through using clinical blood-tested data to verify our prediction, Fuzheng Huayu Capsule was proved to have effects on diabetes and dyslipidemia.

1. Introduction

The traditional Chinese medicine (TCM) ZHENG, also known as TCM syndrome, is the key theory in TCM and the important diagnostic principle for TCM therapy [1]. It is very important to describe ZHENG in molecular level or find the molecular marks in ZHENG identification or classification, and then find the molecular pharmacology of traditional Chinese herbal formulae whose treatment are based the ZHENG.

Most current researches in ZHENG and herbal formulae were guided by the theory of western medicine, their study objects are "disease," not "ZHENG." So these researchers had got a certain "disease," and did some ZHENG identification and ZHENG classification work based on that certain disease [2, 3], though using high-throughput gene microarrays. Similarly, most researches in herbal formulae were limited to find the evidence of herbal formulae's effects on some certain "diseases" [4–8].

As we know, Chinese herbal formulae should aim to "ZHENG," not to "disease." Li et al. [9–11] had designed some systemic network method using public disease and drug component information to analyze the complexity of ZHENG and herbal formulae. For example, they had divided many diseases into cold ZHENG and hot ZHENG.

Since one ZHENG could relate many diseases and herbal formulae aimed to ZHENG, many herbal formulae, whose effect on a certain disease had been confirmed, might also treat other diseases with the same ZHENG (Figure 1).

In order to prove this idea, high-throughput gene microarrays were analyzed. The microarrays were collected from patients with QiXuXueYu ZHENG (Qi-deficiency and

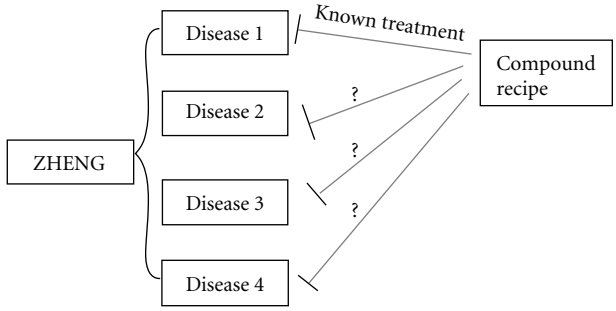


Figure 1: Prediction of herbal formulae's new treatment with the theory of "same ZHENG in different diseases." Many herbal formulae, whose effect on a certain disease had been confirmed, might also treat other diseases with the same ZHENG.

Blood-stasis syndrome) before treatment and treated with Fuzheng Huayu Capsule by a high-throughput drug similarity comparison method, we called it pathway-based similarity comparison (PBSC).

QiXuXueYu is a ZHENG whose patients suffer important energy deficiency and blood stasis. It is related with many different diseases such as diabetes mellitus [12, 13], dyslipidemia [14], hypertension [15], hepatitis, and liver cirrhosis [16]. This phenomenon is called "Same ZHENG in different diseases." Fuzheng Huayu Capsule is a recipe on the basis of Chinese medicine theory in treating liver fibrosis [17] with QiXuXueYu ZHENG, but few researches had been done to find its treatment on other diseases above.

The PBSC method was based on a microarray database "Connectivity Map" (cMap) [18], which collect microarrays corresponding to treatment of 164 different small molecules in different human cell lines. In association with the cMap, a lot of groups explored its usage in various applications, including drug resistance analysis [19], and toxicity prediction [20], But no one used this data resource to predict new treatment of Chinese herbal formulae.

We first apply the cMap database consistent with high-throughput expression data to predict new treatment of Chinese herbal formulae. In our results, there were many drug molecules screened out, including antihyperglycemic, antihyperlipidemic, hypotensive, anti-inflammatory, and antifibrosis drugs and some molecules having global effects. By integrating all the molecules' information, a Fuzheng Huayu Capsule mechanism map was obtained and Fuzheng Huayu Capsule had both short-term treatment effect and long-term prevention and healthcare effect. Furthermore, clinical blood-tested data were used to verify our prediction and finding that Fuzheng Huayu Capsule can really relieve the patients suffering liver cirrhosis combined with diabetes mellitus or dyslipidemia.

2. Material and Methods

2.1. Samples. There were six blood samples, in which four samples were from two QiXuXueYu ZHENG patients (patients A and B) in both states of before treatment and being treated with Fuzheng Huayu Capsule (3200 mg $*$ 3 times/day, 24 weeks). The rest two samples were from QiXuXueYu ZHENG patients (patient C) in both states of before treatment and being treated with placebo (vehicle). All patients were suffering liver cirrhosis from Shanghai Longhua Hospital and had signed an agreement with us. The blood samples were morning fasting venous blood and saved in -20°C with 150 μL EDTA.

Except for the 6 samples, there were additional 360 blood samples from 180 QiXuXueYu ZHENG patients with in both states of before treatment and being treated with Fuzheng Huayu Capsule, and blood tests were taken from these samples to verify our prediction. All the 180 patients were suffering liver cirrhosis. But these samples were at first not collected to prove the effect of Fuzheng Huayu Capsule on hyperglycemia or dyslipidemia, so the samples of patients suffering liver cirrhosis combining hyperglycemia or dyslipidemia were not very abundant. Seventeen patients had higher fasting blood-glucose (GLU), 31 patients had higher postprandial blood sugar (PPG), and 21 patients had higher glycated hemoglobin (Hb1Ac). Fifteen patients' total cholesterols (T-ch) were abnormal. Among them, 7 patients had higher T-ch than the normal range, while 8 patients had lower T-ch than the normal range. Eighteen patients' Total triglycerides (TGs) were abnormal. Among them, 11 patients had higher T-ch than the normal range, while 7 patients had lower T-ch than the normal range.

2.2. RNA Extraction and Microarrays. The TRIzol reagent (Invitrogen Life Technologies Company) was used to extract RNA of leukocyte from the whole blood of the 6 samples, then did a Quality Control with NanoDrop ND-1000.

cDNA was obtained through the Invitrogen first-strand cDNA synthesis using M-MLV RT and added RNA polymerase to degrade RNA. cDNA labelling and hybridizations on NimbleGen Homo sapiens 12×135 K Array (Roche, CAT No. A6484-00-01) were performed according to the manufacturer's protocol.

2.3. Microarray Data Analysis. Microarray data analysis was performed using the GenePix software. Raw expression data were log2-transformed and normalized by quantile normalization. Probes were considered robustly expressed if signal/noise ratio (SNR) < 2.

2.4. Connectivity Map (cMap) Database. "Connectivity Map" is a reference collection of gene-expression profiles from cultured human cells treated with bioactive small mol-ecules or drug molecules [18]. The data set was composed of mRNA expression data for 164 distinct small-mol-ecules and corresponding vehicle controls applied to human cell lines. All these data were by means of Affymetrix GeneChip microarrays. We had downloaded total of 564 gene expression profiles, representing 453 individual instances at http://www.broad.mit.edu/cmap/.

2.5. Pathway Set. Gene sets were needed to sort out genes according to meaningful signal pathways. A set called Sigpathway [21] was used in our method. These gene sets

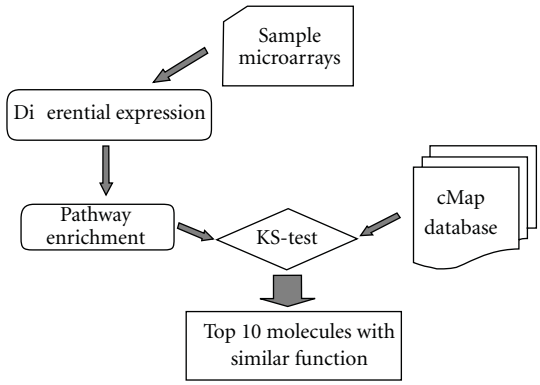


Figure 2: The process of PBSC method. 2-fold change was used as threshold for differential expression, and then Gene Set Enrichment Analysis (GSEA) was performed in every pathway. pathways, whose P-values obtained from GSEA was smaller than 0.05, were selected. Based on the selected Pathways, the expression pattern similarity between the microarrays of ours and in the cMap Database in every pathway was calculated using the KS-test.

are an integration of different pathway databases, including Biocarta, KEGG, BioCyc, pathway-specific microar-ray annotations, and >5,000 gene sets from Gene Ontology. The Sigpathway was available as an R package on http://www.bioconductor.org/packages/devel/bioc/html/sigPathway.html.

2.6. Pathway-Based Similarity Comparison (PBSC) Method. The process of PBSC was showed in Figure 2. At first 2-fold change was used as threshold for differential expression in every sample pair (treated with Fuzheng Huayu Capsule and before treatment), and then Gene Set Enrichment Analysis (GSEA) was performed in every pathway. Pathways, whose P-values obtained from GSEA was smaller than 0.05, were selected. Based on the selected pathways, the expression pattern similarity between the microarrays of ours and in the cMap Database in every pathway was calculated using the KS-test, which was recommended by Li et al. [22].

The progress of KS-test is as follows:

$$p = \max_{j=1}^{t} \left(\frac{j}{t} - \frac{V_j}{N} \right),$$
$$n = \max_{j=1}^{t} \left(\frac{V_j}{N} - \frac{j-1}{t} \right), \quad (1)$$
$$\mathrm{KS} = \begin{cases} P, & (P > n), \\ -n, & n > p. \end{cases}$$

In the formula above, t is the number of genes in either the up- or down-regulated gene groups and j is the jth gene according to the rank of differential expression. N is the number of total genes in array, and the position of the jth gene in the rank ordered whole gene list is $V(j)$.

The result of similarity (KS value) in every pathway would be either positive or negative ("positive" displays the similar effects and "negative" displays the reversed effects). The top 10 reference chemicals which had the most similar pathway (both positive and negative) numbers were selected for each analysis.

All the process above was executed in R (Statistical software).

2.7. Statistic Analysis. From the blood test data of patients suffering liver cirrhosis combining hyperglycemia or dyslipidemia, some indexes related with hyperglycemia or dyslipidemia were extracted, including fasting blood-glucose (GLU), postprandial plasma glucose (PPG), glycated hemoglobin (HbA1c), total cholesterol (T-ch) and total triglyceride (TG). The blood test data were expressed as means ± SD. Comparisons between before treatment and after treatment were performed by Student's t-test. The level of significance was set at $P < 0.05$. t-test was executed in R.

3. Results and Discussion

3.1. Di erential Expression and Pathway Enrichment. The samples from three patients (patients A, B, and C) were, respectively, analyzed by PBSC method. The microarray data of patient B showed more difference expression genes (4375 up, 3066 down) than patient A (1642 up, 1743 down) between being treated with Fuzheng Huayu Capsule and before treatment. In other words, the recipe produced a greater effect on patient B.

Similarly, patient B showed more pathway changes than patient A in the pathway enrichment analysis (67 pathways versus 48 pathways). Many pathways were larger primary metabolic process; some smaller pathways were presented in Table 1. In the smaller pathways, the ubiquitin cycle with the protein catabolic metabolism seemed to be very important in our result. But so many larger primary metabolic processes can also contain, suggested that the effects of Fuzheng Huayu Capsule may be as a whole-regulated mechanism.

Though patient C was treated with placebo, the microarray data also had many differential expression genes (2297 up, 1723 down). But these genes were in disorder and do not enrich many effects. Only 4 pathways were enriched (Table 1).

There were many factors leading to the large difference before and after treatment even for placebo, such as the patients' situation and nursing care during the process of treatment. More repeated microarray examples with repeated experiment would be collected in future to improve the data unbalance.

3.2. The Top 10 Molecules Had Similar Gene Expression Pattern and with Fuzheng Huayu Capsule. After pathway enrichment analysis, the similarity search for every pathway between the microarray data and cMap Database was executed. For each patient, top 10 drug molecules in cMap Database sharing the largest number of significantly affected pathway numbers with Fuzheng Huayu Capsule (patients A and B) or placebo (patient C) were presented in Table 2. "+" indicates the number of pathways positively correlated; "−" indicate the number of pathways negatively correlated.

Table 1: Partial pathway enrichment.

Patient A pathways	Patient B pathways	Patient C pathways
Ubiquitin cycle	Uubiquitin cycle	Cellular protein metabolic process
Leukocyte migration	Apoptosis	Protein metabolic process
Transmembrane receptor protein tyrosine kinase signaling pathway	Ubiquitin-dependent protein catabolic process	Cellular macromolecule metabolic process
Nitrogen compound metabolic process	Regulation of actin polymerization and/or depolymerization	rRNA metabolic process
Regulation of angiogenesis	Nucleocytoplasmic transport	

Table 2: The top 10 drug molecules affected pathways with Fuzheng Huayu Capsule or placebo.

(a) Patient A

cMap ID	Drug molecule	Dose	Pathway counts
169	Tacrolimus	1 uM	22+
383	Cobalt chloride	100 uM	21+
144	Chlorpropamide	100 uM	20+ 1−
641	Benserazide	10 uM	20+
576	Novobiocin	100 uM	20−
487	Pirinixic acid	100 uM	20−
421	Trifluoperazine	10 uM	20+
314	Exisulind	50 uM	20+
284	Tacrolimus	1 uM	20+
268	Genistein	1 uM	20+

(b) Patient B

cMap ID	Drug molecule	Dose	Pathway counts
487	Pirinixic acid	100 uM	53+
161	Verapamil	10 uM	52+
2	Metformin	10 uM	52+
419	Chlorpromazine	10 uM	49+
49+	Sirolimus	100 nM	
49+	Dexverapamil	10 uM	
141	Chlorpropamide	100 uM	49+
122	Alpha-estradiol	10 nM	49+
457	Tetraethylenepentamine	100 uM	47−
124	Mesalazine	100 uM	46+

(c) Patient C

cMap ID	Drug molecule	Dose	Pathway counts
608	NU-1025	100 uM	4−
418	Haloperidol	10 uM	4+
282	Fludrocortisone	1 uM	4+
1072	Trichostatin A	1 uM	3+
984	Acetylsalicylic acid	100 uM	3+
1009	Clozapine	10 uM	3+
1017	Fluphenazine	10 uM	3+
1024	Haloperidol	10 uM	3+
995	Prochlorperazine	10 uM	3−
887	Celastrol	3 uM	3+

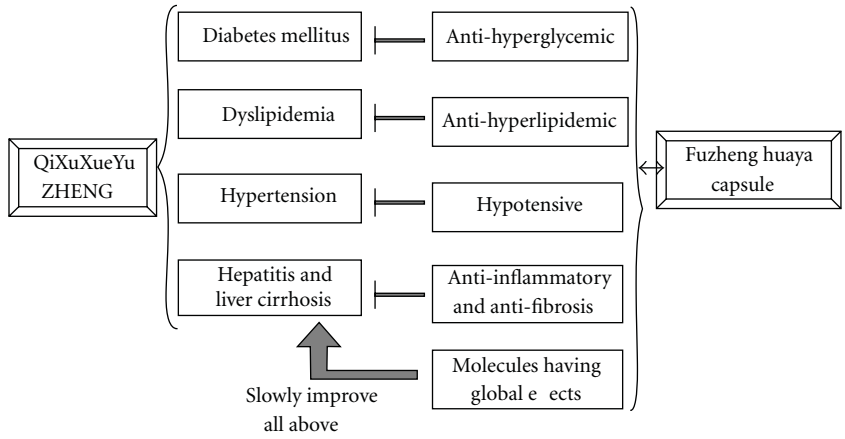


Figure 3: Predicted effects of Fuzheng Huayu Capsule. There were many drug molecules predicted by our method can reflect one part of effects of the formulae, including anti-hyperglycemic (chlorpropamide, metformin), anti-hyperlipidemic (pirinixic acid), hypotensor (verapamil, dexverapamil), anti-inflammatory, and anti-fibrosis drugs (tacrolimus, sirolimus, and mesalazine), molecules having global effects (estrogen, genistein).

Almost all drug molecules presented in Tables 2(a) and 2(B) had positive pathways, so these molecules had similar gene expression pattern and effects with Fuzheng Huayu Capsule in such pathways. These drug molecules could be classified by their effects, including anti-hyperglycemic (Chlorpropamide, Metformin), anti-hyperlipidemic (Pirinixic acid), hypotensor (Verapamil, Dexverapamil), anti-inflammatory and Anti-fibrosis drugs (Tacrolimus, Sirolimus, Mesalazine) and some molecules having global effects (Estrogen, Genistein). The new effects of Fuzheng Huayu Capsule was predicted and summarized in Figure 3.

In anti-inflammatory and Anti-fibrosis drugs, Tacrolimus in Patient A and Sirolimus in Patient B were immunosuppressant drugs. Tacrolimus was a calcineurin inhibitor. Sirolimus inhibits the response to IL-2, and thereby blocks activation of T- and B-cells. They can also ameliorate fibrosis [23, 24]. Mesalazine was also an anti-inflammatory drug [25]. These results showed the known effects of Fuzheng Huayu Capsule.

In Anti-hyperglycemic, Chlorpropamide was the only molecule positive in both patients A and B. It was a drug in the sulphonylurea class used to treat type 2 diabetes mellitus [26]. Sulfonylureas bind to K^+ channel on the cell membrane of pancreatic beta cells, Then depolarization opens voltage-gated Ca^{2+} channels. The rise in intracellular calcium leads to increased fusion of insulin granulae with the cell membrane, and therefore increased secretion of (pro)insulin [26]. Metformin was also a drug used to treat type 2 diabetes mellitus [27].

In hypotensive, verapamil and dexverapamil were calcium channel blockers of the phenylalkylamine class. It had been used in the treatment of hypertension [28]. Calcium channels were present in the smooth muscle that lines blood vessels. By relaxing the tone of this smooth muscle, calcium-channel blockers dilate the blood vessels [28].

In anti-hyperlipidemic, pirinixic acid was a hypolipidemic, peroxisome proliferator-activated receptor [29]. There was a special situation about pirinixic acid in our result. pirinixic acid in patient A was negative to Fuzheng Huayu Capsule, while it was positive in patient B. This means Fuzheng Huayu Capsule could play a role like pirinixic acid to reduce blood lipids and play a reversed role to raise blood lipids. Some other researches had found the bidirectional regulation effect of TCM [30, 31]; it was an unique feature of TCM which was rare in western medicine. We also did some verification on the bidirectional regulation effect of Fuzheng Huayu Capsule in Section 3.4.

In molecules having global effects, genistein was one of several known isoflavones found in leguminous plants, causing effects in the body similar to those caused by the hormone estrogen (estradiol). Isoflavones and estradiol can regulate blood glucose [32], blood fat [33], blood pressure [34], inflammation [35] with many long-term systemic effect.

There were also some molecules in Tables 2(a) and 2(b) that did not have many relationships with the above diseases (diabetes mellitus, dyslipidemia, hypertension, hepatitis and liver cirrhosis). Tetraethylenepentamine was negative to Fuzheng Huayu Capsule in patient B and it was a harmful substance to people, so this result hinted that tetraethylenepentamine would aggravate the illness of patients. Chlorpromazine in Patient B and trifluoperazine in patient A were typical antipsychotic. Exisulind and novobiocin were drugs used to treat cancer. But in Table 2(c), the placebo also shows these effects by some molecules, though the number of pathways was very small. Haloperidol, clozapine, fluphenazine, and prochlorperazine were all antipsychotic. Trichostatin A was an anti-tumor agent. So antipsychotic and anti-tumor were not the main effects of Fuzheng Huayu Capsule. This effect might have some other cause. There might be some bias in examples or the patients may had some comfort mentality after treatment and then show some effects of psychotropic drugs.

3.3. The Potential Multiple-Target Molecular Pharmacology of Fuzheng Huayu Capsule. Integrating all the information

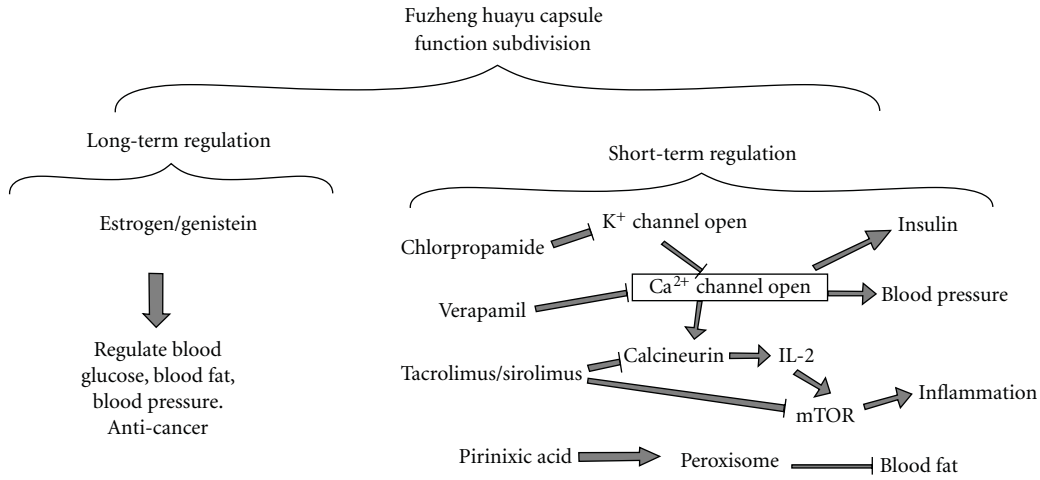


Figure 4: potential multiple-target molecular pharmacology of Fuzheng Huayu Capsule. Genistein and estradiol were assigned to long-term regulation group and other drugs were assigned to short-term regulation group. Ca^{2+} related effects might have core effects in the molecular pharmacology of the short-term group.

Table 3: The laboratory parameters of blood glucose and blood lipid.

Laboratory parameters	Total patient number	Improved patient number	Normal value range	Total average before treatment	Total average after treatment	P value (T test)
GLU (mmol/L)	17	7	3.89–6.1	7.42	6.52	0.068
PPG (mmol/L)	31	12	3.9–7.8	10.84	8.72	0.025
HbA1c (%)	21	16	4.3–6.5	7.48	5.86	0.00002
T-ch (higher) (mmol/L)	7	4	2.86–5.98	7.19	6.49	0.383
T-ch (lower) (mmol/L)	8	7	2.86–5.98	2.66	3.76	0.0207
TG(higher) (mmol/L)	11	7	0.58–1.88	2.61	1.93	0.105
TG (lower) (mmol/L)	7	5	0.58–1.88	0.52	0.94	0.106

above, a mechanism map of Fuzheng Huayu Capsule effects was built up as follows (Figure 4). The drugs in our results were divided into two big groups, long-term regulation group and short-term regulation group. Genistein and estradiol were assigned to long-term regulation group, because they had many sustained effects on our health and we can get them by daily diet or produce them by ourselves.

Chlorpropamide/metformin, tacrolimus/sirolimus, verapamil/dexverapamil, and Pirinixic acid were all assigned to short-term regulation group. The Ca^{2+} related effects had a core effects in the molecular pharmacology of the short-term effects of Fuzheng Huayu Capsule. Ca^{2+} is an important second messenger in many cell primary metabolic processes such as inflammation, metabolism, apoptosis, smooth muscle contraction, intracellular movement, nerve growth, and the immune response.

There was an important point that these small molecules were selected by effects, not by compound structure. The PBSC method could find molecules having similar effects, not similar structure. In fact, many molecules in our result had considerable side effect, but Fuzheng Huayu Capsule do not have considerable side effect.

Therefore, our result did not means there were some molecules in Fuzheng Huayu Capsule having similar structure or drug target with the molecules in our result. They should only have similar effect on downstream mechanism, such as Ca^{2+} related pathway.

3.4. Blood Test Verification. To verify our prediction, we took use of some existing data of blood tests. The data included 360 samples from 180 QiXuXueYu ZHENG patients in both states of before treatment and being treated with Fuzheng Huayu Capsule. But these samples were at first not collected to prove the effect of Fuzheng Huayu Capsule on hyperglycemia or dyslipidemia. So only a part of the patients were suffering hyperglycemia or dyslipidemia, while all the 180 patients were suffering liver cirrhosis. The laboratory values of blood glucose and blood lipid in the data of blood tests were showed in Table 3.

In blood glucose tests, 17 patients had higher GLU before treatment and 7 patients (41%) got back to normal range after treatment with Fuzheng Huayu Capsule. According to the treatment, total average GLU of the 17 patients went down from 7.42 to 6.52, and 12 of 31 patients (38%) got back to normal range. PPG and the total average PPG went down from 10.84 to 8.72. Moreover, 16 of 21 patients (76%) got back to normal range of HbA1c and the total average HbA1c went down from 7.48 to 5.86. There were the significant difference in data of PPG and HbA1c between before and after treatment ($P < 0.05$).

In blood lipid tests, 7 patients had higher T-ch than the normal range, while 8 patients had lower T-ch than the normal range, and 11 patients had higher TG than the normal range, while 7 patients had lower T-ch than the normal range. The average values of all sets of patients tended to normal after treatment. May be it was lack of samples, the data between before and after treatment did not have significant difference except that lower T-ch went up. Interestly, not only the higher T-ch and TG were down regulated, but also the lower T-ch and TG were up regulated by Fuzheng Huayu Capsule, which may be a characteristic of herbal formulae with multi-compounds.

Previous study also reported that Fuzheng Huayu had comprehensive effect on patients suffering liver fibrosis along with Diabetes mellitus [36]. These results suggested that Fuzheng Huayu Capsule could really relieve the patients suffering liver cirrhosis combined with diabetes mellitus and might have biphasic regulation effects on dyslipidemia.

Scince the research was to mainly explore a method to predict new effects of Fuzheng Huayu Capsule through integrat the information of ZHENG, herbal formula, and diseases, the experimental examples were not very abundant. We would carry out studies on large samples in future.

4. Conclusion

We introduced a high-throughput gene microarrays-based method (PBSC) to predict the potential effects of Fuzheng Huayu Capsule, a Chinese herbal formula on liver cirrhosis with QiXuXueYu ZHENG. The predicted results showed that the comprehensive effects of Fuzheng Huayu Capsule might be including Anti-hyperglycemic, anti-hyperlipidemic, hypotensive and anti-inflammatory, and Anti-fibrosis drugs. To verify our prediction, we had also taken the blood tests and got the effectiveness of Fuzheng Huayu Capsule on liver cirrhosis combined with diabetes mellitus or dyslipidemia. Further researches must get more samples to confirm the potential effects of Fuzheng Huayu Capsule.

Our research results suggested that the PBSC method is effective to find small molecules which had similar gene expression patterns and effects with herbal formulae and offer invaluable information for predicting new treatment application of herbal formulae.

Author's Contributions

S. Yu and Z. Guo had equal contributions to this research.

Acknowledgments

This study was supported by National Science and Technology Major Project of China (no. 2012ZX10005001-004 and no. 2009ZX09311-003), Leading Academic Discipline Project of Shanghai Municipal Education Commission (no. J50301) and E-institutes of Shanghai Municipal Education Commission (no. E 03008).

References

[1] A. P. Lu and K. J. Chen, "Integrative medicine in clinical practice: from pattern differentiation in traditional Chinese medicine to disease treatment," *Chinese Journal of Integrative Medicine*, vol. 15, no. 2, p. 152, 2009.

[2] C. Lu, C. Xiao, G. Chen et al., "Cold and heat pattern of rheumatoid arthritis in traditional Chinese medicine: distinct molecular signatures indentified by microarray expression profiles in CD4-positive T cell," *Rheumatology International*, pp. 1–8, 2010.

[3] C. Xiao, L. H. Zhao, and C. Lu, "Study on the differential gene expression of peripheral CD4+ among rheumatoid arthritis patients of cold or heat syndrome type with or without rheumatoid factor," *Zhongguo Zhong xi yi jie he Za Zhi*, vol. 26, no. 8, pp. 689–693, 2006.

[4] S. H. Wu, J. F. Sun, and S. Z. Guo, "Effect of compound recipe Gengniankang (Chinese characters) on senile sexual hormone and expression of estrogen receptor in bone of climacteric female rats," *Chinese Journal of Integrative Medicine*, vol. 11, no. 3, pp. 205–208, 2005.

[5] F. Zuo, Z. M. Zhou, M. Z. Yan et al., "Metabolism of constituents in Huangqin-Tang, a prescription in traditional Chinese medicine, by human intestinal flora," *Biological and Pharmaceutical Bulletin*, vol. 25, no. 5, pp. 558–563, 2002.

[6] Y. Cao, Q. H. Xia, H. Meng, and A. P. Zhong, "Pharmacological effects of serum containing Chinese medicine Bushen Huayu Jiedu compound recipe in lung cancer drug-resistance cells," *Chinese Journal of Integrative Medicine*, vol. 14, no. 1, pp. 46–50, 2008.

[7] Y. T. Wang, Q. R. Tan, L. L. Sun et al., "Possible therapeutic effect of a Traditional Chinese Medicine, Sinisan, on chronic restraint stress related disorders," *Neuroscience Letters*, vol. 449, no. 3, pp. 215–219, 2009.

[8] K. Tanaka and S. Sawamura, "Therapeutic effect of a traditional Chinese medicine, ren-shen-yang-rong- tang (Japanese name: Ninjin'yoeito) on nitric oxide-mediated lung injury in a mouse infected with murine cytomegalovirus," *International Immunopharmacology*, vol. 6, no. 4, pp. 678–685, 2006.

[9] S. Li, B. Zhang, D. Jiang, Y. Wei, and N. Zhang, "Herb network construction and co-module analysis for uncovering the combination rule of traditional Chinese herbal formulae," *BMC Bioinformatics*, vol. 11, supplement 11, p. S6, 2010.

[10] S. Li, B. Zhang, and N. Zhang, "Network target for screening synergistic drug combinations with application to traditional Chinese medicine," *BMC Systems Biology*, vol. 5, supplement 1, p. S10, 2011.

[11] S. Li, Z. Q. Zhang, L. J. Wu, X. G. Zhang, Y. D. Li, and Y. Y. Wang, "Understanding ZHENG in traditional Chinese medicine in the context of neuro-endocrine-immune network," *IET Systems Biology*, vol. 1, no. 1, pp. 51–60, 2007.

[12] G. Z. Miao, X. Z. Liang, and Y. H. Wang, "Clinical observation on treatment of diabetic peripheral neuropathy with qi-sup-

plementing and blood-activating therapy," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 23, no. 11, pp. 826–828, 2003.

[13] H. F. Que, Y. Y. Zhu, Y. F. Wang et al., "Effects of Chinese herbs for replenishing qi and resolving stagnation on hypoxia-inducible factor-1α and vascular endothelial growth factor in granulation tissue of skin ulcers in rats with diabetes," *Journal of Chinese Integrative Medicine*, vol. 5, no. 2, pp. 165–169, 2007.

[14] L. M. Xu and Y. Y. Hu, "Studies on treatment of fatty liver with traditional Chinese medicine," *Zhong xi yi Jie He Xue Bao*, vol. 1, no. 2, pp. 138–141, 2003.

[15] X. Duan, D. Yang, and X. Sun, "Effect of yimai jiangya extract on plasma neuropeptide Y level in patients of senile hypertension with qi-deficiency and blood stasis syndrome," *Zhongguo Zhong xi yi Jie he Za Zhi*, vol. 20, no. 10, pp. 750–752, 2000.

[16] Q. Zhang, P. Liu, and H. W. Zhang, "Study on the patterns of TCM syndrome differentiation of 900 patients with posthepatitic cirrhosis," *Zhongguo Zhong xi yi jie he Za Zhi*, vol. 26, no. 8, pp. 694–697, 2006.

[17] C. Liu, Y. Hu, L. Xu, and P. Liu, "Effect of Fuzheng Huayu formula and its actions against liver fibrosis," *Chinese Medicine*, vol. 4, p. 12, 2009.

[18] J. Lamb, E. D. Crawford, D. Peck et al., "The connectivity map: using gene-expression signatures to connect small molecules, genes, and disease," *Science*, vol. 313, no. 5795, pp. 1929–1935, 2006.

[19] G. Wei, D. Twomey, J. Lamb et al., "Gene expression-based chemical genomics identifies rapamycin as a modulator of MCL1 and glucocorticoid resistance," *Cancer Cell*, vol. 10, no. 4, pp. 331–342, 2006.

[20] J. L. Smalley, T. W. Gant, and S. D. Zhang, "Application of connectivity mapping in predictive toxicology based on gene-expression similarity," *Toxicology*, vol. 268, no. 3, pp. 143–146, 2010.

[21] L. Tian, S. A. Greenberg, S. W. Kong, J. Altschuler, I. S. Kohane, and P. J. Park, "Discovering statistically significant pathways in expression profiling studies," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 38, pp. 13544–13549, 2005.

[22] Y. Li, P. Hao, S. Zheng et al., "Gene expression module-based chemical function similarity search," *Nucleic Acids Research*, vol. 36, no. 20, p. e137, 2008.

[23] F. S. Larsen, B. Vainer, M. Eefsen, P. N. Bjerring, and B. A. Hansen, "Low-dose tacrolimus ameliorates liver inflammation and fibrosis in steroid refractory autoimmune hepatitis," *World Journal of Gastroenterology*, vol. 13, no. 23, pp. 3232–3236, 2007.

[24] E. Patsenker, V. Schneider, M. Ledermann et al., "Potent antifibrotic activity of mTOR inhibitors sirolimus and everolimus but not of cyclosporine A and tacrolimus in experimental liver fibrosis," *Journal of Hepatology*, vol. 55, no. 2, pp. 388–398, 2011.

[25] R. Bergman and M. Parkes, "Systematic review: the use of mesalazine in inflammatory bowel disease," *Alimentary Pharmacology and Therapeutics*, vol. 23, no. 7, pp. 841–855, 2006.

[26] W. El-Andere, A. C. Lerario, and B. L. Wajchenberg, "Effect of short- and long-term chlorpropamide therapy on oral glucose tolerance and erythrocyte insulin receptors in non-obese non-insulin dependent diabetes mellitus," *Hormone and Metabolic Research*, vol. 19, no. 6, pp. 257–263, 1987.

[27] E. Fidan, H. Onder Ersoz, M. Yilmaz et al., "The effects of rosiglitazone and metformin on inflammation and endothelial dysfunction in patients with type 2 diabetes mellitus," *Acta Diabetologica*, pp. 1–6, 2011.

[28] P. Bergson, G. Lipkind, S. P. Lee, M. E. Duban, and D. A. Hanck, "Verapamil block of T-type calcium channels," *Molecular Pharmacology*, vol. 79, no. 3, pp. 411–419, 2011.

[29] A. A. Santilli, A. C. Scotese, and R. M. Tomarelli, "A potent antihypercholesterolemic agent: [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (Wy-14643)," *Experientia*, vol. 30, no. 10, pp. 1110–1111, 1974.

[30] W. Chen, W. An, and J. Chu, "Effect of water extract of Poria on cytosolic free calcium concentration in brain nerve cells of neonatal rats," *Zhongguo zhong xi yi Jie he Za Zhi*, vol. 18, no. 5, pp. 293–295, 1998.

[31] H. Y. Fu and Y. Z. He, "Studies on the hidropoiesis of the decoction of gui-zhi tang in mice and rats," *Zhong xi yi Jie he Za Zhi*, vol. 11, no. 1, pp. 34–6, 1991.

[32] J. S. Lee, "Effects of soy protein and genistein on blood glucose, antioxidant enzyme activities, and lipid profile in streptozotocin-induced diabetic rats," *Life Sciences*, vol. 79, no. 16, pp. 1578–1584, 2006.

[33] L. Nogowski, P. Mackowiak, K. Kandulska, T. Szkudelski, and K. W. Nowak, "Genistein-induced changes in lipid metabolism of ovariectomized rats," *Annals of Nutrition and Metabolism*, vol. 42, no. 6, pp. 360–366, 1998.

[34] H. Si and D. Liu, "Genistein, a soy phytoestrogen, upregulates the expression of human endothelial nitric oxide synthase and lowers blood pressure in spontaneously hypertensive rats," *Journal of Nutrition*, vol. 138, no. 2, pp. 297–304, 2008.

[35] G. Ji, Q. Yang, J. Hao et al., "Anti-inflammatory effect of genistein on non-alcoholic steatohepatitis rats induced by high fat diet and its potential mechanisms," *International Immunopharmacology*, vol. 11, no. 6, pp. 762–768, 2011.

[36] C. Q. Zhao, H. T. Gu, Y. Cheng, and A. et, "Applying Fuzheng Huayu Gantang comprehensive therapeutic program for treatment of post-hepatitis B liver cirrhosis complicated with glyco-metabolic abnormality," *Zhongguo zhong xi yi Jie he Za Zhi*, vol. 28, no. 1, pp. 24–27, 2008.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 132829, 9 pages
doi:10.1155/2012/132829

Research Article

A Damages Learning and Memory in Alzheimer's Disease Rats with Kidney-Yang Deficiency

Dongmei Qi,[1] Yongfa Qiao,[2] Xin Zhang,[1] Huijuan Yu,[1] Bin Cheng,[1] and Haifa Qiao[1,3,4]

[1] *Neuroscience Program, Shandong University of Traditional Chinese Medicine, Changqing University Park, Jinan 250355, China*
[2] *Qingdao Haici Medical Group, 4 Renmin Road, Qingdao 266033, China*
[3] *Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences, 16 Nanxiaojie, Dongzhimeinei, Beijing 100700, China*
[4] *Department of Biomedical Sciences, Florida State University College of Medicine, 1115 West Call Street, Tallahassee, FL 32306, USA*

Correspondence should be addressed to Haifa Qiao, haifa.qiao@med.fsu.edu

Received 9 January 2012; Accepted 18 February 2012

Academic Editor: Shi-Bing Su

Previous studies demonstrated that Alzheimer's disease was considered as the consequence produced by deficiency of Kidney essence. However, the mechanism underlying the symptoms also remains elusive. Here we report that spatial learning and memory, escape, and swimming capacities were damaged significantly in Kidney-yang deficiency rats. Indeed, both hippocampal Aβ_{40} and 42 increases in Kidney-yang deficiency contribute to the learning and memory impairments. Specifically, damage of synaptic plasticity is involved in the learning and memory impairment of Kidney-yang deficiency rats. We determined that the learning and memory damage in Kidney-yang deficiency due to synaptic plasticity impairment and increases of Aβ_{40} and 42 was not caused via NMDA receptor internalization induced by Aβ increase. β-Adrenergic receptor agonist can rescue the impaired long-term potential (LTP) in Kidney-yang rats. Taken together, our results suggest that spatial learning and memory inhibited in Kidney-yang deficiency might be induced by Aβ increase and the decrease of β_2 receptor function in glia.

1. Introduction

Alzheimer's disease (AD), the most common cause of dementia, is a chronic disorder characterized by a progressive decline in cognitive function. Great lines of evidence have verified that the formation of AD is a complicated process. The best-known hypothesis to explain AD is that which involves the role of the accumulation of amyloid-β (Aβ) peptide in the brain. As one of major pathological hallmarks, Aβ was considered as primary cause [1]. Aβ is generated from Aβ precursor protein (APP) via sequential cleavages by β- and γ-secretases [2]. Normally Aβ is physiological product. β-Secretase binds to N-terminal of Aβ at extracellular domain of APP and γ-secretases binds to C terminal of transmembrane domain. The γ-secretase is pivotal, because it determines the ratio of two main Aβ species (Aβ_{40} and Aβ_{42}) [3], and mutations in its catalytic subunit presenilin-1 (PS1) account for most cases of familial Alzheimer's disease (FAD) [4]. Under pathological condition, Aβ_{40} or Aβ_{42} appears to be the major species in the initial parenchymal deposition [5].

Although Alzheimer's disease, as a kind of neurodegenerative disease, was not mentioned in Chinese medicine, the symptoms like learning and memory impairment, dementia, and so forth often appeared in the Traditional Chinese Medicine (TCM) theory. In Chinese medicine, kidney plays an essential role in the pathology of senile dementia. Alzheimer's disease was considered as the consequence produced by deficiency of Kidney essence [6–13]. Therefore, in the clinical treatment and research, tonic kidney herbs were applied as the first choice. However, the published results show that the most studies focus on the alleviation of symptom. Because the animal models lack "common behavior," the nonconsistent or even controversy reports are often published on the different journals. Furthermore, previous investigations which centered on the relationship between dementia and ZHENG (TCM syndrome) were only

limited to the symptom improvement, and the mechanism underlying the ZHENG still remains elusive. Kidney-yang deficiency syndrome (KDS) is one of the primary concepts in TCM. Here we used the older rats which showed the features of Kidney-yang deficiency including profuse urination at night, blur hair, long voiding of clear urine, and low basal metabolic rate as the Alzheimer's disease model and tried to identify the relationship between Kidney-yang deficiency and senile dementia and the underlying mechanism.

2. Materials and Methods

2.1. Animals. Male Sprague Dawley (350–400 g) rats were purchased from the Shandong Laboratory animals Center. In this study, all manipulations and procedures were carried out in accordance with The Guide for Care and Use of Laboratory Animals issued by USA National Institutes of Health and were approved by the Animal Care and Use Committee of Shandong University of Traditional Chinese Medicine. As described previously [14, 15], rats were housed (23 ± 1°C) in groups and maintained under a 12-hour light/dark cycle with food and water available *ad libitum*. The rats with same age whose basal metabolic rate (BMR) was 15% lower than normal value, locomotor activity decreased, fur was blur, and urine was 40% more than normal volume were selected as Kidney-yang deficiency model otherwise, the rats were used as control.

As described previously [16], BMRs were measured with Kalabukhov-skvortsov respirometer. Briefly, the temperature was controlled by water bath (±1°C). KOH was used to absorb CO_2 which the rat produced, and dry silica gel was used to absorb water. After fasting for 4 h, the rest of BMR was measured. Before and after measurement, individual weight (±0.1 g) and anal temperature (±1°C) were measured. The procedure lasted 40 min, and the value was recorded with a 5 min interval. The chamber temperature was 30°C. The average BMR was shown in Supplementary Figure 1 (see Supplementary Material available online at doi: 10.1155/2012/132829).

The locomotor activity was detected in open-field chamber (91.4 × 91.4 cm). Rats were allowed to freely explore the testing chamber for 5 minutes while their distance and jumping activity were recorded through a video which mounted at the above of the chamber. In addition, we also analyzed the time in different zones. The locomotor activity results were shown in Supplementary Figure 2.

2.2. Morris Water Maze Test. Morris water maze test was performed as described [17–20]. A circular, black painted pool (150 cm diameter, 50 cm height) filled to a depth of 35 cm with water was used. The water was maintained at 20 ± 1°C and made opaque by the addition of 30 mL of black ink. The pool was divided into four quadrants with four starting locations called north (N), east (E), south (S), and west (W) at equal distance on the rim. An invisible black platform (10 cm diameter) was submerged 1.5 cm below the water line and placed in the center of the northeast quadrant. Rats were trained and tested for 5 days. The rats were trained in the water maze to find and escape onto the hidden platform with a 120 sec cutoff time. Each rat was gently placed into the water, with the nose pointing toward the wall at one of the starting points. The escape latency, the time required for the rats to climb onto the platform, was recorded as the average of four trials. The searching patterns of animals were also recorded when the platform was removed from the pool on day 6.

2.3. Electrophysiology. In vivo recording of field excitatory postsynaptic potential (fEPSP) was made from the CA1 stratum radiatum of the right hippocampal hemisphere in response to stimulation of the Schaffer collateral-commissural pathway. The electrode was implanted in male Sprague Dawley rats as described previously [19–23]. Briefly, the surgery was carried out under deep urethane (1.5 mg/kg, intraperitoneally) anesthesia. Two small burr holes (1.5 mm diameter) were drilled in the skull for placing the recording electrode and bipolar stimulating electrode. The recording electrode was inserted 3.4 mm posterior to bregma and 2.5 mm right of the midline. The bipolar stimulating electrode was inserted 4.2 mm posterior to bregma and 3.8 mm right of the midline. The electrodes were lowered slowly through the cortex to a depth of 2.5 mm, the final depths were adjusted until the appearance of a negative deflecting excitatory postsynaptic potential (EPSP), then fixed to the bone with acrylic dental cement. The right placement of electrodes in the stratum radiatum of the CA1 region of the dorsal hippocampus was verified by postmortem examination. The recording and stimulating electrodes (0.1 mm diameter) were made by stainless steel needles (0.1 mm) coated with Teflon.

Recording was performed 2 weeks later in freely moving rats after their recovery from surgery. In all experiments, test fEPSP was evoked by stimulating with a square-wave constant current pulse of 50 μs duration at a frequency of 0.033 Hz. At the beginning of each experiment, input-output curves (stimulus intensity versus fEPSP slope) were generated to determine the maximal fEPSP slope, and then the intensity of stimulus was set at a level that evoked an fEPSP slope of 50–60% of the maximum. The slope of fEPSP was measured. LTP was induced by high-frequency stimulation (HFS) using 20 pulses at 200 Hz, repeated three times at a 30 sec interval. Stimuli were delivered from an isolator connected with Stimulator (Nihon Kohden, Tokyo, Japan). All recording was performed using Pclamp 10.1 (Molecular Devices, Sunnyvale, USA). Two consecutive sweeps were averaged.

In vitro acute hippocampal slices from male Sprague Dawley rats were prepared as previously described [20, 24, 25]. Briefly, the slices (400 μm thickness), which were cut acutely in iced and 95% O_2/5% CO_2 oxygenated cutting medium including (mM) 230 sucrose, 2.5 KCl, 10 $MgSO_4$, 1.25 Na_2HPO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, 10 D-glucose, were incubated more than 1 h in the artificial cerebrospinal fluid (ACSF) saturated with 95% O_2/5% CO_2 at 23 ± 1°C. The ACSF contains (in mM) 124 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1.2 KH_2PO_4, 26 $NaHCO_3$, and 10 Glucose. During recording, the slices were continuously superfused with

oxygen-saturated ACSF at room temperature (23 ± 1°C). fEPSPs were recorded using Pclamp 10.1 by placing a glass pipette (3–5 M) filled with NaCl (4 M) in the stratum radiatum of the CA1 region of the hippocampus 100–150 μm away from the cell body layer. Stimuli (200 μs pulse duration) were delivered at 0.017 Hz through a bipolar platinum electrode placed at the level of the Schaffer collaterals from CA3. The response curves evoked by the test stimulus pulse eliciting 50–60% of a maximum fEPSP slope were recorded for 15 min, and LTP was induced with the same stimulating strength by a train of 100 pulses at 100 Hz. One episode of HFS was used. Slices displaying an unstable baseline recording were discarded. All the recordings in hippocampal slices were done at room temperature.

2.4. ELISA for Aβ. As described previously [26], we examined Aβ_{40} and Aβ_{42} with sandwich ELISA kits (BioSource, Grand Island, USA). Rat hippocampus was homogenized and centrifuged at 100,000 g for 1 h. We detected rat Aβ_{40} and Aβ_{42} in supernatants with BNT77/BA27 and BNT77/BC05 sandwich ELISA kits (Wako, San Diego, USA) according to previous reports [27]. All measurements were performed in duplicate.

2.5. Fluorogenic Substrate Assay. We performed the assay as reported [26]. After centrifugation of tissue homogenate aliquots at 13,000 g for 15 min, pellets were resuspended and incubated at 37°C for 2 h in 50 μL of assay buffer (pH 6.5) containing 12 mM fluorogenic substrates (Calbiochem, Philadelphia, USA). The fluorescence was measured using SpectraMax M5 spectrometer (Molecular Devices) with the excitation wavelength set at 355 nm and the emission wavelength set at 440 nm.

2.6. Surface Protein Cross-Linking Assay. As described previously [28, 29], The cell membrane impermeable cross-linker bis(sulfosuccinimidyl) suberate (BS^3) (Pierce, Rockford, USA) was applied to examine internalization of surface proteins. Immediately after cutting hippocampal slices acutely, BS^3 (1 mg/mL) was applied for 40 min at 4°C to link all proteins on the neuronal surface. A thorough wash with PBS was made to remove free BS^3, and then the tissues were homogenized, lysed, and subjected to SDS-PAGE for Western blot analysis to detect proteins which were not on cell surface. Lysates of cells without BS^3 treatment and cytosolic proteins such as actin were probed as controls.

2.7. Data Analysis. All data is expressed as mean ± SEM. Sigma plot 9.0 (Systat Software Inc., Northampton, USA) and SAS software package (Release 6.12, Sas Institute Inc., Cary, USA) was used to plot and analyze data by unpaired t-test for two groups, two-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Spatial Learning Was Impaired in Kidney-Yang Deficiency Rats. In the present study, we first investigated the learning and memory of rats in Morris water maze. One week after finishing BMR measurement, the rats were trained in Morris water maze for 5 days. As shown in Figures 1(a) and 1(b), the escape latency for searching hidden platform of model rats was longer than that of control at different time points. Figure 1(c) showed that the average escape latency of model rats increased significantly compared to control (model: 66.06 ± 5.04 s, $n = 13$; control: 51.17 ± 4.50 s, $n = 16$; $P < 0.05$).

In the test in which the platform was removed, the model rats stayed less time in this quadrant than control group did. As shown in Figures 2(a) and 2(b), the performance of the model rats was poorer than that of control (control: 9.69 ± 0.62 s, $n = 13$; model: 6.36 ± 0.64 s, $n = 16$; $P < 0.01$). At the fifth day, average entering times reduced significantly (control: 7.58 ± 2.61, $n = 13$; model: 4.34 ± 2.18, $n = 16$; $P < 0.05$. Figure 2(c)), suggesting that Kidney-yang deficiency can damage the spatial learning and memory capacity in rats.

3.2. Swimming Capacity Was Damaged in Kidney-Yang Deficiency Rats. In Morris water maze, we also detected the swimming capacity. As shown in Figures 3(a) and 3(b), with the hidden platform in the third quadrant, the average swimming distance of model rats is shorter than that of the control (control: 1.09 ± 0.15 m, model: 0.78 ± 0.13 m; $P < 0.0$, $n = 16$). Figure 3(c) shows the similar results after removing the platform (control: 1.25 ± 0.23 m, model: 0.84 ± 0.21 m; $P < 0.01$, $n = 16$). These results suggested that the motor capacity was damaged by Kidney-yang deficiency.

3.3. Aβ$_{40}$ Increased in Kidney-Yang Deficiency Rats. Previous investigations demonstrated that Amyloid plaque which is largely composed of Aβ in brain is one of the typical pathological characteristics of Alzheimer's disease [30, 31]. Aβ with 40 or 42 amino acid sequences can accumulate easily. Here we thus detected the expression of Aβ_{40} in hippocampus of Kidney-yang deficiency rats. As shown in Figure 4, the expression of Aβ_{40} in model rats (76.43 ± 4.03 pg/mg) is increased significantly compared to that of the control (64.13 ± 6.76 pg/mg, $P < 0.05$).

3.4. Aβ$_{42}$ Was Increased in Kidney-Yang Deficiency Rats. Here we also investigated the expression of Aβ_{42} in hippocampus. As shown in Figure 5, Aβ_{42} increased significantly in rat hippocampus, compared to the control (model: 80.45 ± 5.28 pg/mg; 67.43 ± 5.12 pg/mg, $P < 0.05$). The above results suggested that hippocampal Aβ increase could contribute to the learning and memory impairment of Kidney-yang deficiency rats.

3.5. Activity of Hippocampal γ-Secretase Was Not Changed in Kidney-Yang Deficiency Rats. Subsequently a question was raised: what causes hippocampal Aβ increase in Kidney-yang deficiency? Previous reports showed that Aβ is generated from Aβ precursor protein (APP) via sequential cleavages by β- and γ-secretases [2]. However, the γ-secretase is pivotal, because it determines the ratio of two main Aβ species (Aβ_{40} and Aβ_{42}) [3]. Here we used Fluorogenic substrate

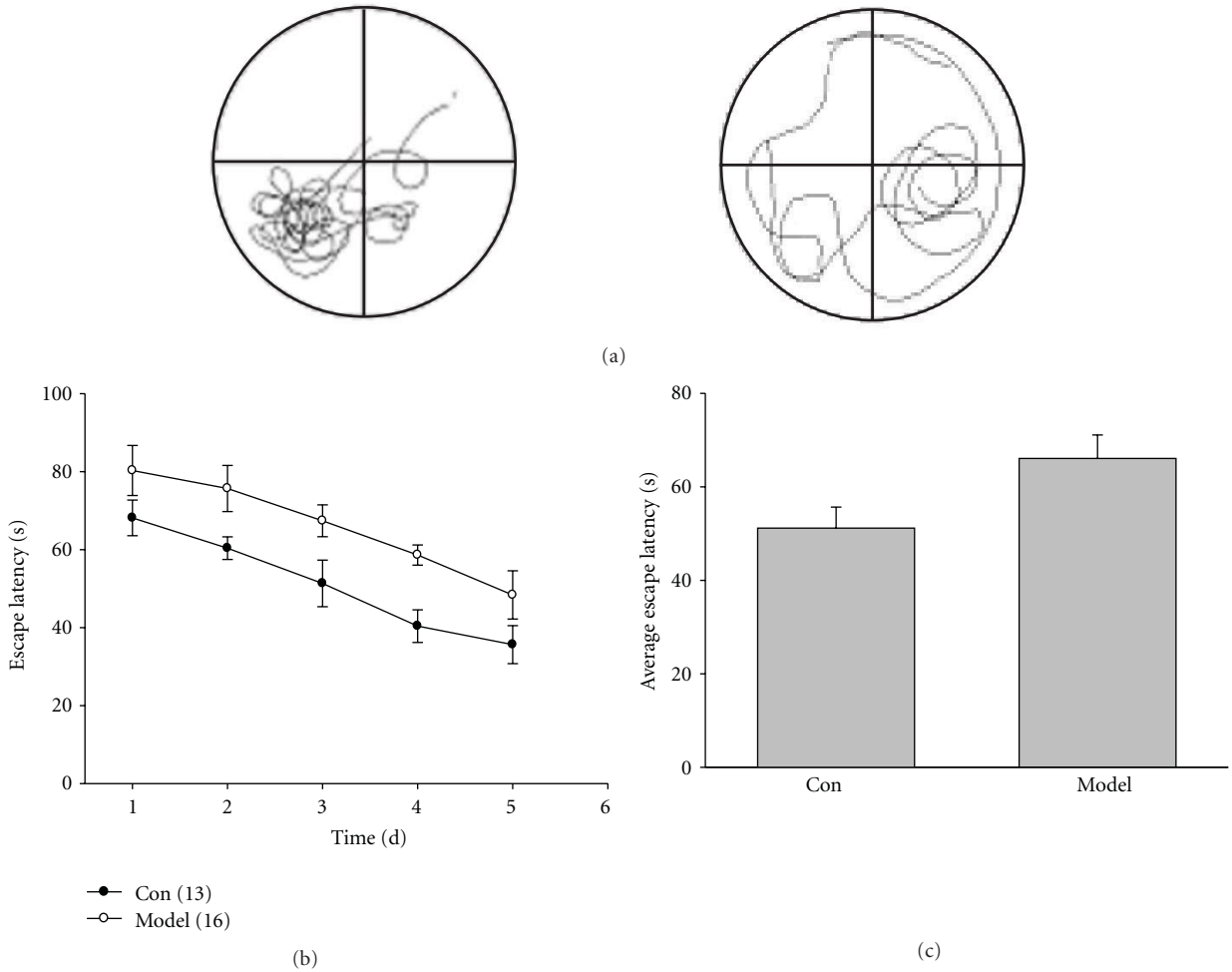


Figure 1: Rats were trained and tested in the Morris water maze with platform hidden at the third quadrant. (a) Representative swimming traces in the Morris water maze. (b) The latency to find the hidden platform at different time points. (c) The average latency of 5 days. Kidney-yang deficiency rats decreased the escape latency significantly, compared to the control ($^*P < 0.05$, unpaired Student's t-test); $n = 13$ for control, 16 for model.

assay to examine the activity of hippocampal γ-secretase. Figure 6 showed that the activity of hippocampal γ-secretase did not change significantly, compared to the control (model: 1.07 ± 0.20; control: 1.05 ± 0.12; $P > 0.05$), suggesting that the increase of hippocampal Aβ expression is not caused by upregulation of γ-secretase in Kidney-yang deficiency rats.

3.6. Learning and Memory Impairment of Kidney-Yang Deficiency is Not Caused by Internalization of Hippocampal NMDA Receptors. It is well documented that Aβ increase could cause NMDA receptor (NMDAR) internalization which is involved in Alzheimer's disease [32]. To determine the role of NMDAR internalization, we used surface protein biotinylation assay to analyze the expression of NMDAR of hippocampal neuron membrane. As shown in Figures 7(a) and 7(b), neuron surface NMDAR expression did not show significant difference between the model and the control (control: 0.18 ± 0.03 model: 0.19 ± 0.02; $P > 0.05$), suggesting that NMDAR internalization did not contribute to the learning impairment of Kidney-yang deficiency.

3.7. Damage of Synaptic Plasticity Is Involved in the Learning and Memory Impairment of Kidney-Yang Deficiency Rats. Previous studies addressed that long-term potentiation (LTP) is associated with learning and memory [33, 34]. Aβ increase can damage hippocampal synaptic plasticity. Here we used extracellular recording *in vivo* to investigate change of the LTP. As shown in Figures 8(a) and 8(b), after high-frequency stimulation (HFS), the LTP was reduced significantly in model than that of the control ($P < 0.05$), suggesting that damage of hippocampal synaptic plasticity could be one of mechanisms responsible for learning and memory impairment.

3.8. β-Adrenergic Receptor Agonist Can Alleviate the Impairment of LTP in Kidney-Yang Rats. β-Adrenergic receptor (β_2-AR) is expressed in the hippocampus and cortex. Activating β_2-AR can enhance the activity of γ-secretase and thus cause an increase in Aβ production. However, as shown in Figure 6, the activity of γ-secretase did not change significantly, suggesting that Aβ expression increase is not caused

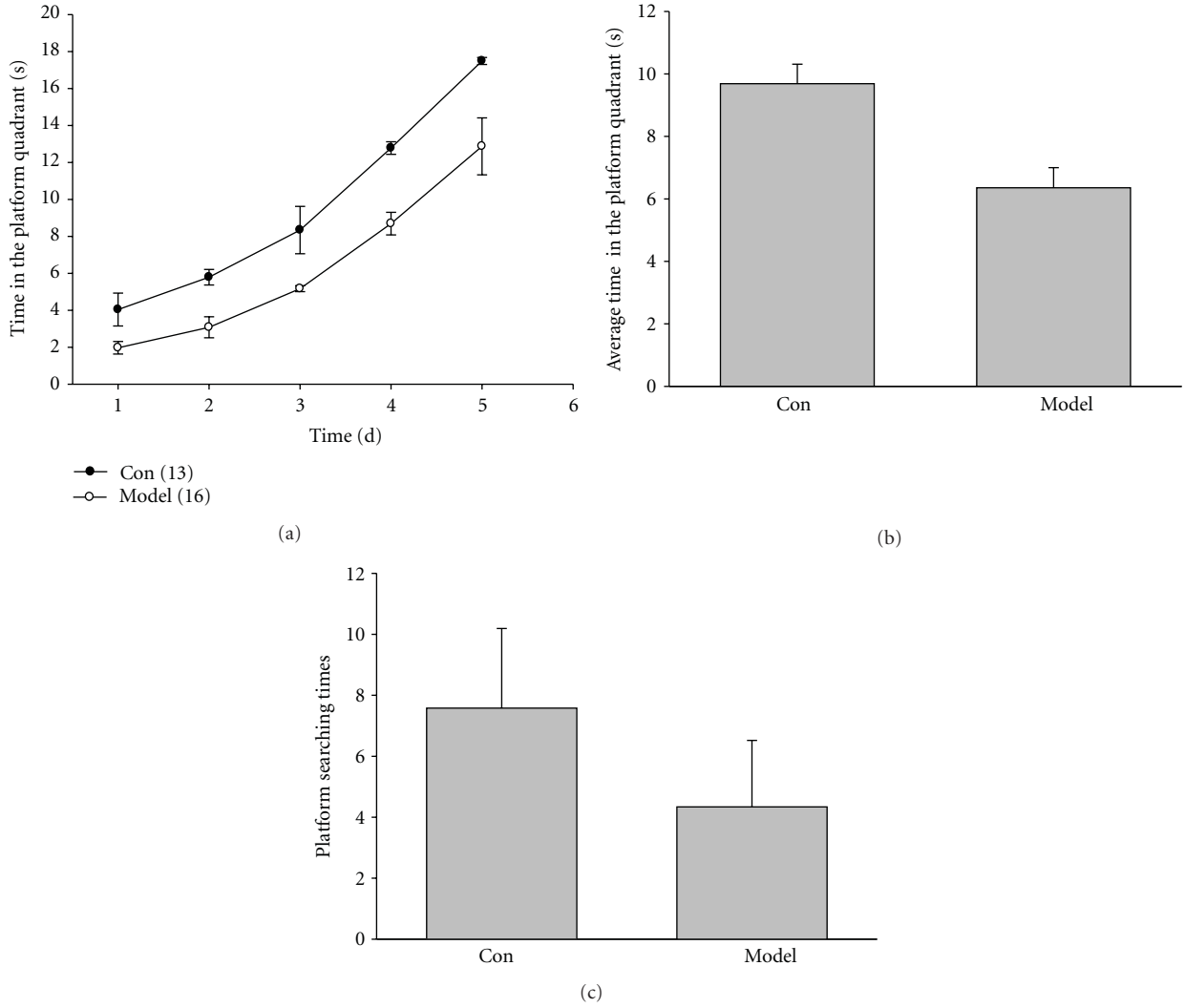


Figure 2: Rats were trained and tested in the Morris water maze without platform. (a) The time spent in the platform quadrant at different time points. (b) The average time spent in the platform quadrant for 5 days. Kidney-yang deficiency rats decreased the time spent in the platform quadrant significantly, compared to the control ($^*P < 0.01$, unpaired Student's t-test). (c) At the fifth day, average entering times to the platform quadrant. The times when Kidney-yang deficiency rats entered platform quadrant after removing the platform decreased significantly ($P < 0.05$, unpaired Student's t-test). $n = 13$ for control, 16 for model.

by upregulating activity of γ-secretase. Here we examine whether β_2-AR activity can affect the hippocampal LTP of Kidney-yang deficiency through recording LTP in acute brain slices. Figures 9(a) and 9(b) showed that the LTP was inhibited in the brain slices from the Kidney-yang deficiency rats ($P < 0.01$), consistent with the *in vivo* recording; specific β_2-AR agonist terbutaline can significantly improve the LTP of the acute hippocampal slice from the model rats ($P < 0.05$), suggesting that glia β_2-AR dysfunction may contribute to the inhibition of LTP in the Kidney-yang deficiency rats.

4. Discussion

In the present study, we firstly selected the Kidney-yang deficiency rats through evaluating the locomotors activity, BMR, total urine volume of 24 h combining with the fur, and demonstrated that spatial learning and escape capacity were significantly impaired in Kidney-yang deficiency rats. We also found that Aβ with 40 and 42 amino acid sequences increased expression in hippocampus of Kidney-yang deficiency rats, consistent with the previous reports in which Aβ enhanced in brain is one of the typical pathological characteristics of Alzheimer's disease [1]. Although the γ-secretase is pivotal in determining the ratio of two main Aβ species (Aβ_{40} and Aβ_{42}), but we demonstrated in Kidney-yang deficiency rats, γ-secretase activity did not change significantly. As previous reports [32], NMDA receptor (NMDAR) internalization induced by hippocampal Aβ increase is involved in Alzheimer's disease; however, the result that neuron surface NMDAR expression did not show significant reduction in Kidney-yang deficiency rules out

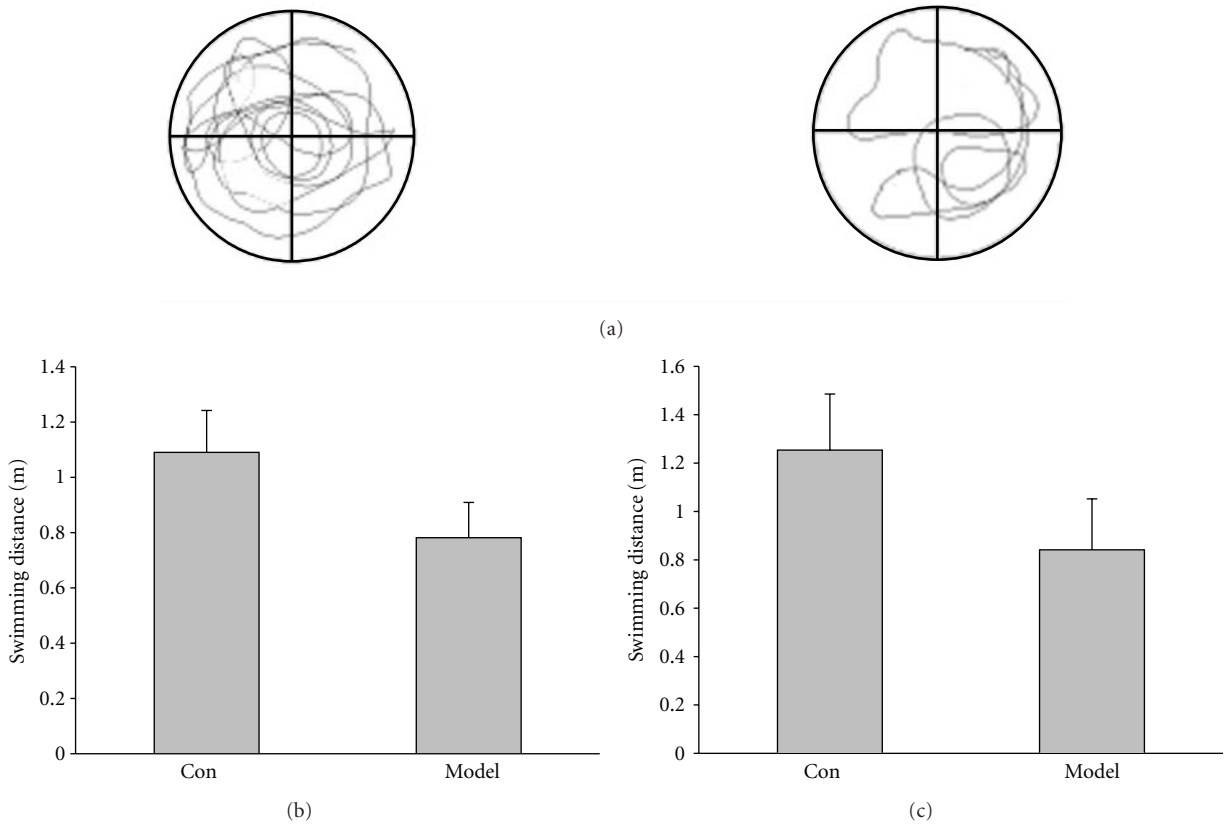


Figure 3: Swimming capacity was detected in the Morris water maze. (a) Representative swimming traces in the Morris water maze after removing the platform. (b) With the hidden platform in the third quadrant, and the average swimming distance of model rats is shorter than that of the control (**$P < 0.01$, unpaired Student's t-test). (c) No platform in the third quadrant, and the average swimming distance of model rats is also shorter than that of the control (**$P < 0.01$, unpaired Student's t test). $n = 13$ for control, 16 for model.

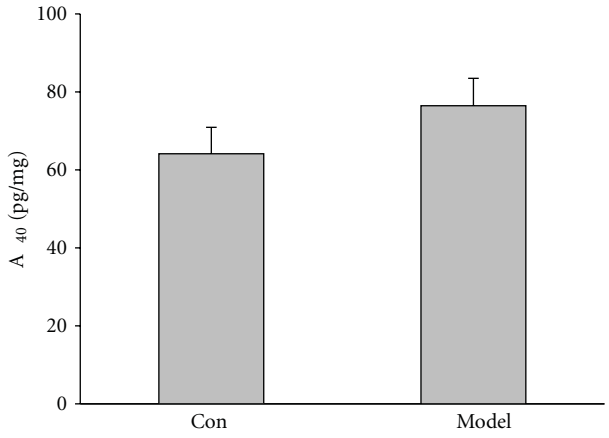


Figure 4: ELISA for Aβ_{40} in the hippocampus. ELISA shows the secreted Aβ_{40} increased significantly in the hippocampus from the Kidney-yang deficiency (*$P < 0.05$, unpaired Student's t-test).

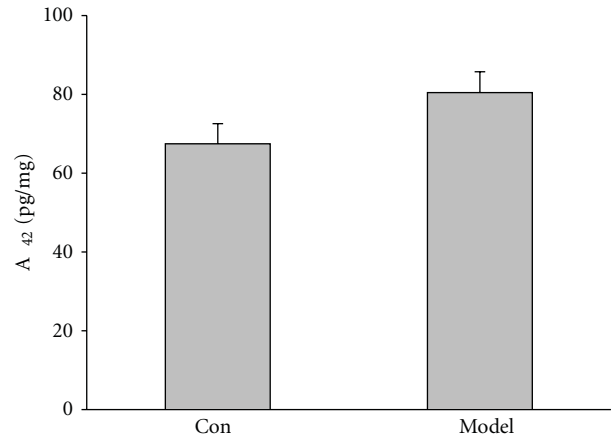


Figure 5: ELISA for Aβ_{42} in the hippocampus. ELISA shows the secreted Aβ_{42} increased significantly in the hippocampus. from the Kidney-yang deficiency (*$P < 0.05$, unpaired Student's t test).

the role of NMDAR internalization in the learning and memory impairment of Kidney-yang deficiency. The damage of synaptic plasticity caused by Aβ increase could be rescued by β_2-AR agonist. Therefore, our studies firstly address that the increase of Aβ may contribute to the learning and memory impairment of Kidney-yang deficiency rats, and β_2-AR inhibition plays an important role in the hippocampal Aβ increase in this model.

Previous studies showed that stress-activated β-ARs not only regulate the secondary message level and subsequently

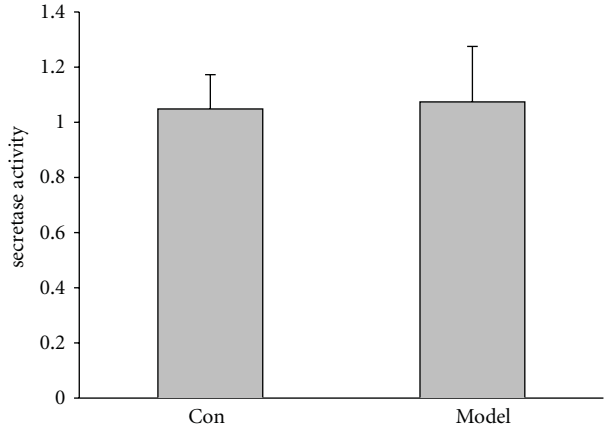


Figure 6: Determination of γ-secretase activity using a Fluorogenic substrate assay. γ-Secretase activity of hippocampus from Kidney-yang deficiency rats did not changed significantly ($P > 0.05$, unpaired Student's t test), $n = 3$ for both groups.

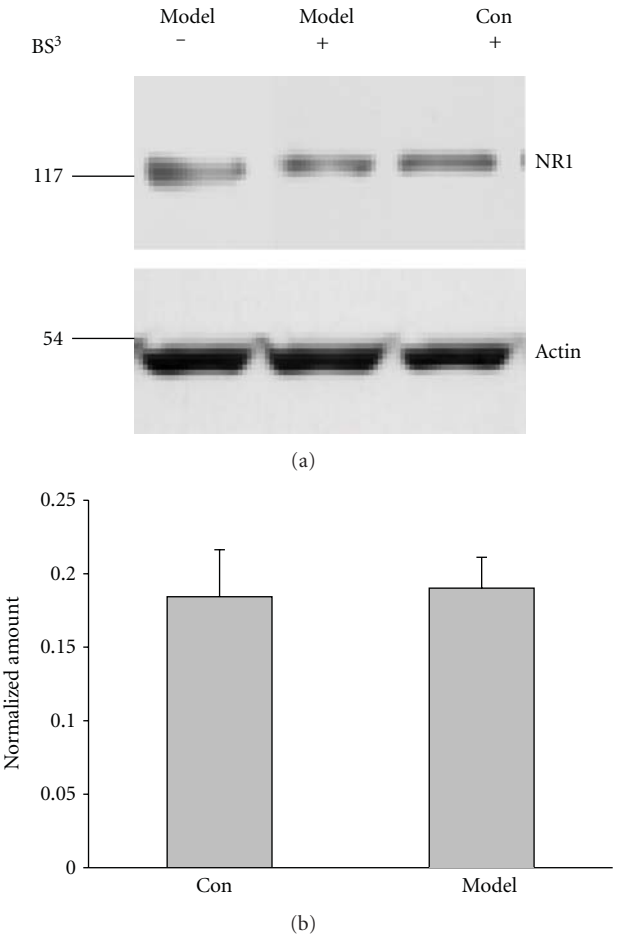


Figure 7: Surface protein biotinylation assay for NMDA receptors (NMDARs) of hippocampal neuron membrane. (a) Representative Western blotting. (b) Summarized data from 4 trials shows that NMDARs expressed on the neuron surface did not change significantly compared to the control ($P > 0.05$, unpaired Student's t-test), $n = 3$ for control, 5 for model.

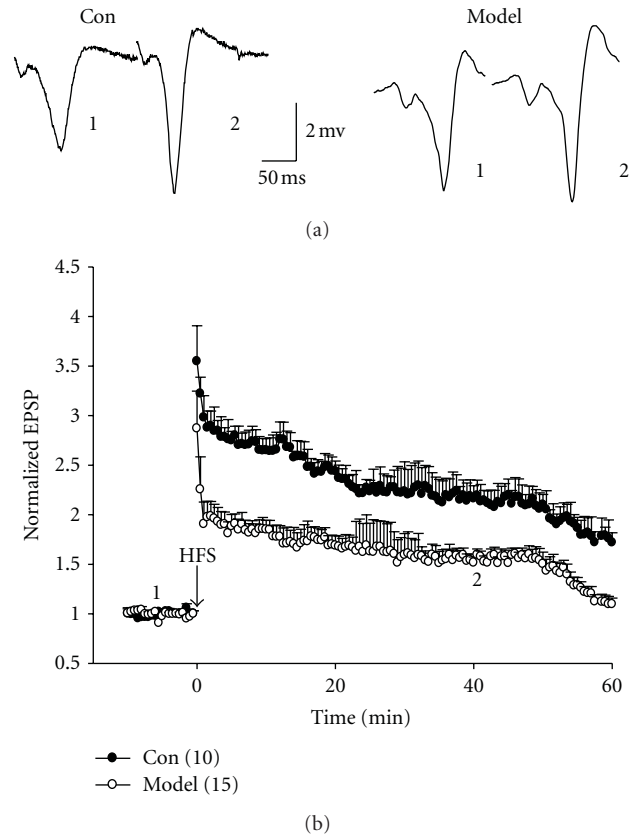


Figure 8: Long-term potential (LTP) recorded *in vivo*. (a) Representative sweeps of field excitatory postsynaptic potential (fEPSP) recorded in the freely moving rats. (b) Summary of averaged normalized fEPSP slope from (a). Compared to the control, the LTP was significantly inhibited in Kidney-yang deficiency rats ($P < 0.05$, two-way ANOVA), $n = 10$ for control, 14 for model. "1" and "2" in (a) and (b) indicate the sweeps recorded separately at 5 min and 40 min before and after high-frequency stimulation.

affect the signal transduction but also play a role in receptor internalization which is associated with the receptor desensitization and signal transduction mediated by clathrin [35, 36]. β_2-ARs express highly in the cortex and hippocampus [37]. The previous reports demonstrated that the activation of β_2-AR promotes the activity of γ-secretase and thus increase Aβ. *In vivo* experiments in AD transgenic mice also verified that after treating with β_2-AR agonist isoproterenol for a long time, the Aβ plaque enhanced in the mouse brain and on the contrary antagonist ICI118551 reduced the plaque [26]. However, the current study shows that although Aβ expression increases significantly in Kidney-yang deficiency rats, the activity of γ-secretase did not change significantly, suggesting that the Aβ increase may not be due to the enhancement of the activity of γ-secretase by overactivating β_2-AR.

As mentioned above, Aβ can affect the neuron surface receptors, NMDAR is one of the affected receptors [38]. Our studies demonstrated that the neuron surface receptors were not internalized in Kidney-yang deficiency rats. Interestingly, the damaged LTP is involved in the decrease of learning

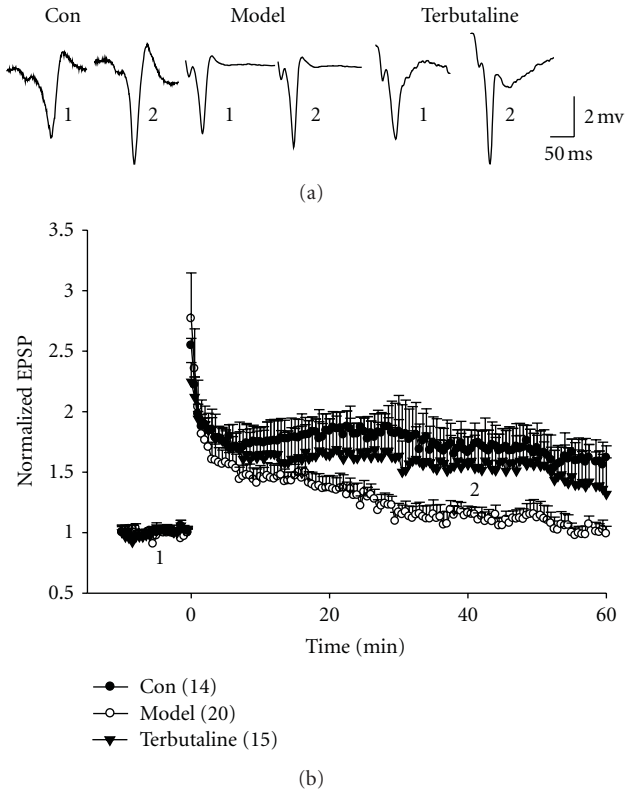


Figure 9: Long-term potential (LTP) recorded in acute hippocampal slices. (a) Representative sweeps of field excitatory postsynaptic potential (fEPSP) recorded in acute hippocampal slices. (b) Summary of averaged normalized fEPSP slope from (a). Compared to the control, the LTP was significantly inhibited in Kidney-yang deficiency rats, but can be rescued by terbutaline, a β_2 receptor agonist ($P < 0.05$, two-way ANOVA), $n = 10$ for control, 14 for model, 11 for terbutaline. "1" and "2" in (a) and (b) indicate the sweeps recorded separately at 5 min and 40 min before and after high-frequency stimulation.

capacity of Kidney-yang deficiency. LTP in acute brain slices showed that β_2-AR agonist can ameliorate the inhibited LTP. However, the question is: What causes Aβ increase in the condition without β_2-AR increase or overactivity? How does Aβ inhibit the LTP? As well known, glia expresses a lot of β_1 and β_2 receptors, and β_2 receptors is primary. Previous investigation demonstrated that LTP could be damaged by increasing TNF release induced by Aβ enhancement [39]. The brain-derived TNF is produced by glia and could be inhibited by β_2 receptor agonist.

5. Conclusions

Taken together, our studies indicated that the spatial learning inhibited in kidney-yang deficiency might be due to hippocampal synaptic plasticity damaged by Aβ_{40} and 42 increase which are associated with the decrease of β_2 receptor function in glia.

Authors' Contribution

D. Qi and Y. Qiao are equally contributed to this work.

Acknowledgments

This work was supported by the Key Program of Shandong Provincial Education Department granted to D. Qi (no. 2007GG2NS02072) and the National Natural Science Foundation of China granted to H. Qiao (no. 30772706).

References

[1] R. E. Tanzi, "The synaptic Aβ hypothesis of Alzheimer disease," *Nature Neuroscience*, vol. 8, no. 8, pp. 977–979, 2005.

[2] N. Takasugi, T. Tomita, I. Hayashi et al., "The role of presenilin cofactors in the γ-secratase complex," *Nature*, vol. 422, no. 6930, pp. 438–441, 2003.

[3] S. S. Sisodia and P. H. St George-Hyslop, "γ-secretase, Notch, Aβ and Alzheimer's disease: where do the presenilins fit in?" *Nature Reviews Neuroscience*, vol. 3, no. 4, pp. 281–290, 2002.

[4] R. E. Tanzi and L. Bertram, "Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective," *Cell*, vol. 120, no. 4, pp. 545–555, 2005.

[5] T. Iwatsubo, A. Odaka, N. Suzuki, H. Mizusawa, N. Nukina, and Y. Ihara, "Visualization of Aβ_{42}(43) and Aβ_{40} in senile plaques with end-specific Aβ monoclonals: evidence that an initially deposited species is Aβ_{42}(43)," *Neuron*, vol. 13, no. 1, pp. 45–53, 1994.

[6] J. He and B. Pu, "Getting Yang from Yang is a basic treatment strategy for Alzheimer's disease," *Liaoning Journal of Traditional Chinese Medicine*, vol. 27, pp. 485–486, 2000 (Chinese).

[7] J. Chen, L. Zhou, and X. Zhang, "Effect of reinforcing bone on receptors and transmitters in Alzheimer's disease rat models," *Modern Journal of Integrated Traditional Chinese and Western Medicine*, vol. 10, pp. 1522–1524, 2001 (Chinese).

[8] J. Hu, Q. Wang, W. Liang et al., "Effect of Tonifying kidney and reinforcing intelligence decoction on somatostatin and neurons with somatostatin mRNA expression of brain in Alzheimer's disease model," *Chinese Journal of Integrated Traditional and Western Medicine*, vol. 20, pp. 533–535, 2000 (Chinese).

[9] P. Wang, J. Mei, L. Zhang et al., "Effect of Restoring consciousness and reinforcing intelligence decoction on hippocampal ultrastructure in SAM-P/10 mice," *Journal Hubei University of Chinese Medicine*, vol. 3, pp. 18–19, 2001 (Chinese).

[10] H. Wang, S. Lai, J. Sun et al., "Effect of tonifying kidney and reinforcing intelligence decoction on behavior of Alzheimer's disease rat models," *Chinese Journal of Integrated Traditional and Western Medicine*, vol. 20, pp. 771–773, 2000 (Chinese).

[11] Q. Yue, S. Zhao, J. Gao et al., "Effect of Daicong decoction on brain M receptors in senile dementia rat models," *Chinese Journal of Integrated Traditional and Western Medicine*, vol. 20, pp. 846–848, 2000 (Chinese).

[12] L. Zhou, X. Zhang, J. Chen et al., "Effect of tonifying kidney and reinforcing intelligence decoction on learning and memory in Alzheimer's rat model," *Traditional Chinese Medicine Research*, vol. 14, pp. 23–25, 2001 (Chinese).

[13] M. Liu and H. Liu, " Current status of reinforcing intelligence herbs," *Chinese Journal of Integrated Traditional and Western Medicine*, vol. 15, pp. 59–61, 1995 (Chinese).

[14] Y. Q. Li, B. Zhu, P. J. Rong, H. Ben, and Y. H. Li, "Neural mechanism of acupuncture-modulated gastric motility,"

World Journal of Gastroenterology, vol. 13, no. 5, pp. 709–716, 2007.

[15] X. Y. Gao, S. P. Zhang, B. Zhu, and H. Q. Zhang, "Investigation of specificity of auricular acupuncture points in regulation of autonomic function in anesthetized rats," *Autonomic Neuroscience*, vol. 138, no. 1-2, pp. 50–56, 2008.

[16] A. Gorecki, "Klabukhov-skvorstsov respiremeter and resting metabolic rate measurement," in *Methods for Ecological Bio-Energetic*, W. Grondzinsky, Ed., IBP Handbook 24, pp. 309–313, Bllokwell Scientific, Oxford, UK, 1975.

[17] R. G. M. Morris, E. Anderson, G. S. Lynch, and M. Baudry, "Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5," *Nature*, vol. 319, no. 6056, pp. 774–776, 1986.

[18] H. Zhou, X. Ge, L. Z. Wang, L. Ma, and G. Pei, "Attenuation of morphine tolerance and dependence in scopolamine-treated rats," *NeuroReport*, vol. 10, no. 10, pp. 2007–2010, 1999.

[19] L. Pu, G. B. Bao, N. J. Xu, L. Ma, and G. Pei, "Hippocampal long-term potentiation is reduced by chronic opiate treatment and can be restored by re-exposure to opiates," *Journal of Neuroscience*, vol. 22, no. 5, pp. 1914–1921, 2002.

[20] D. Qi, Y. Zhu, L. Wen, Q. Liu, and H. Qiao, "Ginsenoside Rg1 restores the impairment of learning induced by chronic morphine administration in rats," *Journal of Psychopharmacology*, vol. 23, no. 1, pp. 74–83, 2009.

[21] C. Doyle, C. Hölscher, M. J. Rowan, and R. Anwyl, "The selective neuronal NO synthase inhibitor 7-nitro-indazole blocks both long-term potentiation and depotentiation of field EPSPs in rat hippocampal CA1 in vivo," *Journal of Neuroscience*, vol. 16, no. 1, pp. 418–424, 1996.

[22] L. Xu, R. Anwyl, and M. J. Rowan, "Behavioural stress facilitates the induction of long-term depression in the hippocampus," *Nature*, vol. 387, no. 6632, pp. 497–500, 1997.

[23] X. Y. Wang and J. T. Zhang, "Effects of ginsenoside Rg1 on synaptic plasticity of freely moving rats and its mechanism of action," *Acta Pharmacologica Sinica*, vol. 22, no. 7, pp. 657–662, 2001.

[24] S. Yang, H. Qiao, L. Wen, W. Zhou, and Y. Zhang, "D-Serine enhances impaired long-term potentiation in CA1 subfield of hippocampal slices from aged senescence-accelerated mouse prone/8," *Neuroscience Letters*, vol. 379, no. 1, pp. 7–12, 2005.

[25] H. F. Qiao, S. Yang, W. X. Zhou, and Y. X. Zhang, "NT-1, an active constituent extracted from Tiaoxin Recipe, enhances long-term potentiation of CA1 subfield in rat hippocampal slices," *Life Sciences*, vol. 79, no. 1, pp. 8–15, 2006.

[26] Y. Ni, X. Zhao, G. Bao et al., "Activation of β2-adrenergic receptor stimulates γ-secretase activity and accelerates amyloid plaque formation," *Nature Medicine*, vol. 12, no. 12, pp. 1390–1396, 2006.

[27] M. Ramsden, A. C. Nyborg, M. P. Murphy et al., "Androgens modulate β-amyloid levels in male rat brain," *Journal of Neurochemistry*, vol. 87, no. 4, pp. 1052–1055, 2003.

[28] R. A. Hall, A. Hansen, P. H. Andersen, and T. R. Soderling, "Surface expression of the AMPA receptor subunits GluR1, GluR2, and GluR4 in stably transfected baby hamster kidney cells," *Journal of Neurochemistry*, vol. 68, no. 2, pp. 625–630, 1997.

[29] G. Broutman and M. Baudry, "Involvement of the secretory pathway for AMPA receptors in NMDA-induced potentiation in hippocampus," *Journal of Neuroscience*, vol. 21, no. 1, pp. 27–34, 2001.

[30] W. Ji and I. Ha, "Drug development for Alzheimer's disease: recent progress," *Experimental Neurobiology*, vol. 19, pp. 120–131, 2010.

[31] A. M. Hall and E. D. Roberson, "Mouse models of Alzheimer's disease," *Brain Research Bulletin*. In press.

[32] P. Kurup, Y. Zhang, J. Xu et al., "Aβ-mediated NMDA receptor endocytosis in alzheimer's disease involves ubiquitination of the tyrosine phosphatase STEP61," *Journal of Neuroscience*, vol. 30, no. 17, pp. 5948–5957, 2010.

[33] T. V. P. Bliss and G. L. Collingridge, "A synaptic model of memory: long-term potentiation in the hippocampus," *Nature*, vol. 361, no. 6407, pp. 31–39, 1993.

[34] T. V. Bliss, G. L. Collingridge, and R. G. Morris, "Introduction. Long-term potentiation and structure of the issue," *Philosophical Transactions of the Royal Society B*, vol. 358, pp. 607–611, 2003.

[35] M. González-Gaitán and H. Stenmark, "Endocytosis and signaling: a relationship under development," *Cell*, vol. 115, no. 5, pp. 513–521, 2003.

[36] S. K. Shenoy, M. T. Drake, C. D. Nelson et al., "β-arrestin-dependent, G protein-independent ERK1/2 activation by the β_2 adrenergic receptor," *Journal of Biological Chemistry*, vol. 281, no. 2, pp. 1261–1273, 2006.

[37] A. Russo-Neustadt and C. W. Cotman, "Adrenergic receptors in Alzheimer's disease brain: selective increases in the cerebella of aggressive patients," *Journal of Neuroscience*, vol. 17, no. 14, pp. 5573–5580, 1997.

[38] E. M. Snyder, Y. Nong, C. G. Almeida et al., "Regulation of NMDA receptor trafficking by amyloid-β," *Nature Neuroscience*, vol. 8, no. 8, pp. 1051–1058, 2005.

[39] Q. W. Wang, M. J. Rowan, and R. Anwyl, "Inhibition of LTP by beta-amyloid is prevented by activation of β_2 adrenoceptors and stimulation of the cAMP/PKA signalling pathway," *Neurobiology of Aging*, vol. 30, no. 10, pp. 1608–1613, 2009.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 268542, 11 pages
doi:10.1155/2012/268542

Review Article

Current Situation and Perspectives of Clinical Study in Integrative Medicine in China

Jie Wang and Xingjiang Xiong

Department of Cardiolorgy, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, 5 Bei Xian Ge Street, Xi Cheng District, Beijing 100053, China

Correspondence should be addressed to Xingjiang Xiong, 5administration@163.com

Received 14 November 2011; Revised 19 January 2012; Accepted 24 January 2012

Academic Editor: Aiping Lu

Integrative medicine is not only an innovative China model in clinical practice, but also the bridge for TCM toward the world. In the past thirty years, great achievements have been made in integrative medicine researches, especially in clinical practice. The clinical achievements mainly include the following three: innovating methodology of disease-syndrome combination, excavating the classical theory in traditional Chinese medicine (TCM), preventing and curing refractory diseases. The development ideas and strategies of integrative medicine for future mainly include (a) standing on frontier field of international medicine and improving the capability of preventing and curing refractory diseases; (b) moving prevention and control strategy forward and improving the curative effect of common and frequent disease; (c) excavating the classical theory of TCM and broadening the treatment system of modern medicine; (d) improving the innovation level of new high effective drugs on the basis of classical prescriptions and herbs in TCM; (e) rerecognizing the theory of formula corresponding to syndrome in TCM and enhancing the level of clinical research evidence based on evidence-based medicine. Integrative medicine will do obtain greater achievements in creating new medicine and pharmacology and make more tremendous contributions for the great rejuvenation of the Chinese nation and human health care.

1. Introduction

The coexistence of western medicine (WM) and traditional Chinese medicine (TCM) began to appear when WM was introduced to China from the middle of 16th century. The tendency of "confluence of Chinese and western medicine" appeared as the two medical systems contacting and influencing with each other since then. With the development of modern medical technology, intercourse, and cooperation between TCM and WM, integrative medicine was established in the 1980s. Under the guidance of "system learning, comprehensively mastering, sorting, and improving," predecessors of integrative medicine have been exploiting the complementary advantages of macro and micro, global and local, structure and function, traditional and modern, disease differentiation, and syndrome differentiation in WM and TCM, in order to create new medicine and pharmacology theory. Through unremitting efforts of integrative medicine staffs at home and abroad, remarkable achievements have been made in health care, teaching, researching, academic development, discipline construction, talent training, and so forth. So we can say that integrative medicine is not only an innovative China model in clinical practice, but also the bridge for TCM toward the world [1–3]. Now the clinical achievements of the past 30 years and developing strategies of integrative medicine are described as follows.

2. Clinical Achievements

2.1. Innovating Methodology of Disease-Syndrome Combination: A New Mode for Syndrome Research. The relationship between disease and syndrome is thought to be one of the most significant problems in TCM clinical and basic practice. As a new mode for syndrome research, disease-syndrome combination mainly refers to absorbing the idea and theory of disease differentiation in western medicine (WM) as

well as syndrome differentiation in TCM. The mode, which originates from the medical practice since more than half a century ago, has realized mutual compensation of advantages of TCM and WM [1, 2]. Combining whole thinking, imagery thinking, and dialectical thinking in TCM with materialism of modern medical sciences, the mode can be regarded as a good cut-in point and successful control pattern for integrative medicine [3]. It has complementary advantages of WM and TCM and marks a new era created by integrative medicine in clinical researches. Seminar on the academician Chen Keji's academic thinking about "the new mode of disease-syndrome combination and its application in clinical practice" was held in Beijing on May 23, 2011. Professor Chen and his students discussed the scientific connotation of this new mode and its application in diagnosis, treatment, and scientific research together. China news of traditional Chinese medicine, a famous domestic media, made follow-up report on the symposium, which had evoked large repercussions.

Academician Chen Keji pointed out that modern view of disease-syndrome combination includes six aspects: (a) mode of disease differentiation by WM combined with syndrome differentiation by TCM; (b) mode of syndrome differentiation and treatment combined with specific prescription for certain illness; (c) mode of treating according to disease staging; (d) mode of differentiation of the basic pathogenesis combined with syndrome differentiation and treatment in TCM; (e) mode of treating according to syndrome differentiation rather than disease differentiation when there's no disease can be diagnosed in WM; (f) mode of treating according to disease differentiation rather than syndrome differentiation when there's no syndrome can be diagnosed in TCM [4]. The emphasis of the mode could be played on either syndrome or disease. As the connotation of syndrome in TCM is significantly different from disease in WM, laying special emphasis on syndrome means that syndrome is just the basis of therapeutic scheme. On the contrary, effective therapeutic plans should be formulated according to disease differentiation when special emphasis is laid on disease. This new mode is beneficial to the original innovation in diagnosis and treatment. The advantages of the mode include four aspects as follows. (a) *Definitely diagnosing*. As the disease diagnosis in TCM is vague and extensive, it is entirely necessary and possible to absorb some relevant achievements of disease diagnosis in WM for definitely diagnosing. (b) *Targeted treating*. As the new mode pays more attention to the therapeutic evaluation of disease, it could achieve more definite therapeutic targets and stable curative effect compared with syndrome differentiation mode alone. (c) *Accurately prognosing*. Summarization of clinical phenomena is the principal judgment basis for prognosis in TCM, therefore, the prognosis judgment is always not very accurate. However, the new mode has vital guidance value for treatment and prognosis judgment. (d) *Deepening classics*. Due to the succinctness and conciseness of TCM classics, the essential features of the disease and syndrome could be rerecognized and deepened through combining with the modern cognition of pathology, diagnostics and pharmacology study in WM.

Syndrome is not only the core of TCM basic theory and syndrome differentiation, but also the bridge to associate disease and formula. Different from diagnosis based on pathological mechanism, syndrome is a classification according to subjective symptom and objective sign collected by physical examination [5–7]. Premise studies on disease-syndrome combination lies in syndrome diagnostic criteria and therapeutic evaluation system. Researches on syndrome diagnostic criteria aim at establishing the scientific and normative diagnosis system, while researches on therapeutic evaluation aim at constructing an objective evaluation system. Under the leadership of academician Chen Keji, we are the first to study and report on blood stasis syndrome in coronary heart disease based on the new mode. Contributed to the diagnosis of blood stasis syndrome in coronary heart disease, 19 items such as precordial pain, dark purple tongue color, and erythrocyte deformability were selected based on the calculation analysis of 48 kinds of examination items in 92 cases patients with coronary heart disease. And the clinical diagnosis accordance rate was 89%. 6 items giving the greatest contribution to diagnosis such as blood viscosity and total cholesterol (TC) were confirmed by a stepwise regression analysis for 21 items such as hemorheology and blood lipid [8]. Correlation analysis of blood stasis syndrome and pathological changes shown in coronary angiography with coronary heart disease showed that the blood stasis syndrome score was significantly correlated to the maximal stenosis degree and coronary lesion score demonstrated by coronary angiography before percutaneous coronary intervention (PCI), and the correlation was increased along with the increasing of the patients' age and the course of the disease. Conclusions were also verified in our related researches [9–11].

Through mathematical statistics method and computational intelligence approach, it was found out that the major syndrome factors of coronary heart disease are blood stasis, qi deficiency, turbid phlegm, qi stagnation, heat deposition, yang deficiency, yin deficiency, and cold coagulation based on calculation analysis of 5099 cases patients reported on literatures and 1069 cases patients with coronary heart disease validated by coronary angiographic. We also constructed the diagnosis scales of blood stasis syndrome and its accompanied syndromes in coronary heart disease, such as qi deficiency and blood stasis syndrome and qi stagnation and blood stasis syndrome [12–14]. In the study of therapeutic evaluation system, taking coronary heart disease as example, important indexes such as syndrome evaluation scale, clinical critical events, and quality of life were selected on the basis of completely evaluating the present indexes through application of clinical epidemiology-/evidence-based medicine method. Meanwhile, high validity and reliability of therapeutic evaluation system of coronary heart disease was constructed through comprehensive analysis of various index by the hall for workshop of metasynthetic engineering. Clinical efficacy scale of TCM syndrome and the primitive entry pool of scale for patient-reported outcomes of coronary heart disease were established by our team [15].

2.2. Excavating the Classical Theory in TCM. It is meaningful to promote the original innovation in integrative medicine researches through further understanding the connotation of syndrome diagnosis, therapeutic principle, and classical prescription by modern science and technology. Among these studies, three researches below are honored.

The first one is blood stasis syndrome theory and the clinical application of the method of promoting blood circulation and removing blood stasis. Blood stasis syndrome theory is first recorded in *The Songs of Chu*, a classical literature written in ancient China. *Shuo Wen Jie Zi* (Text Notes and Word Explanations) written by Xu Shen in the Eastern Han Dynasty explained that blood stasis is hematocele. And it was frequently mentioned in TCM classics such as *the Canon of Internal Medicine*, *Treatise on Febrile Diseases*, and *Synopsis of Golden Chamber*. Inspired by the prominent TCM doctor Guo Shikui's experience in treating angina pectoris by Decoction for Removing Blood Stasis, a classic formula developed by Wang Qingren in Qing Dynasty, academician Chen Keji advocated treating coronary heart disease by activating blood circulation to dissipate blood stasis principally. His team was the first to study the diagnostic criteria of blood stasis syndrome and report the quantitative scoring method, which had been extensively used in domestic and was the first study in using objective quantitative method in TCM syndrome study. Objectified study of abdomen diagnosis on blood stasis syndrome was also superior to the research methods of Japan in the same period. Standards of syndrome differentiation and therapeutic evaluation of coronary heart disease were formulated according to above-mentioned researches, which had already become national standards. The essence of blood stasis syndrome and mechanism of treating coronary heart disease by activating blood circulation to dissipate blood stasis had been elucidated at various levels of intact animal, tissue, cell, molecule, and gene protein expression. As coronary restenosis after coronary artery balloon injury and stent placement have been considered an international difficult problem, academician Chen firstly treated it by decoction for removing blood stasis and optimized the prescription to a more simplified and effective recipe, *Xiong Shao* Capsule (XSC). A randomized controlled trial (RCT) about XSC showed that the restenosis rate in XSC group treated by XSC on the basis of routine western therapy was decreased by 45% compared with routine western therapy group, and the experimental studies showed that XSC could suppress the gene expression of proliferation of vascular smooth muscle cells [16–19]. With important academic value and clinical significance, the study has promoted the academic development of TCM greatly, which had been awarded the first award of national science and technology progress in 2003.

The second study is the theory of dispelling interior pathogenic factors and purgation and its application in the treatment of acute abdomen. According to the theoretical basis of "the six fu-viscera function well when unobstructed," academician Wu Xianzhong began to explore integrative medicine therapy on acute abdomen in early years. Through unifying standard for syndrome differentiation and defining operative indication, his team accumulated a large number of valid acute abdomen cases with the therapeutic method of expelling pathogens by purgation. Under the leadership of Professor Wu, the multidisciplinary and prospective researches included the effects of dispelling interior pathogenic factors and purgation on the splanchnic blood flow and caecal single smooth muscle cell, the clinical characteristics of multiple organ dysfunction syndrome (MODS) caused by several different kinds of elements, the changes of nerve-endocrine-immunological network in MODS and the effects of purgative herbs on information transmission mechanism of immune cells through four times of different scale joint research in the seventh to tenth five-year plan period. After years of efforts, significant progress had been made and the operation rate had been reduced in the treatment of acute abdomen, such as severe acute cholangitis, acute severe pancreatitis, and complicated biliary stones, which was awarded the second award of national science and technology progress in 2003 [20–22].

The third one is the theory of "treating the toxifying disease with poisonous agents" and researches for arsenic trioxide (AS_2O_3) treating acute promyelocytic leukemia (APL). The theory is a traditional simple understanding of hypertoxic drug treating difficult and complicated diseases. It was recorded in *Compendium of Materia Medica* that the herbal nature of arsenolite is very hot and poisonous, while white arsenic sublimed from arsenolite is more poisonous. White arsenic is a traditional external drug for removing the necrotic tissue and promoting granulation plaster, the effective component of which is AS_2O_3. The research was enlightened by the prominent TCM doctor's experienced external prescription for treating skin cancer. On the basis of verification of curative effects and optimizing prescription, researchers developed the arsenous acid injection from the experienced external prescription, which had definitive curative effect for the patients with APL and reached the top level in the world [23–26]. The mechanism of arsenous acid treating APL was illustrated from the perspective of molecular oncology, including degradation of PML/RARa fusion proteins, downregulating gene expression of Bcl2 and inducing apoptosis in leukemia cells. Arsenous acid became the first antileukemia drug of inducing apoptosis in the world arousing the medical research fever of arsenic trioxide [27–29]. It was honored as "ancient remedy performs new tricks" in 1996 by Science [30]. Sloan-Kettering and his coworkers reported that 12 patients with recurrence of APL after conventional chemotherapies were treated with AS_2O_3, and 11 cases of them relieved completely in 1996. This paper, published in New England Journal of Medicine, directly led to the widely acceptance of AS_2O_3 in the treatment of APL in the international medical field [31].

2.3. Preventing and Curing Refractory Diseases. As the frontier field and hot issue of cardiovascular diseases, restenosis after percutaneous coronary intervention and myocardial ischemia reperfusion injury (MIRI) during open heart surgery of cardiopulmonary bypass has become the best innovative points of clinical studies in integrative medicine.

Researches showed that restenosis after percutaneous coronary intervention was closely related to blood stasis syndrome. Predominantly evaluated by restenosis (RS) rate estimated by coronary angiography (CAG), a prospective randomized controlled study was carried out on RS after PCI to observe the intervention effect of *Xiong Shao* Capsule (XSC). Compared with the control group, the incidence of RS rate in the XSC group was significantly lower (24.1% versus 48.5%, $P < 0.05$) and the extent of angiostenosis and diameter of the culprit arteries, determined by CAG, also significantly reduced after patients had been treated for 6 months with [(2.21 ± 0.85) mm versus (1.72 ± 0.99) mm, $P < 0.05$], and [(26.58 ± 20.72) % versus (41.19 ± 30.92) %, $P < 0.05$], respectively. The incidence of clinical end-point event was significantly lower in the XSC group than that in the control group (11.7% versus 27.6%) and the P value was close to statistical significance ($P = 0.051$). Comparing with the control group, the blood-stasis syndrome score in the XSC group was also significantly lower ($P < 0.01$). The results showed that XSC had a wide range of therapeutic effects including effectively preventing RS after PCI in combination with conventional western medical treatment, decreasing the attack of angina pectoris and improving the blood stasis syndrome. Experimental researches on blood activating herbs showed that it can significantly inhibit pathological vascular remodeling after balloon injury, thus reduce late lumen loss and prevent restenosis [32–36].

As the establishing the cardiopulmonary bypass of open heart surgery is key point of successful operation, myocardial ischemia reperfusion injury (MIRI), which is very obvious during the recovery of circulation, has become the hot issue needed to be resolved. Some scholars found that the pathogenesis of MIRI during open heart surgery of cardiopulmonary bypass is deficiency of heart qi in the origin and excess of heart blood stasis and internal turbid toxin in the superficiality and the therapeutic principles are boosting qi and nourishing heart, activating blood circulation and resolving toxin simultaneously. It was proposed that of astragalus injection and tetramethylpyrazine injection for boosting qi and activating blood circulation should be given by vein injection during operation and *Hu Xin Bao* (compatibility of extracts of ginseng and panax notoginseng with taurine) for boosting qi, activating blood circulation, and resolving toxin should be given by oral administration before operation. The research showed that astragalus injection combined with tetramethylpyrazine injection could reduce the content of MDA and myocardial enzymes' release and improve the activity of SOD, NO, and NOS. Serial studies demonstrated that boosting qi combined with activating blood circulation have significantly synergetic effects, and boosting qi, activating blood circulation, combined with resolving toxin were superior to those simple boosting qi, activating blood circulation, resolving toxin, and boosting qi combined with activating blood circulation [37, 38].

Multiple organ dysfunction syndrome (MODS) is one of the difficult problems in the field of the critical care medicine, which is characterized by acute onset, rapid progress, and extremely high mortality. Since the 1970s of 20th century, some scholars began to take vigorous action to explore a new way of preventing and treating MODS by integrative medicine and a new theory of "bacteria and bacterial toxin treated simultaneously" was presented ultimately. They also perfected schemes for the diagnosis procedure and treatment standard of MODS by both TCM and integrative medicine. And four therapeutic principles for the main types of syndromes were put forward, such as activating blood circulation to dissipate blood stasis therapy on blood stasis syndrome, clearing heat and toxin therapy on heat toxin syndrome, reinforcing the vital energy and consolidating the constitution therapy on acute deficient syndrome, and dispelling interior pathogenic factors and purgation therapy on Yangming fu-organ syndrome. Integrative medicine therapy can effectively improve the clinical efficacy and shorten the course of the disease thus reducing mortality. A famous injection of Chinese medicine, "*Shen Nong* 33," with the effect of activating blood circulation to dissipate blood stasis and antiendotoxin, was developed, which has reduced the mortality of international recognized infectious four or more organs failure from 100% to 50% and reached the international advanced level. Furthermore, a new strategy of "bacteria, bacterial toxin, and inflammatory mediator treated simultaneously" was put forward on the basis of the theory of "bacteria and bacterial toxin treated simultaneously." *Xue Bi Jing* injection, the first Chinese medicine preparation in emergency medicine, was developed, which have made great contributions to the advancement of critical care medicine [39–43].

Chronic hepatitis B is the common disease in China, as well as in the world, causing great affliction to patients. It has become the major issue in the treatment of chronic liver disease. The progression of chronic hepatitis B may lead to liver cirrhosis and hepatocellular carcinoma. Hepatic fibrosis is the common pathological end stage of various chronic liver diseases regardless of the etiology, and blocking the occurrence and development of fibrosis of liver is very important in chronic hepatic diseases' treatment and prognosis. TCM has become the important therapy in treating chronic hepatitis, liver fibrosis, and liver cirrhosis. Some scholars put forward the hypothesis that liver fibrosis and early liver cirrhosis can be reversed. They found out that the basic pathogenesis of liver fibrosis is weakened body resistance and blood stasis, so therapeutic method of strengthening body resistance and dispelling stasis was established, and "*Fu Zheng Hua Yu* Capsules," a new drug for treating liver fibrosis, was developed. Predominantly evaluated by liver tissue fibrosis, clinical researches were carried out to observe the curative effect of the therapeutic method of strengthening body resistance and dispelling stasis. The total inversion rate of liver tissue fibrosis was 52% to 58.3% compared before and after treatment, which also confirmed that liver fibrosis can be reversed and treated. The mechanism includes significantly inhibiting lipid peroxidation, the proliferation of hepatic stellate cell and activation of collagen expression, reducing inflammation of hepatocytic injury model, increasing the activity of matrix metalloproteinases, promoting the degradation of pathological liver collagen, and so on [44–46].

Combining the macroscopic view with microscopic view, syndrome differentiation with disease differentiation,

regional with global, taking stopgap measures with taking radical measures, supporting healthy aspects with eliminating pathogens, tumor treatment model by integrative medicine emphasizes contriving individual treatment plan and evaluation standard on the basis of biological characteristics and the course of disease. Malignant tumors could be treated by TCM therapies such as reinforcing the vital energy and consolidating the constitution, supplementing qi and nourishing yin, and clearing away heat and toxic materials, combined with conventional therapies such as radiotherapy, chemotherapy, and surgery. TCM treatment has significances in decreasing toxicity and increasing efficacy on radiotherapy and chemotherapy. Integrative medicine theory has a remarkable effect in alleviating symptoms such as dry mouth in hyperpyrexic consumption of yin syndrome and deficiency of both qi and yin syndrome caused by head and neck cancer after radiotherapy, relieving symptoms such as cough caused by acute radiation pneumonitis, improving immune function, and survival quality of postoperative patients, preventing the tumor from recurrence or metastasis and prolonging survival time. The new model of combining TCM and modern cancer treatment has attracted widespread attention in the world, which is known as "China Model for Cancer Treatment" [47]. In addition, screening of tumor inhibition from more than 3,000 species of Chinese herbs and nearly 300 Chinese herbal compound, effective components having directly killing effect on cancer cell such as indirubin, camptothecin, vinblastine, matrine, and aclitaxel were extracted. Some Chinese herbs, having the effect of immunological enhancement and biological response modifier-like action such as polyporus, poria cocos, and mushroom, were also found out.

APL is a special type of acute leukemic (AL). TCM suggests that the pathogenesis of APL is weakened body resistance and excessiveness of pathogen, so therapeutic method of eliminating pathogenic factors and strengthening body resistance was established. Some scholars developed the Compound Realgar Natural Indigo Tablets (Realgar, Indigo Naturals, Salvia and Radix pseudostell) on the basis of clearing away heat and toxic materials and supplementing qi with activating blood circulation and promoting hemogenesis method. 155 cases of APL patients were treated by the Compound Realgar Natural Indigo Tablets and the remission rate was 97.42% after treating for 6 months. No side effect, serious infection, bleeding, and DIC were found during the treatment course. It was also characterized by higher negative conversion rate of PML—RARα fusion gene and simple application. The results demonstrated that the complete remission rate of treatment of the Compound Realgar Natural Indigo Tablets were 10–15% higher than that of all-trans retinoic acid (ATRA). On this basis, the effect of post-remission therapy mainly with Compound Realgar Natural Indigo Tablets on long-term survival of 74 cases patients with APL showed that the median remission time was 48 months with recurrence rate only 14.86% and 10-year survival probability was 75.38% [48–50].

Since the 1970s of 20th century, the basic syndrome of type 2 diabetes included yin deficiency with internal excessive heat, deficiency of both qi and yin, and deficiency of both yin and yang, therefore, III-type differentiation of type 2 diabetes was established and developed. It had already been adopted by national guidelines for new drug in the late 1980s. As deficiency of both qi and yin was the important basic syndrome of the disease, "*Jiang Tang Jia* tablets," a new Chinese herb of supplementing qi and nourishing yin, could improve insulin resistance, islet β-cell function, and the level of glucose and lipid metabolism, the total effective rate of which was 76.54%. In addition, researches of *Tang Wei Kang* capsule treating early diabetic nephropathy and *Tang Xin Ping* treating diabetic cardiopathy have gotten progress [51, 52]. Some scholars also found out that blood stasis was another significant pathogenesis of type 2 diabetes due to the changes of hemorheology with different degree were found. So they advocated treating the disease by promoting blood circulation and removing blood stasis principally. Based on this idea, promoting blood circulation by removing blood stasis recipes, such as nourishing yin and activating blood recipes and *Xian Zhen* tablet of reinforcing kidney and activating blood, were developed. Those recipes have multilevel and multitarget effects, including improving symptoms, reducing blood glucose, improving blood rheology and blood flow, lowering triglycerides (TGs), and malondialdehyde (MDA), enhancing activity of erythrocyte SOD, Na^+-K^+-ATP enzyme and Ca^{2+}-Mg^{2+}-ATP enzyme, and so forth. The experimental studies showed that the effect of *Xian Zhen* tablet includes lowering blood glucose and glycosylated hemoglobin, decreasing urine protein excretion, improving renal function, reducing the pathological changes of glomerular mesangial expansion and basement membrane thickening, decreasing AGEs amounts of renal cortex, and downregulating RAGE-mRNA expression in renal cortex and endothelia of heart vessel. It provided a new idea for preventing and treating diabetic and chronic vascular complications [53, 54].

Severe pancreatitis, namely, acute hemorrhagic necrotizing pancreatitis, is characterized by acute onset, rapid progress, high mortality, and poor prognosis. 65% of the death cases are due to complicating with acute respiratory distress syndrome (ARDS). According to the theoretical basis that "the six fu-viscera function well when unobstructed" and "the lung and the large intestine are interior-exteriorly related," acute pancreatitis is treated by expelling pathogens by purgation, and the average cure rate reached to 97%, while the average cure rate of severe pancreatitis was 80%. Compared with our country and abroad, the mortality has reached the lowest level. *Qing Yi* decoction, a famous antipyretic and purgative prescription, protected the lung from injury in many aspects, by preserving the damage of gut barrier function, reducing or eliminating endotoxemia derived from the gut, inhibiting the production, and release of TNF, IL-6, and the translocation of bacteria. The results may fully show the superiority of integrative medicine in treating serious diseases [55, 56].

A certain progress was also made on dermatosis and burn medicine by integrative medicine therapy. Vitiligo was effectly treated by taking modified *Tao Hong Si Wu* decoction, external application of compound tar traditional Chinese rubbing-drugs and melagenine extracted from placenta.

243 patients with vitiligo were treated by modified *Tao Hong Si Wu* decoction and the total effective rate was 68.2%, the mechanism of which was related to upregulation of tyrosinase activity, increasing the melagenine content, and promoting melanocyte proliferation [57]. Moist exposed burn therapy (MEBT), a new therapeutic system of burn medicine in integrative medicine, has become the leading enabler throughout the world. It is found out that the burn wound should be kept in a moist but not macerated environment in order to promote in nature recovery and generation of the skin rather than in traditional dry environment. And the exact curative effect was obtained by MEBT and moist exposed burn ointment (MEBO) [58].

Severe acute respiratory syndrome (SARS) has aroused international attention for strong infectiousness, rapid progression, poor prognosis, and high mortality, which has no special effective therapy yet. 524 patients of SARS in China were divided into integrative medicine treatment group ($n = 318$) and western medicine treatment group ($n = 206$). The existence rates for the symptoms of weakness, short breath, dyspnea in the first group were significantly lower than that in the second group after treatment. The duration of weakness was averagely shortened by 1.5 days in the first group. And short breath, dyspnea, and muscle aching pain were averagely shortened by 2 days, 1 day, and 2 days, respectively. Researches showed that the effect of integrated therapy of TCM and WM for treating SARS was superior to WM treatment alone, and the integrative medicine could improve clinical symptoms such as weakness, short breath, and dyspnea [59–61]. The exact clinical curative effect was also recognized by World Health Organization (WHO).

3. Developing Strategies

3.1. Standing on Frontier Field of International Medicine and Improving the Capability of Preventing and Curing Refractory Diseases. Previous achievements in clinical researches of integrative medicine showed that it is absolutely necessary to keep a foothold at frontier field of international medicine and life science and derive the wisdom and new theories from these subjects in order to find the innovation and breakthrough from subject cross and osmosis. Aiming at the hot issues and knotty problems confronted in clinical medicine, we could put forward scientific hypotheses in exploring the etiology and pathogenesis of the disease and seek for the effective therapeutic principles and classical prescriptions. Basing on the research mentioned above, the clinical efficacy should be objectively evaluated by randomized controlled trials (RCT), and the potential mechanism should be illustrated ultimately. By summarizing the clinical regularity in time, it will contribute to the innovation of the medical theory and guide clinical practice.

Taking coronary heart disease as example, despite great advancements in the fields of basic and clinical researches made by modern medicine, there are still some issues to be resolved, such as acute coronary syndrome complicated by microvascular thrombosis, myocardial ischemia-reperfusion injury, no-reflow phenomenon, stent thrombosis, obvious subjective symptoms such as hypodynamia and shortness of breath remained after percutaneous coronary intervention (PCI), and ventricular remodeling following myocardial infarction [62–64]. Previous study showed that the prospects of integrative medicine is brightening in treatment for coronary stent thrombosis and protecting the myocardial ischemia-reperfusion injury.

In addition, as viral infectious disease belonged to the category of epidemic febrile disease in TCM thousands years ago, Chinese ancients had accumulated rich experience and formed a systematic and complete theory in treatment. Currently, better therapeutic efficacy of viral infectious diseases could be achieved by combining two medical systems, especially in SARS, N1H1, and bird flu. Also, more similar breakthrough points of integrative medicine can be found, for instance, improving the low success rate of assisted reproductive technology (ART) by combining ART with TCM therapeutic method of reinforcing kidney and activating blood, and so forth.

3.2. Moving Prevention and Control Strategy Forward and Improving the Curative Effect of Common and Frequent Disease. "Moving prevention and control strategy forward" is a national macrohealth policy, which well adapted to the new medical model, "physiological-psychological-social-environmental" model. It means that the focal point of medicine will be transferred from treating disease to health care, and disease prevention will be paid more attention to. Therefore, the policy of "prevention first" will be carried out instead of traditional ideological concept "treatment is more major than prevention." It is similar to the TCM theory of "preventive treatment of disease," including principles of "preventing measure taken before the occurrence of disease" and "preventing measure taken after the occurrence of disease" in *Canon of Internal Medicine*. Concrete measures of "moving prevention and control strategy forward" include concept forward, funding forward, emphasis of the researches forward, and measures to be carried out forward. It could reduce the incidence of the major diseases from the origin and effectively control the medical expense and save resources in medicine and health. Integrative medicine researches should also observe the principles above and pay more and more attention to improve the curative effect of common and frequent diseases.

Taking cardiovascular disease, for example, there are about 30% of the population in the world died from cardiovascular and cerebrovascular events, among which 62% of stroke and 49% of cardiovascular events were directly caused by hypertension [65]. According to the China cardiovascular reports (2008-2009), the occurrence and mortality of cardiovascular disease is still increasing in our country, and it is estimated that the number of patients with cardiovascular disease is at least 230 million. It also demonstrated that there were about 200 million hypertensive patients in China with more than 10 million patients increased annually. As the primary cardiovascular risk factor, the risk level of hypertension is equivalent to three other cardiovascular risk factors together. That is why more

emphasis should be taken on prevention and intervention of earlier-stage hypertension in clinical researches of integrative medicine. Additionally, hyperlipidemia, hyperglycemia, obesity, and other risk factors also should be paid more attention to. It is reported by World Health Organization (WHO) that if risk factors were controlled as early as possible, 80% of the disease can be prevented effectively, such as coronary heart disease, stroke, and diabetes. Furthermore, paying 1 *yuan* in prevention will save 7-8 *yuan* in treatment.

3.3. Excavating the Classical Theory of TCM and Broadening the Treatment System of Modern Medicine. JAMA, an international authoritative journal, have commented that traditional medicine should joint tracks with modern medicine. It suggested that not only should we inherit traditional academic thoughts but also keep an eye on modernization of TCM and study it in a scientific and systematic way [66]. The target is to fully digest the traditional Chinese medicine, and apply it to modern medical system. How to do it? The first is further understanding of the essence in TCM, while giving up the dross. The second is illustrating the mechanism of the traditional therapy by using advanced scientific technology in order to improve the safety of the treatment and alleviate the toxicity adverse effect.

Viscera, meridians, prescriptions, and syndromes, the precious wealth in TCM left by our ancients, are worth deeply researching into. However, as the theory is profound, classical, and concise, combining with clinical practice is the unique way to understand the connotation. For example, the lung and the large intestine are interior-exteriorly related, that is to say, the lung was associated with the large intestine by meridians. In the clinical practice, dysfunction of the large intestine conduction could cause no descending of the lung qi, conversely, no descending of the lung qi also can result in obstruction of fu-qi. It is reported that introducing fu-unblocking and purgation therapy into adults' acute pneumonia is a rapid and effective treatment. In the 70th of the last century, Professor Wang had applied *Liang Ge San* (Cool Diaphragm Powder) to treat SIRS and MODS, which mainly manifested as Yangming visceral substantive syndrome. The result demonstrated that the respiratory function of 80% patients with respiratory failure was rapidly improved, and the recovery was greatly promoted. Among the patients with acute pancreatitis accompanied with MODS, the therapy also received superior efficacy. All of these theories mentioned above, including blood stasis syndrome theory, theory of dispelling interior pathogenic factors and purgation, and theory of "treating the toxifying disease with poisonous agents," were all worth deeply excavated, which have being greatly broadened the treatment system of modern medicine.

3.4. Improving the Innovation Level of New High Effective Drugs on the Basis of Classical Prescriptions and Herbs in TCM. Compared with TCM theory, Chinese herbs are much easier to be modernized and recognized. Therefore, it is of great significance in promoting modernization, industrialization, and industrialization of the Chinese herbs, by ways of combining modern technology with fully understanding of classical prescriptions and herbs.

China is a great power with rich herb resources. According to the records in Formula Dictionary of Traditional Chinese Medicine, there are approximately 100 thousand prescriptions, including special prescriptions and herbs for certain diseases. The classical prescriptions and herbs provided with definite clinical indications are of more meaning to be developed. That will provide an effective shortcut to improve the capability of developing new drugs, which is characterized by definite chemical structure, explicit action mechanism, obviously curative effect, advanced formulations, convenience for taking, and low price.

At present, the effective fractions and monomers extracted from Chinese herbs had obtained reliable clinical benefits. For instance, artemisinin extracted from Sweet Wormwood (*Artemisia annua L.*) have a definite effect in the treatment of falciparum malaria, which had been confirmed by multinational joint researches. The new therapy developed by Professor Tu Youyou has saved millions of lives across the globe, especially in the developing world, which had also been listed in the catalog of "essential medicines" by the World Health Organization (WHO) [67–71]. Therefore, Professor Tu was awarded the 2011 Lasker~DeBakey Clinical Medical Research Award for the discovering artemisinin and its utility for treating malaria. The research will raise a new global round of climaxes of modernization and internationalization of TCM.

In addition, indirubin extracted from indigo naturals in *Danggui Long Hui* Wan (Pill of Angelica sinensis, Gentian and Aloe) could treat leukemia. Diterpenonid versicolaction extracted from Tripterygium Wilfordii Hook can be used as immunosuppressive agents for treating rheumatoid arthritis (RA). Biphenyl dimethyl dicarboxylate (DDB) extracted from *Schisandra chinensis* could decrease the ALT and AST activity. Tetramethylpyrazine extracted from Ligusticum Chuanxiong Hort has showed a good effect for ischemic cerebrovascular disease. Cantharidin extracted from Mylabris could treat liver cancer. Moreover, other active components such as ginsenoside, total puerarin flavonoids, polyporus umbellate polysaccharides, ganoderma lucidum polysaccharide, anisodamine, tanshinone, trichosanthin, tetrahydropalmatine, tetrandrine, rubidate, ilexonin A, and ferulic acid sodium.

3.5. Rerecognizing the Theory of Formula Corresponding to Syndrome in TCM and Enhancing the Level of Clinical Research Evidence Based on Evidence-Based Medicine. Evidence-based medicine (EBM) is a new subject quickly developed in the clinical medicine field in the 1990s. The core thinking is to combine evidence, personal experiences, and patients' actual situation to formulate scientific measures for preventing diseases, promoting the recovery and improving life quality. Among them, clinical evidence originates mainly from randomized controlled trial (RCT), systematic review, and meta-analysis. With medical science transforming from traditional experience medicine into evidence-based medicine, fundamental changes have taken

place in clinical medicine. Therefore, following the principle of respecting science and evidence, it is of the utmost importance to enhance the level of clinical research evidence in TCM and integrative medicine [72–74].

It is noteworthy that the treatment concept, formula corresponding to syndrome, lied in classical works of TCM, is similar to the ideas of EBM. However, it had been ignored for a long time. The theory of syndrome differentiation and formula corresponding to syndrome are two characteristic inheritance veins in TCM. Generally speaking, the former is always the mainstream ideology in TCM, while the latter has been paid little attention. Clinical medication based on pathogenesis is the core idea of syndrome differentiation, while clinical medication based on formula syndrome is not exactly the same as it. The most significant difference between them is whether giving attention to the objective evidences of formula utilization. The theory of formula corresponding to syndrome attaches great importance to the objective indications of herbs, which mainly comes from long-term, large-scale and repeated clinical trials by Chinese ancients.

As the indications of herbs are objective and concrete, clinical effect could be repeated at anytime, anywhere, and for anybody. So it is suggested that, facing with one patient, 10 TCM physicians may prescribe the identical prescription and get rapid treatment effect simultaneously according to the indications of formulae and herbs. This is just the reason why the significant curative effect can be by classical prescriptions get in treating severe and lingering illness. The extractive process of indications is similar to the evidence-based research, and indications of herbs have probably exceeded the category of expert experience in EBM. Therefore, carrying out clinical studies under guidance of EBM and formula corresponding to syndrome is helpful to summarize the indications of formulae and herbs, and enhance the level of clinical research evidence in TCM [75–79].

4. Summary

The history of man's science development showed that the crossing and blending of two kinds of knowledge systems will be able to set up a new knowledge system. Integrative medicine, an unprecedented task in present world, is a new pattern of medicine, which is formed by the integration of TCM and WM. The current situation of integrative medicine career was highly evaluated by academician Han Qide. He pointed out that integrative medicine is an inevitable choice for the development of Chinese medicine and the breakthrough point of development for modern medicine, which have unique advantages and will play an important role in China. With changing of the disease chart, increasing of metabolic disease, malignant tumor, iatrogenic disease and drug-induced disease, and the coming of senile society as well as the change of people's views on health and medical mode, both opportunities and challenges have been brought to the development of integrative medicine. Thus we believed that under the guidance of "pay equal attention to both WM and TCM" and "implementing the integrative medicine and developing TCM," integrative medicine will obtain great achievements in creating new medicine and pharmacology, which builds on the combination of both WM and TCM, and make tremendous contributions for the great rejuvenation of the Chinese nation and human health care [80].

Conflict of Interest

All authors manifest that there is no conflict of interests.

Acknowledgments

The current work was partially supported by the National Basic Research Program of China (973 Program no. 2003CB517103) and the National Natural Science Foundation Project of China (no. 90209011). The first author and the corresponding author contributed equally in this paper.

References

[1] K. J. Chen, "Mao ZD and integrative medicine," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 13, no. 2, pp. 711–713, 1993.

[2] A. P. Lu and K. J. Chen, "Chinese medicine pattern diagnosis could lead to innovation in medical sciences," *Chinese Journal of Integrative Medicine*, vol. 17, no. 11, pp. 811–817, 2011.

[3] H. Xu and K. Chen, "Integrative medicine: the experience from China," *Journal of Alternative and Complementary Medicine*, vol. 14, no. 1, pp. 3–7, 2008.

[4] K. J. Chen, Y. R. Jiang, and Y. H. Xie, "Past and present of combination of disease differentiation and syndrome differentiation," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 31, no. 4, pp. 437–443, 2011.

[5] M. F. Mei, "A systematic analysis of the theory and practice of syndrome differentiation," *Chinese Journal of Integrative Medicine*, vol. 17, no. 11, pp. 803–810, 2011.

[6] A. P. Lu and K. J. Chen, "Pondering on syndrome differentiation of diseases," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 25, no. 9, pp. 843–845, 2005.

[7] A. S. Ferreira and A. J. Lopes, "Chinese medicine pattern differentiation and its implications for clinical practice," *Chinese Journal of Integrative Medicine*, vol. 17, no. 11, pp. 818–823, 2011.

[8] J. Wang, K. J. Chen, and W. L. Wa, "Computerized stepwise regression for blood stasis symptom-complex in coronary heart diseases," *Zhong Xi Yi Jie He Za Zhi*, vol. 11, no. 1, pp. 47–49, 1991.

[9] H. Xu, X. Y. Lu, and K. J. Chen, "Study on correlation of blood-stasis syndrome and its accompanied syndromes with pathological changes showed in coronary angiography and restenosis after percutaneous coronary intervention," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 27, no. 1, pp. 8–13, 2007.

[10] X. C. Ma, T. Y. Yin, and K. J. Chen, "Relationship between coronary angiography and syndrome differentiation type of TCM," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 21, no. 9, pp. 654–656, 2001.

[11] J. Wang, Q. Y. He, and C. S. Ma, "Research on relationship between coronary lesion and blood stasis syndrome based on coronary angiography," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 28, no. 12, pp. 1074–1077, 2008.

[12] J. Wang, F. Y. Chu, J. Li et al., "Study on Syndrome Element characteristics and its correlation with coronary angiography

in 324 patients with coronary heart disease," *Chinese Journal of Integrative Medicine*, vol. 14, no. 4, pp. 274–280, 2008.

[13] Q. Y. He, J. Wang, K. W. Yao, Y. W. Xing, and J. Li, "Prospective research on the deficiency-syndrome of the viscera of 1069 patients of coronary heart disease with multiple rotating center," *Zhong Hua Zhong Yi Yao Za Zhi*, vol. 24, no. 1, pp. 31–33, 2009.

[14] J. Li and J. Wang, "Study on syndrome elements and their association law of angina pectoris with 5099 cases reported on literatures," *Zhongguo Zhong Yi Ji Chu Yi Xue Za Zhi*, vol. 13, no. 12, pp. 926–927, 2007.

[15] Q. Y. He, J. Wang, K. W. Yao, and C. S. Ma, "Establishment and primary screening of primitive entry pool of rating scale for patient-reported outcomes of coronary heart disease angina," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 31, no. 1, pp. 15–18, 2011.

[16] K. J. Chen, D. Z. Shi, H. Xu et al., "XS0601 reduces the incidence of restenosis: a prospective study of 335 patients undergoing percutaneous coronary intervention in China," *Chinese Medical Journal*, vol. 119, no. 1, pp. 6–13, 2006.

[17] H. Xu, D. Shi, and K. Chen, "Inhibition of vascular remodelling in a porcine coronary injury model by herbal extract XS0601," *Chinese Medicine*, vol. 1, article 2, 2006.

[18] Q. H. Shang, H. Xu, X. Y. Lu, C. Wen, D. Z. Shi, and K. J. Chen, "A multi-center randomized double-blind placebo-controlled trial of Xiongshao Capsule in preventing restenosis after percutaneous coronary intervention: a subgroup analysis of senile patients," *Chinese Journal of Integrative Medicine*, vol. 17, no. 9, pp. 669–674, 2011.

[19] Z. Y. Gao, H. Xu, D. Z. Shi, C. Wen, and B. Y. Liu, "Analysis on outcome of 5284 patients with coronary artery disease: the role of integrative medicine," *Journal of Ethnopharmacology*, 2011.

[20] X. Z. Wu, "Modern research on purgation method of traditional Chinese medicine—application of the method in acute abdominal diseases and experimental studies," *Chinese Journal of Integrative Medicine*, vol. 4, no. 1, pp. 5–8, 1998.

[21] Q. Zhao, N. Q. Cui, J. K. Li, and X. Z. Wu, "Clinical and experimental study of effect on acute phase protein of multiple organ dysfunction syndrome treated with Dachengqi decoction," *Chinese Journal of Integrative Medicine*, vol. 4, no. 4, pp. 251–254, 1998.

[22] X. Z. Lin, Y. Zheng, D. L. Ma, W. L. Xie, S. Zhao, and X. Z. Wu, "Effect of Dachengqi granules on level of endotoxin in ente rogenous endotoxemia in rats," *Chinese Journal of Integrative Medicine*, vol. 8, no. 4, pp. 229–301, 2002.

[23] T. D. Zhang, P. F. Zhang, S. R. Wang, and T. Y. Han, "Clinical observations on 6 cases of leukemia treated by Ailing injection," *Heilongjiang Yi Yao*, no. 3, pp. 66–67, 1973.

[24] T. D. Zhang, "Study on Chinese herbs containing arsenic treating leukemia," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 18, no. 10, p. 581, 1998.

[25] T. D. Zhang, "Studies on treatment of leukemia with traditional Chinese drugs containing arsenic. On treatment of leukemia with Ailing No.1," *Chinese Journal of Integrative Medicine*, vol. 5, no. 2, pp. 89–94, 1999.

[26] T. D. Zhang, G. Q. Chen, Z. G. Wang, Z. Y. Wang, S. J. Chen, and Z. Chen, "Arsenic trioxide, a therapeutic agent for APL," *Oncogene*, vol. 20, no. 49, pp. 7146–7153, 2001.

[27] G. Q. Chen, X. G. Shi, W. Tang et al., "Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): I. As_2O_3 exerts dose-dependent dual effects on APL cells," *Blood*, vol. 89, no. 9, pp. 3345–3353, 1997.

[28] Z. X. Shen, G. Q. Chen, J. H. Ni et al., "Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (AFL): II. Clinical efficacy and pharmacokinetics in relapsed patients," *Blood*, vol. 89, no. 9, pp. 3354–3360, 1997.

[29] G. Q. Chen, J. Zhu, X. G. Shi et al., "In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML- RARα/PML proteins," *Blood*, vol. 88, no. 3, pp. 1052–1061, 1996.

[30] J. Mervis, "Ancient remedy performs new tricks," *Science*, vol. 273, no. 5275, p. 578, 1996.

[31] S. L. Soignet, P. Maslak, Z. G. Wang et al., "Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide," *New England Journal of Medicine*, vol. 339, no. 19, pp. 1341–1348, 1998.

[32] X. Y. Lu, D. Z. Shi, and H. Xu, "Clinical study on effect of xiongshao capsule on restenosis after percutaneous coronary intervention," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 26, no. 1, pp. 13–17, 2006.

[33] H. Xu, D. Shi, and K. Chen, "Clinical effect of Xiongshao capsule on preventing restenosis post-PTCA or/and stenting," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 20, no. 7, pp. 494–497, 2000.

[34] F. Q. Xu, H. Xu, and J. G. Liu, "Effect of xiongshao capsule on vascular remodeling in rabbit with experimental atherosclerosis," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 24, no. 4, pp. 331–335, 2004.

[35] D. Z. Shi, F. Q. Xu, X. C. Ma et al., "Prevention and treatment of restenosis after PTA in rabbit with Xuefu Zhuyu concentrated pills," *Zhong Yi Za Zhi*, vol. 24, no. 4, pp. 331–335, 2004.

[36] H. Xu, D. Z. Shi, and K. J. Chen, "Effect of xiongshao capsule on vascular remodeling in porcine coronary balloon injury model," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 21, no. 8, pp. 591–594, 2001.

[37] X. Y. Lu, W. G. Zhang, S. N. Zhou, W. Shao, Y. Z. Chen, and Z. H. Yin, "Clinical study on myocardium protection of Huxinbao during open heart surgery of cardiopulmonary bypass," *Zhongguo Zhong Yi Yao Xin Xi Za Zhi*, vol. 10, no. 2, pp. 16–18, 2003.

[38] S. N. Zhou, W. G. Zhang, W. Shao et al., "Clinical study on effection of replenishing qi, activating blood circulation and the compatibility of replenishing qi and activating blood circulation in myocardial ischemia reperfusion injury during open heart surgery of cardiopulmonary bypass," *Shandong Sheng Wu Yi Xue Gong Cheng*, vol. 18, no. 3, pp. 40–42, 1999.

[39] J. D. Wang, N. J. Cui, T. Y. Gao et al., "Investigation on the therapeutic effect of the treatment with the principle, 'Bacteria and bacterial toxin treated simultaneously' in 50 patients with infections multiple system organ failure (MSOF): a new theory and verification of its clinical value," *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue*, vol. 1, no. 1, pp. 5–8, 1989.

[40] J. D. Wang and L. Xue, "Bacteria, bacterial toxin and inflammatory mediator treated simultaneously is a new strategy for the treatment of severe sepsis," *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue*, vol. 10, no. 6, pp. 323–325, 1998.

[41] S. H. Cao, J. D. Wang, and Y. P. Li, "From 'Bacteria and bacterial toxin treated simultaneously' to 'four therapeutic principles and methods treating four main types of syndromes': developing and perfection of syndrome differentiation of multiple organ dysfunction syndrome by integrative medicine," *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue*, vol. 17, no. 11, pp. 641–643, 2005.

[42] L. Xue, "Research of the new strategy for the treatment of systemic inflammatory response syndrome and mutiple organ

dysfunction syndrome: an experimental study of the effect of traditional Chinese herb—'Xuebijing," *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue*, vol. 9, no. 12, pp. 720–722, 1997.

[43] S. H. Cao and J. D. Wang, "Protective effects of Xuebijing on tissue and endothelial cells in rats with septic multiple organ dysfunction," *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue*, vol. 14, no. 8, pp. 489–491, 2002.

[44] P. Liu, C. H. Liu, C. Liu, and L. M. Xu, "Serum pharmacological effects of Fuzheng Huayu Decoction on ito cell proliferation and collagen synthesis in rats," *Chinese Journal of Integrative Medicine*, vol. 4, no. 2, pp. 118–122, 1998.

[45] P. Liu, "Clinic and mechanism study of health-supporting and stasis-resolving decoction on chronic hepatitis B," *Shi Jie Hua Ren Xiao Hua Za Zhi*, vol. 6, no. 7, pp. 60–61, 1998.

[46] P. Liu, C. Liu, and Y. Y. Hu, "Effect of fuzheng huayu recipe in treating posthepatitic cirrhosis," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 16, no. 8, pp. 459–462, 1996.

[47] J. Li and H. S. Lin, "Integrative medicine: a characteristic China model for cancer treatment," *Chinese Journal of Integrative Medicine*, vol. 17, no. 4, pp. 243–245, 2011.

[48] S. L. Huang, A. X. Guo, Y. Xiang, X. B. Wang, H. X. Lin, and L. Fu, "Clinical study on the treatment of acute promyelocytic leukemia mainly with Composite indigo naturalis tablets," *Zhong Hua Xue Ye Xue Za Zhi*, vol. 16, no. 1, pp. 26–28, 1995.

[49] Y. Xiang, X. H. Chang, Y. B. Cheng et al., "Effect of post-remission therapy mainly with compound Huangdai tablet on long-term survival of patients with acute promyelocytic leukemia," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 30, no. 12, pp. 1253–1256, 2010.

[50] Y. Xiang, S. L. Huang, A. X. Guo et al., "The influence on long-term survey of the patients with acute promyelocytic leukemia treated alternatively with compound huangdai tablets and chemotherapy," *Lin Chuang Xue Ye Xue Za Zhi*, vol. 16, no. 5, pp. 204–206, 2003.

[51] X. F. Yan, Q. Ni, S. B. Chen, Y. X. Xiao, and L. Kang, "Study on Prof. Lin Lan's theory of III-type differentiation of type 2 diabetes," *Zhong Yi Za Zhi*, vol. 46, no. 12, pp. 885–887, 2005.

[52] L. Lin, Q. Ni, and Q. J. Gao, "Clinical observation on treatment of diabetic nephropathy by Tangweikang capsule," *Chinese Journal of Integrative Medicine*, vol. 8, no. 1, pp. 19–23, 2002.

[53] D. Y. Tang, S. S. Guo, and R. Y. Sun, "Effect of xianzhen tablet on content of advanced glycosylation end products (AGEs) and mRNA expression of AGE-specific cellular receptor in renal cortex of diabetic rats," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 25, no. 1, pp. 60–63, 2005.

[54] M. Z. Pan, S. S. Guo, X. C. Liang, D. Y. Tang, J. W. Zhang, and R. Y. Sun, "Regulative effects of Xianzhen tablets on the expression of RAGE-mRNA in the aorta in diabetic rats," *Beijing Zhong Yi Yao Da Xue Xue Bao*, vol. 25, no. 4, pp. 21–25, 2002.

[55] Q. P. Wen, H. L. Chen, and F. L. Guan, "Effect of Qingyi decoction on rats with acute lung injury caused by severe acute pancreatitls," *Zhongguo Zhong Xi Yi Jie He Wai Ke Za Zhi*, vol. 9, no. 4, pp. 52–56, 2003.

[56] Q. P. Wen, H. L. Chen, and F. L. Guan, "Effect of Qingyitang on acute lung injury induced by severe acute pancreatitis in rats," *World Chinese Journal of Digestology*, vol. 12, no. 6, pp. 1341–1345, 2004.

[57] A. Xu, Y. W. Li, and X. D. Wei, "Curative efficacy and mechanisms of modified tao-hong-si-wu-tang, a traditional Chinese medicine, in the treatment of vitiligo," *Pigment Cell Research*, vol. 3, p. 248, 2007.

[58] R. X. Xu, "Chinese medical concept of moist exposed burn therapy," *Zhongguo Shao Shang Chuang Yang Za Zhi*, vol. 8, no. 2, pp. 249–250, 2007.

[59] Y. M. Xie, J. Q. Hu, W. L. Wen et al., "Effect of integrated therapy of TCM and WM on clinical symptoms in 318 cases of SARS," *Zhong Yi Za Zhi*, vol. 45, no. 9, pp. 671–674, 2004.

[60] R. B. Wang, J. M. Liu, Y. Y. Jiang et al., "Preliminary study on clinical efficacy of integrative Chinese and western medicine in treating severe acute respiratory syndrome (SARS)," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 23, no. 7, pp. 492–493, 2003.

[61] Z. Y. Jiang, X. D. Tang, W. S. Qi et al., "Efficacy evaluation of TCM in treatment of patients with severe acute respiratory syndrome (SARS) in recovery stage," *Zhong Yi Za Zhi*, vol. 45, no. 9, pp. 671–674, 2004.

[62] G. Heusch, P. Kleinbongard, D. Böse et al., "Coronary microembolization: from bedside to bench and back to bedside," *Circulation*, vol. 120, no. 18, pp. 1822–1836, 2009.

[63] A. Prasad, G. W. Stone, D. R. Holmes, and B. Gersh, "Reperfusion injury, microvascular dysfunction, and cardioprotection: the "dark side" of reperfusion," *Circulation*, vol. 120, no. 21, pp. 2105–2112, 2009.

[64] F. H. Zhao and K. J. Chen, "Prevention of no-reflow phenomenon in acute coronary syndrome," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 30, no. 4, pp. 341–342, 2010.

[65] H. Redwood, "Hypertension, society, and public policy," *European Heart Journal, Supplement*, vol. 9, pp. B13–B18, 2007.

[66] K. J. Chen, "Promoting the integration of TCM and WM by Fully utilizing modern science and technology," *Yi Xue Yan Jiu Za Zhi*, vol. 35, no. 1, p. 1, 2006.

[67] Qinghaosu Coordinating Research Group, "A new sesquiterpene lactone-qinghaosu," *Chinese Science Bulletin*, no. 3, p. 142, 1977.

[68] China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials, "Clinical studies on the treatment of malaria with qinghaosu and its derivatives," *Journal of Traditional Chinese Medicine*, vol. 2, no. 1, pp. 45–50, 1982.

[69] China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials, "Chemical studies on qinghaosu (artemisinine)," *Journal of Traditional Chinese Medicine*, vol. 2, no. 1, pp. 3–8, 1982.

[70] Y. Y. Tu, "The discovery of artemisinin (qinghaosu) and gift from Chinese medicine," *Nature Medicine*, vol. 17, no. 10, pp. 19–22, 2011.

[71] Y. Y. Tu, *Artemisinin (Qinghaosu) and Artemisinin Derivatives*, Beijing Chemical Industry Press, Beijing, China, 2009.

[72] K. J. Chen, "Evaluation of clinical therapeutic effect for traditional Chinese medicine," *Zhong Xi Yi Jie He Xue Bao*, vol. 3, no. 1, pp. 1–2, 2005.

[73] K. J. Chen, T. Q. Li, and B. H. Wei, "Evidence-based medicine and integration of traditional Chinese, and Western medicine," *ZhongGuo Zhong Xi Yi Jie He Za Zhi*, vol. 22, no. 1, pp. 8–13, 2002.

[74] C. Berle, D. Cobbin, N. Smith, and C. Zaslawski, "An innovative method to accommodate Chinese medicine pattern diagnosis within the framework of evidence-based medical research," *Chinese Journal of Integrative Medicine*, vol. 17, no. 11, pp. 824–833, 2011.

[75] J. Wang, X. J. Xiong, Q. Y. He, and S. H. Wang, "Connotation and principle of formulae corresponding to syndromes," *Zhong Yi Za Zhi*, vol. 50, no. 3, pp. 197–199, 2009.

[76] X. J. Xiong, F. Y. Chu, H. X. Li, and Q. Y. He, "Clinical application of the TCM classic formulae for treating chronic bronchitis," *Journal of Traditional Chinese Medicine*, vol. 31, no. 1, pp. 69–72, 2011.

[77] X. J. Xiong, "Connotation of syndrome in the theory of prescriptions corresponding to syndromes," *Zhong Yi Lin Chuang*, vol. 32, no. 1, pp. 53–57, 2011.

[78] X. J. Xiong, "Formula syndrome differentiation is different from syndrome differentiation," *Zhong Yi Lin Chuang*, vol. 31, no. 3, pp. 374–376, 2010.

[79] X. J. Xiong and J. Wang, "Discussion of related problems in herbal prescription science based on objective indications of herbs," *Zhong Xi Yi Jie He Xue Bao*, vol. 8, no. 1, pp. 20–24, 2010.

[80] H. Xu and K. J. Chen, "Integrating traditional medicine with biomedicine towards a patient-centered healthcare system," *Chinese Journal of Integrative Medicine*, vol. 17, no. 2, pp. 83–84, 2011.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 970985, 6 pages
doi:10.1155/2012/970985

Research Article

Diagnosis Analysis of 4 TCM Patterns in Suboptimal Health Status: A Structural Equation Modelling Approach

Li-Min Wang,[1] Xin Zhao,[1] Xi-Ling Wu,[1] Yang Li,[2,3,4] Dan-Hui Yi,[2,3] Hua-Ting Cui,[1] and Jia-Xu Chen[1,5]

[1] *School of Preclinical Medicine, Beijing University of Chinese Medicine, No. 11, Beisanhuan Donglu, Chaoyang District, Beijing 100029, China*
[2] *Center for Applied Statistics, Renmin University of China, 59 Zhongguancun Avenue, Haidian District, Beijing 100872, China*
[3] *School of Statistics, Renmin University of China, 59 Zhongguancun Avenue, Haidian District, Beijing 100872, China*
[4] *School of Public Health, Yale University, 60 College Street, New Haven, CT 06511, USA*
[5] *Department of Basic Theory in Chinese Medicine, Henan University of Traditional Chinese Medicine, Zhengzhou 450008, China*

Correspondence should be addressed to Jia-Xu Chen, chenjiaxu@hotmail.com

Received 5 January 2012; Accepted 24 January 2012

Academic Editor: Aiping Lu

Background. We illustrated an example of structure equation modelling (SEM) in the research on SHS to explore the diagnosis of the Sub optimal health status (SHS) and provide evidence for the standardization of traditional Chinese medicine (TCM) patterns in SHS. And the diagnosis of 4 TCM patterns in SHS was evaluated in this analysis. *Methods*. This study assessed data on 2807 adults (aged 18 to 49) with SHS from 6 clinical centres. SEM was used to analyze the patterns of SHS in TCM. Parameters in the introduced model were estimated by the maximum likelihood method. *Results*. The discussed model fits the SHS data well with CFI = 0.851 and RMSEA = 0.075. The direct effect of Qi deficiency pattern on dampness pattern had the highest magnitude (value of estimate is 0.822). With regard to the construct of "Qi deficiency pattern", "fire pattern", "stagnation pattern" and "dampness pattern", the indicators with the highest load were myasthenia of limbs, vexation, deprementia, and dizziness, respectively. It had been shown that estimate factor should indicate the important degree of different symptoms in pattern. *Conclusions*. The weights of symptoms in the respective pattern can be statistical significant and theoretical meaningful for the 4 TCM patterns identification in SHS research. The study contributed to a theoretical framework, which has implications for the diagnosis points of SHS.

1. Introduction

Suboptimal health state (SHS) is a physical state between health and disease and is characterized by the perception of health complaints, general weakness, and low energy [1]. In the related discussion, it is shown as energy reduction, symptoms of function, and adaptability diminishing but has not met the current diagnostic criteria for disease yet. From the view of TCM theory, *Yin* (things associate with the physical form of an object and have less energetic qualities) and *Yang* (things associate with energetic qualities), *Qi* (*Qi* is life-force, which animates the forms of the world) and *Xue* (*Xue* is a dense form of body fluids that have been acted upon and energized by *Qi*), and *Zang* (*Zang* consists of the heart including the pericardium, lung, spleen, liver, and kidney; *Zang* organs mainly manufacture and store essence: *qi*, blood, and body fluid) and *Fu* (*Fu* consists of gall bladder, stomach, large intestine, small intestine, urinary bladder, and the *Sanjiao* (three areas of the body cavity); Fu organs mainly receive and digest food, absorb nutrient substances, and transmit and excrete wastes) are in an unbalanced state though no any organic pathological changes have been found in the body when people have a subhealth state.

Recent years, SHS has become a new public health challenge all over China. The number of people who were reported suboptimal health in the absence of a diagnosable condition increased [2]. Research on classification and standardization of patterns of suboptimal health status is a hot topic in recent years [3–5]. Unfortunately, the quantitative analyses about the symptoms in different patterns of SHS

in TCM are limited. However, with increasing economic development, the prevalence of SHS is expected to escalate. Studies on intervention and prognosis for SHS are expected to become increasingly important, especially in TCM clinical research. Consequently, the existence of a pattern differentiation to assess SHS will be essential. Therefore, the present study was based on the multicentral large sample clinical epidemiological investigation, and Structural Equation Model (SEM) was used to make analysis on the patterns of SHS.

2. Methods

2.1. Clinical Data Collection. The participants were cluster sampled from six clinical centres participating in this project. The centres are the Beijing Guanghua Hospital Medical Center in Beijing (BJ for short), the Hanzhong People's Hospital Medical Center in Shanxi Province (SX for short), The Hospital affiliated to Changchun University of Chinese Medicine Medical Center in Jilin Province (JL for short), the Shenzhen Second People's Hospital in Guangdong Province (GD for short), the Zhenjiang People's Hospital Medical Center in Jiangsu Province (JS for short), and the Huangshi Aikang Hospital in Hubei Province (HB for short).

The participants from the 6 clinical centres, which were sampled from over 1 million people, consisted of 2807 sub-health samples, in which 1286 were male (45.81% of the total number of cases, age 31.07 ± 0.235 years), and 1521 were female (54.19% of the total number of cases, age 32.26 ± 0.213 years). As shown in Table 1, further information on the samples was provided. Ethical approval for the research protocol and written informed consent were obtained from the ethics committee prior to the study initiation. Written informed consent was obtained from all of respondents. Self-administered questionnaire has good reliability, and validity [6, 7]. Data were collected during October 2009–March 2010.

2.2. Diagnostic Criteria of SHS Include the Following Two Items. More than three-month recurring illness state and efficiency decline because of persistent or excessive fatigue; and no major organic diseases and physiological or mental diseases. Case which must strictly meet the previous two criteria should be diagnosed as SHS.

2.3. Inclusion Criteria of SHS Also Include the Three Items as Follows. Each case must accord with the SHS diagnostic criteria; age should be from 18 through 49 years; each case must be attached with an informed consent form (ICF) signed by the respondent. Case which must all be consistent with the previous 3 items can be concluded in.

2.4. Additionally, Exclusion Criteria of Sub-Health State Have Five Items. Any case who do not accord with inclusion criteria; Women who are pregnant, breast-feeding, or intend to pregnant; any case who do not sign an informed consent form; any case whose questionnaire [6, 7] is incomplete filled (the absence and omitting of self-administered items except general information should not beyond 5% or no interview); and any patient who catches metabolic syndrome. Any case which meets the previous items must be excluded.

Consecutive samples with a single center are used in present study. In other words, the participants who met the inclusion criterion while not being rejected for exclusion criterion were all included, for inducing selection bias. Clinical investigators were trained so that they were fully understood the epidemiological survey programs and standard operating procedures. Epidata 3.02 was used to verify the data parallel double-inputted.

2.5. Statistical Analysis. A basic structure equation model consists of two components: the measurement model which describes how indicator variables related to the latent variables and the structural model which analyzes the relationships among latent variables. The models proposed were estimated using the AMOS 16.0 program. Confirmatory factor analysis (CFA) was used to construct the measurement model structural mode, by maximum likelihood method to estimate parameters. Goodness of fit for our model was two indices of practical fit: the comparative fit indices (CFIs) and the root mean square error of approximation (RMSEA), which were in wide use and known to be relatively unaffected by sample size [8]. The model is well fitted for RMSEA being equal and less than 0.05, middle matched for RMSEA being greater than 0.08 and less than 0.1, and unmatched for RMSEA being greater than 0.1. The value of CFI is between 0 and 1. The value is bigger while model fits better [9]. Figure 1 showed the flow chart for building structure equation model of SHS.

2.6. Theoretical Model. Based on results of the summary research and the experts' counselling, we build the theoretical model for the basic patterns of sub-health state [10–13] and the understanding of patterns transfer regulation. The liver governs free coursing, which refers to liver *qi*'s physiological function of ensuring smooth free flow (of *qi* and Blood), so the dysfunction may lead to Stagnation pattern of liver. And long-term stagnation causes the heat; that is, the stagnation of live-*qi* can lead to the fire pattern of liver. Deficiency of spleen *qi* causes the dysfunction in water transportation and then results in Dampness pattern. The dampness obstructing long-term can cause heat and fire, so dampness pattern can lead to fire pattern. Dampness hampering *qi* movement can lead to stagnation pattern.

Figure 2 showed the theoretical model tested. The latent variables were represented by the ellipses. The exogenous variable "Qi deficiency pattern" was composed of 6 directly observed variables, fatigue, degree of fatigue, weakness, shortness of breath, lazy speech, and dizziness. The variable "Stagnation pattern" was measured with 7 indicators, emotional depression, irritability, nervousness, anxiety, often heaving a deep sigh, hypochondriac pain, and the lower abdomen pain. The variable "fire pattern" was a latent variable with 6 indicators, bitter taste in mouth, dry pharynx, upset, deep-colored urine, constipation, and swollen sore throat. Four directly observed variables, including dizziness,

Table 1: Characteristics of the samples in different areas.

	BJ	SX	JL	GD	JS	HB
Sample size	717	452	463	486	563	666
No(%) of sub	564 (78.7%)	418 (58.3%)	448 (62.5%)	431(60.1%)	445 (62.1%)	501 (69.9%)
Mean age (SD) of sub	30.41 ± 0.298	33.19 ± 0.39.	34.13 ± 0.389	30.72 ± 0.369	33.81 ± 0.446	28.78 ± 0.298

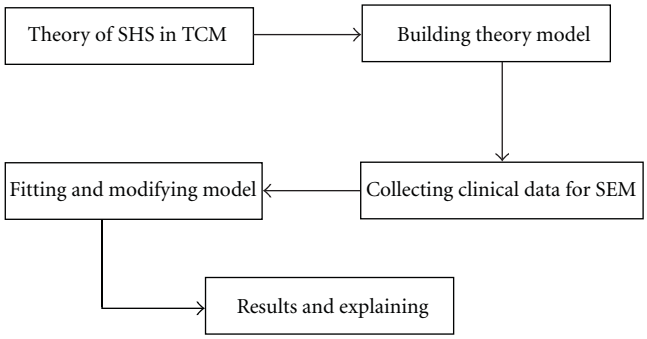


Figure 1: Flow chart for building SEM of SHS.

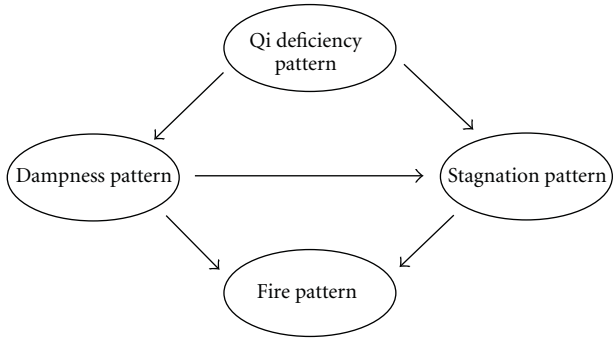


Figure 2: Theoretical model tested using structural equations.

sticky mouth, limpness, and drainage difficulty, were used to construct the latent variable "dampness pattern".

3. Results

3.1. Measurement Model. The first step in the structural equation analysis was the construction of the measurement model. The initial measurement model was constructed on the understanding of patterns transfer regulation in SHS. The factor loadings of the indicators of the latent construct "Qi deficiency pattern" were all higher than 0.60, the two inverse items (x12 and x02) excepted. The indicator with the highest load for this construct was myasthenia of limbs. This indicates that the latent variable adequately predicted the variability of the observed variable (Figure 2). With regard to the constructs "fire pattern", "stagnation pattern", and "dampness pattern", the indicators with the highest load were vexation, deprementia, and dizziness, respectively. In the main symptoms of stagnation pattern, the load coefficient of emotional depression and nervousness was higher than that of hypochondriac pain and lower abdomen pain. It was shown that emotional symptoms for diagnosis of stagnation pattern have greater weight. That was different from the other stagnation patterns of disease status; hypochondriac pain and lower abdomen pain had the greater weight [14, 15].

3.2. Structure Model. Standardized coefficients of the structural model obtained for the SHS were presented in Table 2. These coefficients indicated the impact on the response variable relative to the variation of one standard deviation unit in the explanatory variable. The direct effect of Qi deficiency pattern on dampness pattern was of the highest magnitude (value of estimate is 0.822), and then on the stagnation pattern (value of estimate is 0.351). This implied that for each variation of one standard deviation in Qi deficiency pattern there was a significant increase of 0.822 standard deviation in dampness pattern.

In the same way, the direct effect of Qi deficiency pattern on myasthenia of limbs was of the highest magnitude (value of estimate is 0.686), and then on fatigue (value of estimate is 0.664). This implied that for each variation of one standard deviation in Qi deficiency pattern there was a significant increase of 0.686 standard deviation in myasthenia of limbs and of 0.664 standard deviation in fatigue. The fit of our model provided a middle fit to our data with CFI = 0.851 and RMSEA = 0.075. All of the paths in the final model were highly significant. The final model was represented in Figure 3 and the factor loadings of the measurement model were shown in Table 3.

4. Discussion

TCM pattern is a generalization of various symptoms and signs occurring in a certain stage of a disease, investigating causes, pathogenesis, pathological manifestation, location, and nature of disease. Pattern is an abstraction idea based on the symptoms or signs. It is similar to latent variable which should be quantified and made objective. Pattern identification is a method of thinking which provides evidence for treatment by synthesizing and analyzing clinical data and differentiating patterns on the basis of TCM theories.

Structural equation modelling integrates the idea of factor analysis, correlation analysis, and regression analysis. It can inference on the direct and indirect effects among variables [16–18] besides the analysis of the observation latent variables and measurable variables. With data mining technology widely used in TCM diagnosis [19] and clinical

Table 2: The standardized coefficients of the structural model.

Effects		Estimate
Y4 dampness syndrome	←Y1 Qi deficiency pattern	.822
Y3 fire syndrome	←Y4 dampness pattern	.577
Y2 stagnation syndrome	←Y4 dampness pattern	.520
Y3 fire syndrome	←Y2 stagnation pattern	.407
Y2 Stagnation syndrome	←Y1 Qi deficiency pattern	.351

Table 3: Shows the factor loadings of the measurement model.

Effects		Estimate
x03 myasthenia of limbs	←Y1 Qi deficiency pattern	0.686
x01 fatigue	←Y1 Qi deficiency pattern	0.664
x19 disinclination to say	←Y1 Qi deficiency pattern	0.649
x04 short breath	←Y1 Qi deficiency pattern	0.632
x12 inferiority	←Y1 Qi deficiency pattern	−0.143
x02 degree of fatigue	←Y1 Qi deficiency pattern	−0.149
x41 vexation	←Y3 fire pattern	0.689
x36 dry pharynx	←Y3 fire pattern	0.623
x44 swollen sore throat	←Y3 fire pattern	0.554
x35 bitter taste of mouth	←Y3 fire pattern	0.549
x39 constipation	←Y3 fire pattern	0.525
x40 deep-colored urine	←Y3 fire pattern	0.508
x28 deprementia	←Y2 stagnation pattern	0.721
x30 nervous	←Y2 stagnation pattern	0.717
x32 be apt to breathe	←Y2 stagnation pattern	0.669
x31 anxiety	←Y2 stagnation pattern	0.644
x33 hypochondriac distension and pain	←Y2 stagnation pattern	0.585
x34 abdominal distension and pain	←Y2 stagnation pattern	0.571
x47 dizziness	←Y4 dampness pattern	0.731
x49 limpness	←Y4 dampness pattern	0.722
x48 sticky mouth	←Y4 dampness pattern	0.629
x50 drainage difficulty	←Y4 dampness pattern	0.585

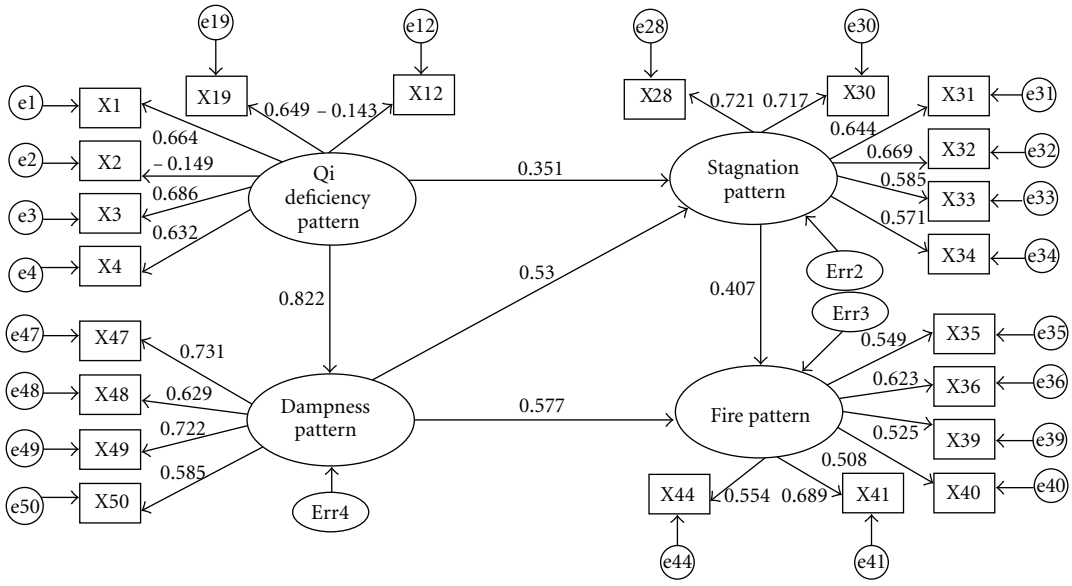


Figure 3: Structural equation model of SHS.

research, SEM was also applied in the study of TCM syndrome standards [18–21].

The results of this study indicate that the SHS model provided middle fit to the data obtained from a large cross-sectional clinical epidemiological investigation. It would be helpful to know for both clinical and research purposes, for example, which variable (symptom) is important to the SHS pattern identification.

Our findings were consistent with the theory of TCM pattern. Effects of Qi deficiency pattern on dampness pattern (0.822) were greater than those on stagnation pattern (0.351). The fact of Qi deficiency of spleen leading to dampness pattern was more obvious than the fact of Qi deficiency of liver leading to stagnation pattern, which was related to the fact of Qi deficiency of spleen being more popular than Qi deficiency of liver and consistent with the fact of liver stagnation and Qi deficiency of spleen pattern being the popular pattern of SHS [10]. Effects of dampness pattern on fire pattern (0.577) were greater than those on stagnation pattern (0.520). It is shown that the dampness obstructing long-term can cause heat and fire. Further, effects of stagnation pattern on fire pattern (0.407) were less than those of stagnation pattern on fire pattern (0.577). It was probably due to effects of Qi deficiency pattern on dampness pattern being greater than those on stagnation pattern, which had indirect effect on the degree of influence of dampness pattern and stagnation pattern on fire pattern.

Furthermore, to a certain degree, the study presented here revealed that the weights of symptoms in the respective pattern represent importance to the pattern identification in SHS. The symptoms of different patterns showed the specific standardized factor loadings, which indicate the weights in their respective patterns and the exact diagnosis of patterns. The exogenous variable "Qi deficiency pattern" was composed of 6 directly observed variables, fatigue, degree of fatigue, weakness, shortness of breath, lazy speech, and dizziness. The variable "stagnation pattern" was measured with 7 indicators, emotional depression, irritability, nervousness, anxiety, often heaving a deep sigh, hypochondriac pain, and the lower abdomen pain. In the main symptoms of stagnation pattern, the load coefficient of emotional depression and nervousness was higher than that of hypochondriac pain and lower abdomen pain. It was shown that emotional symptoms for diagnosis of stagnation pattern had greater weight. That was different from the other stagnation patterns of diseases; hypochondriac pain and lower abdomen pain had the greater weight [14, 15]. In general, the weights of symptoms in the respective pattern can be significant for 4 TCM patterns identification in SHS.

One of the limitations of this study was that all variables were assessed using questionnaires [6, 7]; results may have been biased by the common method variance. This level of bias was a real cause for concern in survey studies because the common method variance may enhance the observed correlation between variables [22]. Another limitation in our present study was the rejection of subpatterns related to Qi deficiency pattern and fire pattern, which should have a certain influence to thoroughly analyze SHS patterns. Despite the afore mentioned limitations, the overall findings of the study suggested that the use of SEM enables us to find and support the possible cause-effect relationship between latent variables (patterns) and measurable variables (symptoms) in SHS. Therefore, by using SEM analysis, we can provide establishing of diagnostic criteria patterns of SHS. In future studies, it would therefore be valuable to test the quantification diagnosis of SHS subpatterns within the clinical setting.

5. Conclusions

In conclusion, we have demonstrated that the use of SEM enables us to find and support the impossible cause-effect relationship between latent variables (patterns) and measurable variables (symptoms) in SHS. The study contributed to a theoretical framework, which had implications for the diagnosis points of SHS. To a certain degree, the weights of symptoms in the respective pattern represented importance to the pattern identification in SHS. It was shown that emotional symptoms for diagnosis of stagnation pattern have greater weight in SHS.

Conflict of Interests

The author's declare that they have no conflict of interests.

Authors' Contributions

L. M. Wang carried out many of the experiments and drafted the paper. Y. Li and D. H. Yi analyzed and interpreted the data. X. Zhao, H. T. Cui, and X. L. Wu performed some of the experiments and contributed to the drafting of the paper. J. X. Chen was involved in the conception and design of the study and the supervision of experiments and contributed to its correction. All authors read the manuscript, contributed to its correction, and approved the final version. L. M. Wang, X. Zhao, Y. Li, and D. H. Yi contributed equally to this work.

Acknowledgments

This work was supported by Hi-Tech Research and Development Program of China (863 Program) (2008AA02Z406), China National Funds for Distinguished Young Scientists (30825046), Program for Innovative Research Team in Beijing University of Chinese Medicine (2011CXTD-07), and MOE Project of Key Research Institute of Humanities and Social Sciences in Universities (2009JJD910002).

References

[1] Y. X. Yan, Y. Q. Liu, M. Li et al., "Development and evaluation of a questionnaire for measuring suboptimal health status in urban Chinese," *Journal of Epidemiology*, vol. 19, no. 6, pp. 333–341, 2009.

[2] Y. Zhou, L. M. You, J. B. Zhang, and N. H. Guan, "Causes and prevention of sub-health," *Chinese Journal of Health Education*, vol. 18, pp. 714–715, 2002 (Chinese).

[3] B. Y. Liu, L. Y. He, Y. M. Xie et al., "Epidemiologic study on TCM syndrome characteristics of sub-healthy people group

in Beijing area," *Journal of Beijing University of Traditional Chinese Medicine*, vol. 30, pp. 130–135, 2007 (Chinese).

[4] C. Q. Yu, X. L. Wang, B. L. Zhang, H. F. Ding, S. F. Chang, and B. L. Zhang, "Discriminatory analysis on mainly syndrome characteristic of sub-health state," *Journal of Liaoning College of Traditional Chinese Medicine*, vol. 8, pp. 129–131, 2006 (Chinese).

[5] China Association of Chinese Medicine, *Clinical Guidelines of Chinese Medicine on Sub-Health*, China Press of Traditional Chinese Medicine, Beijing, China, 2006.

[6] H. Zhao, J. X. Chen, W. H. Xiong et al., "Evaluation of reliability and validity of TCM syndrome questionnaire of sub-health state," *China Journal of Traditional Chinese Medicine and Pharmacy*, vol. 1, pp. 61–65, 2011 (Chinese).

[7] H. Zhao, J. X. Chen, W. H. Xiong et al., "Design of TCM syndrome questionnaire of sub-health state," *Journal of Beijing University of Traditional Chinese Medicine*, vol. 1, pp. 38–41, 51, 2011 (Chinese).

[8] C. Y. Wang, D. P. Wang, X. W. Zhao, Q. W. Fang, and Y. Liu, "The comparison of goodness index of structural equation model," *Modern Preventive Medicine*, vol. 37, pp. 7–9, 2010 (Chinese).

[9] F. M. Huang, *Structural Equation Modeling-Theory and Application*, China Tax Publishing House, Beijing, China, 2005.

[10] H. Zhao and J. X. Chen, "Approach to subhealth state from theory of liver governing discharging," *Shanghai Journal of Traditional Chinese Medicine*, vol. 43, pp. 47–48, 2009 (Chinese).

[11] H. Zhao and J. X. Chen, "Several issues relating to sub-health state," *Shandong Journal of Traditional Chinese Medicine*, vol. 27, pp. 583–584, 2008 (Chinese).

[12] J. Yu and Y. M. Xie, "Exploration on treatment of sub-health state," *Shandong Journal of Traditional Chinese Medicine*, vol. 25, pp. 438–440, 2006 (Chinese).

[13] C. Q. Yu, B. L. Zhang, and H. Ma, "Cognition on etiology and mechanism of sub-health state in traditional Chinese medicine," *Tianjin Traditional Chinese Medicine*, vol. 22, pp. 198–199, 2005 (Chinese).

[14] Y. P. Ma, T. F. Wang, X. L. Xue et al., "Symptom characteristic of stagnation of liver-QI with deficiency of the spleen syndrome and modern literature study on standards of syndrome differentiation," *China Journal of Traditional Chinese Medicine and Pharmacy*, vol. 21, pp. 89–92, 2006 (Chinese).

[15] S. X. Wu, S.Q. Fang, Y. H. Wang, F. M. Lian, and P. Liu, "Expert-questionnaire on symptom distribution and characteristics of syndrome of liver-depression and spleen-deficiency," *Journal of Beijing University of Traditional Chinese Medicine*, vol. 30, pp. 854–856, 2007 (Chinese).

[16] J. T. Hou, Z. L. Wen, and Z. J. Cheng, *Structural Equation Model and its Application*, Educational Science Publishing House, Beijing. China, 2004.

[17] E. Y. Mun, A. von Eye, and H. R. White, "An SEM approach for the evaluation of intervention effects using pre-post-post designs," *Structural Equation Modeling*, vol. 16, no. 2, pp. 315–337, 2009.

[18] H. W. Marsh, K. T. Hau, and Z. Wen, "In search of golden rules: comment on hypothesis-testing approaches to setting cutoff values for fit indexes and dangers in overgeneralizing Hu and Bentler's (1999) findings," *Structural Equation Modeling*, vol. 11, no. 3, pp. 320–341, 2004.

[19] F. F. Xue and J. X. Chen, "Research on data mining application in the diagnostics of TCM," *Journal of Traditional Chinese Medicine*, vol. 50, pp. 200–202, 2009 (Chinese).

[20] Q. G. Chen, C. T. Shen, H. Q. Zhang et al., "Application of structural equation model in standardization of syndrome differentiation," *Chinese Journal of Health Statistics*, vol. 22, pp. 2–4, 2005 (Chinese).

[21] S. P. Xie, J. S. Chen, Q. L. Xu, Y. P. Hu, M. J. Hou, and Z. Y. Diao, "Analysis on structural equation model of HIV/AIDS syndrome differentiating and quantification diagnosis," *Chinese Journal of Basic Medicine in Traditional Chinese Medicine*, vol. 16, pp. 577–579, 2010 (Chinese).

[22] P. M. Podsakoff, S. B. MacKenzie, J. Y. Lee, and N. P. Podsakoff, "Common method biases in behavioral research: a critical review of the literature and recommended remedies," *Journal of Applied Psychology*, vol. 88, no. 5, pp. 879–903, 2003.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 835686, 8 pages
doi:10.1155/2012/835686

Review Article

Management of Hepatic Encephalopathy by Traditional Chinese Medicine

Chun Yao,[1] Nong Tang,[1] Guoxiang Xie,[2] Xiaojiao Zheng,[2] Ping Liu,[3] Lei Fu,[1] Wu Xie,[1] Fan Yao,[1] Houkai Li,[2] and Wei Jia[2]

[1] *Guangxi College of Traditional Chinese Medicine, Nanning, Guangxi 530001, China*
[2] *Department of Nutrition, University of North Carolina at Greensboro, North Carolina Research Campus, Kannapolis, NC 28081, USA*
[3] *Institute of Liver Diseases, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*

Correspondence should be addressed to Wei Jia, w_jia@uncg.edu

Received 3 February 2012; Accepted 9 February 2012

Academic Editor: Aiping Lu

In spite of the impressive progress in the investigation of hepatic encephalopathy (HE), the complex mechanisms underlying the onset and deterioration of HE are still not fully understood. Currently, none of the existing theories provide conclusive explanations on the symptoms that link liver dysfunction to nervous system disorders and clinical manifestations. This paper summarized the diagnostic and therapeutic approaches used for HE in modern medicine and traditional Chinese medicine and provided future perspective in HE therapies from the viewpoint of holistic and personalized Chinese medicine.

1. Introduction

Hepatic encephalopathy (HE), also known as portosystemic encephalopathy, is defined as a spectrum of neuropsychiatric abnormalities in patients with liver dysfunction, after exclusion of other known brain diseases [1–3]. The aggravation of HE will result in hepatic coma or coma hepaticum, which may ultimately lead to death [4]. It is believed that the increase of harmful substances entering brain from blood is the main cause of HE, and currently the identified causal factors for HE are ammonia [5, 6], γ-aminobutyric acid (GABA) [7, 8], false neurotransmitters [9, 10], and the imbalance of certain amino acids in plasma [11, 12]. In spite of the impressive progress in research aiming to uncover the etiology of HE, the complex mechanisms underlying the onset and deterioration of HE and related conditions are still not fully understood. Currently, none of the existing theories provide conclusive explanations on the symptoms that link liver dysfunction to nervous system disorders and clinical manifestations. Generally, it has been accepted that high blood ammonia, which is not properly metabolized in and removed from liver because of the hepatic dysfunction, is closely associated with dysregulation of central nervous system (CNS). The elevation of neurotoxins such as ammonia in blood and CNS impairs the related neurotransmitter system and leads to the functional disorder of CNS. Such a complicated pathology of HE implies the possibility of systematic involvement of multiple organs in orchestrating the development of HE. Therefore, it is necessary to adopt a systems strategy with interdisciplinary studies to understand how dysregulated metabolites disturb the organ-organ (liver-brain) interactions and eventually to uncover the mechanisms of HE at a systems level.

Traditional Chinese medicine (TCM) typically involves a personalized diagnosis and the use of herbal formulae of between 10–20 separate herbal ingredients selected from material medica of several thousand herbs that are prepared either as a boiled decoction, as dried herbal extracts, or taken as pills [13]. Diagnostic and therapeutic treatment principles are framed according to the TCM understanding of pathological processes. A good practice of TCM is usually considered to require a TCM pattern identification based on clinical manifestation followed by the use of individualized herbal decoctions that are adapted to address

the particular TCM pattern of each patient [14]. Since the treatment will change following the changing TCM patterns and clinical manifestations. TCM is a dynamic and highly responsive system of medicine that resonates strongly with the increasing emphasis within systems biology strategy for the use of both multiple approaches to achieve optimum diagnosis and individualized treatments to take into account variable responses to modern drugs.

In the theory of TCM, the onset of HE is due to the invasion of damp and heat in triple burners which leads to phlegm and stagnation of Qi, which eventually causes the disability of thinking in HE patients. Such a traditional theory appears to be consistent with the modern theories of HE etiology. Given the holistic and personalized nature of TCM, HE and its clinical manifestations are divided into various TCM patterns (phenotypes) with different characteristics, which necessitate different therapeutic methods in TCM. In this paper, we summarized the theories and therapeutic methods of HE both in modern medicine and TCM and provided future perspective in HE therapies from the viewpoint of systems biology.

2. The Diagnosis of HE in Modern Medicine

To date, there are no gold-standard diagnostic procedures for HE with high sensitivity and specificity in modern medicine. HE patients usually have advanced chronic liver disease and thus have many of the physical and laboratory stigmata associated with severe hepatic dysfunction. Physical features may include muscle wasting, jaundice, ascites, palmar erythema, edema, spider telangiectasias, and fetor hepaticus [15, 16]. However, some of these features (such as muscle wasting, spider telangiectasias, and palmar erythema) are usually absent in HE patients with fulminant hepatic failure who are previously healthy, because the development of these features requires a relatively longer period of hepatic dysfunction. As a result, substantial technical and laboratory examinations are needed for diagnosis of HE patients, which include psychological test, electrophysiological test, and imaging.

2.1. Psychological Test. The most common tests used in clinics are number connection test (NCT), line tracking test (LTT), serial dotting test (SDT), continuous reaction time (CRT), critical frequency scintillometer (CFS), and wisconsin Ccard sorting test (WCST). The combination of these tests will increase the accuracy of HE determination, avoiding false diagnosis based on a single test. For example, HE psychological testing, a combined test group including NCT, DST, LTT and SDT, has become a rapid and practical procedure that takes less than 20 minutes and achieves 96% sensitivity and 100% specificity.

2.2. Electrophysiological Test. Electrophysiological and electropsychological changes can be tested by electroencephalogram (EEG) and brain electrical activity mapping (BEAM). EEG can be used not only as an evaluation tool but for early diagnosis as well. However, it may be difficult to evaluate the disease objectively as it lacks specificity [17]. Recently, based on the EEG assessment, a test called artificial neural network-expert system (ANNES) with the computer analysis technology is proposed as an expert system to overcome this problem [18]. Additionally, analysis of the EEG utilizing a spatiotemporal decomposition technique (SEDACA) provides significantly more diagnostic information on the neuropsychiatric status of HE patients than obtained conventionally [19].

2.3. Imaging. The imaging methods for HE include computerized tomography (CT) and magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), single photon emission computed tomography (SPECT), and positron emission tomography (PET) [20]. CT and MRI are mainly used to detect brain morphology, such as hydrocephalus in the acute HE patients and encephalatrophy, especially in lobus frontalis in the chronic HE patients [21]. MRS is mainly used to detect the changes of compounds in cells, analyzing the concentrations of such metabolites as glycoconjugates, amino acids, cholines, phospholipids, and creatine to help early diagnosis. Both SPECT and PET are used extensively to assess brain perfusion, which is typically less "active" in HE patients than in healthy people.

3. Therapies of HE in Modern Medicine

The treatments for HE typically include (1) elimination or correction of the underlying factors participating in HE, (2) restoring metabolic homeostasis, (3) promoting regeneration of liver cells, (4) antibiotic agents that inhibit mucosal glutaminase in the intestine to reduce ammonia production in the gut, and (5) artificial liver support or liver transplantation [22–26]. Most patients show clinical signs of improvement in the symptoms of HE within 24–48 hours of initiation of treatment. Serum levels of ammonia might lag behind the clinical response.

3.1. Dietary Regulation of Homeostasis. Restriction of protein intake in diet is preferred for HE therapy. It is advised to consume more calories from vegetable and dairy protein, because vegetable protein is rich in branched-chain amino acids and nonabsorbent fiber, which are beneficial for the balance of normal gut microbiota and acidifying the intestinal tract. It is also necessary to uptake sufficient carbohydrate and vitamins, that is, vitamin C can reduce the level of pH in blood and divert ammonia from brain to blood [27–29]. On the other hand, drinking sufficient water is helpful for maintaining homeostasis of the body, which protects body from hypokalemia, hyperkalemia, hyponatremia, hypocalcemia, hypomagnesemia, and metabolic alkalosis. Additionally, some other ways are available for keeping the homeostasis such as plasma or albumin transfusion, to increase plasma colloid osmotic pressure, improve hypoxemia and hydrocephalus, and prevent hemorrhage and bacterial infection.

3.2. Elimination of Blood Ammonia. Elimination of blood ammonia is critical for HE treatment. Lactulose is widely

used as a standard medicine to evaluate the effect of new drug for HE [30]. Besides, Lactitol is also well practiced for HE therapy with comparable effect to lactulose, but with better tolerance [31, 32]. Although Neomycin is effective for HE patients, long-term usage is prohibited for its toxicity [33, 34]. Oral administration of L-7-ornithine-aspartate (OA) can effectively eliminate the level of blood ammonia [35]. Recent studies show that the concentration of blood ammonia in HE patients was significantly decreased by Rifaximin, as well as amelioration of the patients' condition [36–38].

3.3. Supplementation of Branched-Chain Amino Acids. The administration of branched-chain amino acids (BCAAs) may help adjust the abnormal ratio of BCAAs to aromatic amino acids (AAAs) crossing the blood-brain barrier (BBB), so that the symptom of HE can be improved. A recent meta-analysis has shown that patients with cirrhosis who receive BCAAs are more likely to recover from HE than those who do not receive this supplement [39]. BCAAs improve levels of serum albumin, increase progression-free survival, and reduce both the number of hospitalizations and the length of hospital stays in patients with cirrhosis [40]. These amino acids can be administered orally as well as intravenously.

3.4. Use of False Neurotransmitter Antagonist. According to the theory of false neurotransmitter for HE development [2, 10], antagonists of false neurotransmitter could be used for HE therapy, including bendopa, dopamine agonist Bromocriptine, and opium receptor Narcon. Bendopa could pass the BBB and flow into brain tissue and produce dopamine and norepinephrine by enzymatic catalysis, which are substitutes for the false neurotransmitter and help to recover nerve function. Bromocriptine could agitate postsynaptic dopamine receptor to upregulate prolactin with nerve transmitting function strengthened. Using Bromocriptine alone or with Lactulose together is especially effective to those chronic HE patients who are insensitive to Neomycin or Lactulose. Narcon can cross BBB easily and attenuate the inhibited effect on CNS caused by redundant opioid peptides. Clinical data showed that Narcon is helpful to improve the consciousness of HE patients. However, evidence has indicated that obvious variations exist in therapeutic effectiveness of these medicines on HE patients [41, 42].

4. Treatment of HE in TCM

In TCM, the phenotype of HE is the result of impaired resistance to damp and heat environment (two of the six exogenous pathogens in TCM including wind, cold, heat, damp, dryness, and fire), along with the reduced function of middle burner (the middle part of triple burners in TCM, referring mainly to the organs located between diaphragm and navel, including stomach and spleen) to excrete toxic substances. The accumulated toxic substances in the middle burner spread to the triple burners (including upper, middle, and lower burner, covering all of the organs) and affect the upper orifices (upper orifices are the openings on the face, such as the eyes, ears, nose, and mouth). The essential substances of the organs are distributed through these orifices, so any pathologic change of these orifices contributes to a diagnosis of the disorders of these organs. The pathogenesis in TCM view is consistent with the modern medicine in that abnormal accumulation of metabolites, especially the production, absorption, and distribution of endotoxin in the patients with liver failure will cause the metabolic imbalance in blood. As a result, the therapeutic strategy for HE under TCM includes purgation and eliminating stasis in organs and inducing resuscitation, which is holistic and dynamic in nature.

4.1. Treatment of HE according to TCM Patterns. TCM pattern differentiation is a method to analyze and characterize the clinical manifestations of a disease, a process in which the geographical location, nature, occurrence, and development of the diseased and pathogenic factors are taken into account. Once a specific pattern of an HE patient is identified, a specific treatment strategy will be used to correct or mitigate the pattern of the patient. Table 1 provides a summary of typical TCM patterns of HE and their subsequent treatments.

4.1.1. TCM Pattern of HE—Invasion of Pericardium by Excessive Heat Toxin. The main clinical manifestation of this pattern is characterized as follows. High fever appears at night, severe jaundice with clear yellowing of the body and deteriorating fast, either coma and unconsciousness or disturbed emotion even delirium, constipation inducing distension and ascites, hemorrhinia, hematemesis, hematochezia, bright red tongue substance with yellow and dry tongue coating, taut thready or taut rapid pulse. Treatment should be cleansing the heat toxin and inducing resuscitation. TCM prescriptions commonly used in clinic are Purple Snowy Powder [43], Qing Ying Liang Xue Tang and Cow-bezoar Bolus for Resurrection [44], Antipyretic and Antitoxic Decoction [45], Coptidis Decoction for Detoxification, combined with Rhubarb and Treasured Bolus [46], and a new prescription made with some of the herbs from the three prescriptions Herbae Artemisiae Capillariae Decoction, Antiphlogistic Decoction of Five Drugs, and Cornus Rhinoceri and Rehmannia Decoction [45].

4.1.2. TCM Pattern of HE—Dampness and Phlegm Accumulation Causing Mental Confusion. The main clinical manifestation of this syndrome is characterized as follows. Apparent symptom of jaundice, dark complexion, coma with nausea and vomiting, abdominal distension, high fever at the same time, urine with yellow color and small amount, exhaustion, chest distress, abdominal flatulence, bitterness in the mouth, dark red tongue substance with white greasy or yellow greasy tongue coating, soft and rolling pulse or soft and thready pulse. Treatment should be clearing away dampness, dispelling Phlegm and inducing resuscitation. TCM prescriptions commonly used in clinics are Herbae Artemisiae Capillariae Decoction [47], Artemisiae Scopariae and Poriae Powder [48], Phlegm-removing decoction, combined with

Table 1: The typing, prescription, and treatments for different syndromes of HE.

Prescription	Medicinal herb ingredients
Invasion of pericardium[a] by excessive heat and toxin	
Zi Xue Pill	*Gypsum Fibrosum, Gypsum Rubrum, Magnetitum, Talcum, Bubali Cornu, Saigae Tataricae Cornu, Aucklandiae Radix, Aquilariae Lignum Resinatum, Cimicifugae Rhizoma, Glycyrrhizae Radix et Rhizoma, Caryophylli Flos, Natrii Sulfas, Moschus, Cinnabaris*
Qing Ying Liang Xue Decoction	*Bubali Cornu, Salviae Miltiorrhizae Radix et Rhizoma, Artemisiae Scopariae Herba,*
An Gong Niu Huang Pill	*Imperatae Rhizoma, Bergenia Herba, Paeoniae Radix Rubra, Rehmanniae Radix, Moutan Cortex, Gardeniae Fructus Praeparatus, Rhei Radix et Rhizoma*
Qing Wen Bai Du Oral Solution	*Bubali Cornu, Coptidis Rhizoma, Scutellariae Radix, Artemisiae Scopariae Herba, Lysimachiae Herba, Gypsum Fibrosum, Anemarrhenae Rhizoma, Platycodonis Radix, Rhei Radix et Rhizoma, Gardeniae Fructus, Smilacis Glabrae Rhizoma, Alismatis Rhizoma, Plantaginis Semen, Aurantii Fructus Immaturus, Forsythiae Fructus, Rehmanniae Radix, Lophatheri Herba, Scrophulariae Radix*
Huang Lian Decoction for Detoxification Plus Zhi Bao Pill	*Coptidis Rhizoma, Phellodendri Chinensis Cortex, Scutellariae Radix, Gardeniae Fructus, Rhei Radix et Rhizoma* *Bubali Cornu, Bovis Calculus, Eretmochelys imbricata, Ambrum, Cinnabaris, Realgar, Moschus, Benzoinum*
Yin Chen Hao Decoction	*Artemisiae Scopariae Herba, Gardeniae Fructus, Rhei Radix et Rhizoma*
Wu Wei Detoxification Oral Liquid	*Lonicerae Japonicae Flos, Taraxaci Herba, Violae Herba, Begonia Fimbristipula Herba, Eupolyphaga or Steleophaga*
Xi Jiao Di Huang Decoction	*Bubali Cornu, Rehmanniae Radix, Paeoniae Radix Rubra, Moutan Cortex, Arnebiae Radix*
Pattern of mental confusion by dampness and phlegm[b] accumulation	
Yin Chen Wu Ling Dispersing agent	*Artemisiae Scopariae Herba, Polyporus, Alismatis Rhizoma, Atractylodis Macrocephalae Rhizoma, Poria, Cinnamomi Ramulus*
Phlegm-removing Decoction with Da Huang	*Arisaematis Rhizoma, Pinelliae Rhizoma, Aurantii Fructus Immaturus Poria, Citri Exocarpium Rubrum, Cinnabaris, Acori Tatarinowii Rhizoma, Atractylodis Macrocephalae Rhizoma, Caryophylli Flos, Aquilariae Lignum Resinatum, Santalum album,*
Su He Xiang Pill	*Olibanum, Piperis Longi Fructus, Bubali Cornu, Benzoinum, Aucklandiae Radix, Cyperi Rhizoma, GinsengRadix et Rhizoma, Bambusae Caulis In Taenias, Glycyrrhizae Radix et Rhizoma, Rhei Radix et Rhizoma, Styrax, Moschus, Borneolum Syntheticum*
Ju Fang Zhi Bao Pill	*Bubali Cornu, Bovis Calculus, Eretmochelys imbricata, Ambrum, Cinnabaris, Realgar, Moschus, Benzoinum*
Yin Chen Si Ling Decoction	*Artemisiae Scopariae Herba, Poria, Alismatis Rhizoma, Polyporus, Gardeniae Fructus*
Chang Pu Yu Jin Decoction	*Acori Tatarinowii Rhizoma, Curcumae Radix, Arisaema Cum Bile, Pinelliae Rhizoma, Magnoliae Officinalis Cortex, Myristicae Semen, Polygalae Radix, Forsythiae Fructus, Pogostemonis Herba*
Pattern of Yin[c] deficiency of liver and kidney and Yang[c] excess of Liver	
Yi Guan Decoction	*Rehmanniae Radix, Angelicae Sinensis Radix, Lycii Fructus, Glehniae Radix, Ophiopogonis Radix, Toosendan Fructus*

Table 1: Continued.

Prescription	Medicinal herb ingredients
Ling Yang Jiao Decoction	*Saigae Tataricae Cornu, Testudinis Carapax Et Plastrum, Rehmanniae Radix, Ligustri Lucidi Fructus, Eclipse Prostrala Herba, Dendrobii Caulis, Margaritifera Concha, Moutan Cortex, Paeoniae Radix Rubra, Bupleuri Radix, Prunellae Spica, Chrysanthemi Flos, Haliotidis Concha, Carthami Flos, Persicae Semen, Angelicae Sinensis Radix, Chuanxiong Rhizoma, Trogopteri xanthipes stool, Cyperi Rhizoma, Corydalis Rhizoma, Artemisiae Scopariae Herba*
Pattern of exhaustion of Yin and Yang, and disturbance in spirit	
Pulse-Activating Powder	*Ginseng Radix Et Rhizoma, Ophiopogonis Radix, Schisandrae Chinensis Fructus*
Xi Jiao Di Huang Decoction	*Bubali Cornu, Rehmanniae Radix, Paeoniae Radix Alba or Paeoniae Radix Rubra, Moutan Cortex*
Shen Fu Long Mu Decoction	*Ginseng Radix Et RhizomaRubra, Aconiti Lateralis Radix Praeparata, Astragali Radix, Ostreae Concha, Corni Fructus, Polygonati Rhizoma, Rehmanniae Radix, Rehmanniae Radix Praeparata, Schisandrae Chinensis Fructus, Scrophulariae Radix, Ophiopogonis Radix, Adenophorae Radix*

[a]Pericardium refers to an anatomical membrane surrounding heart, and physiologically it protects the heart. When exogenous pathogens invade the heart, the pericardium is always the first to be attacked. Invasion of the pericardium by pathogenic heat gives rise to symptoms of mental disturbances such as coma and delirium in TCM.
[b]Phlegm is usually secreted by dysfunction of lung and spleen, and occasionally by the consumption of body fluids by fire and heat evils. A disharmony of body fluids can produce either external, visible phlegm, such as sputum secreted by the respiratory tract, or internal, invisible phlegm.
[c]The Yin-Yang theory believes that the normal life activities of the human body result from the harmonious relation of the unity of opposites between Yin and Yang. The imbalance between Yin and Yang is one of the basic pathogenesis of a disease. All the pathological changes can be summarized as excess or deficiency of Yin or Yang. To be more concrete, "Yang excess leads to heat syndrome while Yin excess causes cold syndrome"; "Yang deficiency results in cold syndrome while Yin deficiency causes heat syndromes"; "Yang deficiency affects Yin while Yin deficiency affects Yang."

Rhubarb and Storax Pill [46], Jufang Zhibao Dan [47], Yin Chen Si Ling Decoction, and Changpu Yujin Decoction [45].

4.1.3. TCM Pattern of HE—Yin Deficiency of Liver and Kidney Coupled with Yang Excess of Liver. The main clinical manifestation of this syndrome is characterized as follows. Swarthy complexion, thin shape, faintness, coma, distracted, jerking movements in the extremities, red and dry tongue substance with little tongue coating, taut thready or taut rapid pulse. Treatment should be nourishing liver and kidney, and expelling wind and heat. TCM prescriptions commonly used in clinic are Du Xiao Ke Li [49], Yiguan Decoction [50], Cornu Satgae Decoction, and Subphrenic Recesses [45].

4.1.4. TCM Pattern of HE—Exhaustion of Yin and Yang Coupled with Disturbance in Spirit. The main clinical manifestation of this syndrome is characterized as follows. Dottiness, coma, pale complexion, cold extremities, carphology, syncope with convulsion, slow reaction, weak breath, diaphoresis, incontinence of urine and feces, pale tongue substance without tongue coating, feeble and impalpable pulse. Treatment should be supplementing Qi and nourishing Yin, reduce resuscitation, and recuperate depleted Yang. TCM prescriptions commonly used in clinic are Pulse-Activating Powder or Cornus Rhinoceri and Rehmanniae Decoction [51], Shen Fu Long Mu Tang, or Ginseng Decoction [45].

4.2. TCM Treatmen—Purging Organs and Opening Orifices. The pathogenesis of HE mostly includes the deficiency of liver and kidney, phlegm retention and blood stasis, failure of Yang and Yin to raise and fall, respectively, which could be regarded as the declining function in distributing nutrients to the organ and excretion out of the organ, leading to the symptoms like coma, convulsion, and mental confusion [52]. The TCM pattern of the excess phlegm and serum stasis with the deficiency in both Yin and Yang of Qi and blood affect the mental stability. A clinical TCM retrospective survey with a large number of HE patients ($n = 1072$) and a prospective survey with 133 HE subjects revealed that the main cause of liver failure is a combination of toxin, phlegm, and blood stasis entangled in the body along with dampness, heat, and pestilence invasion [53]. Therefore, the TCM treatment approaches involve removing toxin, expelling blood stasis, and eliminating phlegm have been applied in the clinical treatment of HE. Several representative clinical studies are described using this approach in the following.

Rhubarb (*Rhei Radix and Rhizoma*) is a potent herb with purging activity, which can relieve internal heat and promote blood circulation by removing blood stasis and normalizing gallbladder to cure jaundice [54–56]. Li and Ma [57] applied a decoction of a single medicinal plant, *Rhubarb*, through colon infusion in 30 HE patients. About

30 g of Rhubarb was prepared to decoction in a 200 mL of water as an enema. This decoction was administered 1-2 times daily for 10 days as a course of treatment. Six patients experienced "complete remedy" (CR, defined as reaching and maintaining a conscious and lucid state of mind for 3 weeks after dose), 18 patients experienced "partial remedy" (PR, significant improvement of the symptoms), while 6 patients had no effect. The total efficacy (CR + PR) was 80%.

Lv and Li [58] applied a TCM agent, Tongfu Xiere Decoction, containing *Rhubarb, Dandelion, Magnolia Bark, Citri Immaturus, and Fructus Mume*, to 64 HE patients, with an attempt to relieve internal heat, and cool, promote blood flow, and eliminate phlegm and freeing channels. The patients were divided into two groups, a control group in which all subjects received intravenous infusion of 40 mL of Qingkailing (a TCM drug) injection, 250 mL of BCAA or 10 g of Hepa Merz, once a day, and a TCM group in which Tongfu Xiere Decoction was applied in addition to the treatments in the control group. The decoction was prepared as an enema and administered through colon infusion at 250 mL a day. Therapeutic efficacy in TCM group reached 93.94% while the control group reached 80.65%.

A combined Narcon and *Rhubarb* therapy for 62 HE patients was conducted by Huang [59] recently. The patients were randomly divided into two groups, conventional therapy group ($N = 24$) with an integrated approach comprising antibiotics treatment, balancing electrolytes, amino acids, and pH in body fluid, and so forth, and the treatment group ($N = 38$), in which intravenous infusion of Narcon and colon infusion of Rhubarb decoction were applied in addition to the approach used in the conventional therapy group. Narcon was infused at a dose of 4 mg in 500 mL of 5% Glucose, at 0.3 mg/h. The decoction of 30 g of *Rhubarb* in 500 mL water was applied once a day. The results showed a significant improvement in effectiveness in the treatment group with 94.7% efficacy (defined as showing a conscious state <48 h after dose) compared to an efficacy of 66.7% in the conventional therapy group.

A similar clinical investigation was conducted using the decoction of *Rhubarb* as an enema to treat 60 HE patients at the First Hospital affiliated to Guangxi University of Traditional Chinese Medicine. The patients were randomly divided into two groups, conventional therapy group ($N = 30$) with an integrated approach, and the treatment group ($N = 30$) with colon infusion of a decoction of *Rhubarb* and *Mume Fructus* were applied in addition to the approach used in the conventional therapy group. The decoction of Rhubarb and *Fructus Mume* (30 g : 30 g in 100 mL water) was applied once a day. After a 3-day course of treatment, the total effective rate (defined as HE symptoms improved by one stage (0–4 stages) within 48 h after dose) in the treatment group was 83.33%, higher than that (56.67%) in conventional therapy group [60].

5. Summary and Prospect

5.1. Summary of Therapies for HE with Modern Medicine and TCM. The current diagnoses of HE are still based on experimentalism lacking accurate and objective evaluation of the pathology in modern medicine due to the complexity of the HE pathogenesis. Current treatment of HE is focused on a comprehensive management of disease symptoms and improvement of patients' quality of lives, with less satisfactory effectiveness in reversing the pathological course of HE. On the other hand, long-term exposure to therapeutic drugs also results in drug resistance and dependence. As a result, no universally effective treatment has been generated in modern medicine. However, the successful use of TCM therapeutic approaches over the past decades suggests that alternative approaches be taken into consideration for HE therapy with holistic and personalized views and a multilevel and multipathway adjustment strategy. For example, the treatment with the strategy of purging organs and removing blood stasis has been increasingly accepted for HE therapy in TCM. Nevertheless, more well-designed studies should be conducted to further evaluate the clinical efficacy of TCM approaches and elucidate the complicated mechanisms of TCM treatment for HE patients.

5.2. Prospective. The brain and liver are key targets for damage induced by dysregulated metabolites often associated with gut-generated signals. Thus the gut-liver-brain axis is crucial for coordinating homeostasis and health. Therefore, interdisciplinary studies of how dysregulated metabolites disturb the gut-liver-brain interactions will uncover novel mechanisms of HE, which are essential for understanding the pathogenesis at a systemic level. Such knowledge is the basis for development of effectively preventive and therapeutic strategies in most-at-risk populations.

Recent studies suggest that HE seems to be the result of the energy metabolism defects in brain, neurotransmitter abnormity, and mutation of the receptors on the membrane of neuron. Thus, the pathogenesis of HE might be a result of systematic dysfunctions in multiple organs. Metabolomics, an important element for systems biology with genomics, transcriptomics, and proteomics, has been increasingly applied in identifying and quantifying significantly altered metabolites in cell, tissue, organ, or organism, as the end products of biological processes reflecting pathological change of diseases or the effects of medicine to the body. Serum metabolite profiling with 1H-NMR has been implemented in patients with normal, cirrhosis, or minimal HE, in which substantial differentiated metabolites have been identified among different groups [61]. The application of metabolomics to the study of HE will help understand the pathogenesis and provide a new method for early diagnosis of this disease. Metabolomics may be an effective technique linking quantitative changes of metabolites to syndromes of TCM because the various syndromes of TCM may result from global metabolic imbalances in the patients. As a result, metabolomics can be applied as a holistic profiling tool to unveil the veil of TCM diagnosis and therapies of HE. Such a novel clinical approach coupled with TCM strategies is expected to make breakthrough discoveries in the areas of characterizing metabolic phenotypes of HE, developing diagnostic and treatment biomarkers, and identifying herbal medicines suitable for HE treatment.

References

[1] A. T. Blei and J. Córdoba, "Hepatic encephalopathy," *American Journal of Gastroenterology*, vol. 96, no. 7, pp. 1968–1976, 2001.

[2] R. F. Butterworth, "Hepatic encephalopathy: a neuropsychiatric disorder involving multiple neurotransmitter systems," *Current Opinion in Neurology*, vol. 13, no. 6, pp. 721–727, 2000.

[3] S. vom Dahl, G. Kircheis, and D. Häussinger, "Hepatic encephalopathy as a complication of liver disease," *World Journal of Gastroenterology*, vol. 7, no. 2, pp. 152–156, 2001.

[4] P. Amodio and A. Gatta, "Neurophysiological investigation of hepatic encephalopathy," *Metabolic Brain Disease*, vol. 20, no. 4, pp. 369–379, 2005.

[5] R. F. Butterworth, J. F. Giguère, J. Michaud, J. Lavoie, and G. P. Layrargues, "Ammonia: key factor in the pathogenesis of hepatic encephalopathy," *Neurochemical Pathology*, vol. 6, no. 1-2, pp. 1–12, 1987.

[6] R. F. Butterworth, "Pathophysiology of hepatic encephalopathy: a new look at ammonia," *Metabolic Brain Disease*, vol. 17, no. 4, pp. 221–227, 2002.

[7] S. Ahboucha and R. F. Butterworth, "Pathophysiology of hepatic encephalopathy: a new look at GABA from the molecular standpoint," *Metabolic Brain Disease*, vol. 19, no. 3-4, pp. 331–343, 2004.

[8] E. Roberts, "The γ-aminobutyric acid (GABA) system and hepatic encephalopathy," *Hepatology*, vol. 4, no. 2, pp. 342–345, 1984.

[9] W. A. Fogel, W. Andrzejewski, and C. Maslinski, "Neurotransmitters in hepatic encephalopathy," *Acta Neurobiologiae Experimentalis*, vol. 50, no. 4-5, pp. 281–294, 1990.

[10] R. F. Butterworth, "Neurotransmitter dysfunction in Hepatic Encephalopathy: new approaches and new findings," *Metabolic Brain Disease*, vol. 16, no. 1-2, pp. 55–65, 2001.

[11] R. F. Butterworth, "Neuroactive amino acids in hepatic encephalopathy," *Metabolic Brain Disease*, vol. 11, no. 2, pp. 165–173, 1996.

[12] B. Als-Nielsen, R. L. Koretz, L. L. Kjaergard, and C. Gluud, "A systematic review on branched-chain amino acids for hepatic encephalopathy," *Journal of Hepatology*, vol. 34, supplement 1, article 62, 2001.

[13] W. Jia, W. Gaoz, and L. Tang, "Antidiabetic herbal drugs officially approved in China," *Phytotherapy Research*, vol. 17, no. 10, pp. 1127–1134, 2003.

[14] W. Jia, W. Y. Gao, Y. Q. Yan et al., "The rediscovery of ancient Chinese herbal formulas," *Phytotherapy Research*, vol. 18, no. 8, pp. 681–686, 2004.

[15] J. Vaquero, J. Polson, C. Chung et al., "Infection and the progression of hepatic encephalopathy in Acute Liver Failure," *Gastroenterology*, vol. 125, no. 3, pp. 755–764, 2003.

[16] K. D. Mullen, P. Ferenci, N. M. Bass, C. B. Leevy, and E. B. Keeffe, "An algorithm for the management of hepatic encephalopathy," *Seminars in Liver Disease*, vol. 27, no. 2, pp. 32–47, 2007.

[17] S. J. Munoz, "Hepatic encephalopathy," *Medical Clinics of North America*, vol. 92, no. 4, pp. 795–812, 2008.

[18] P. Amodio, A. Pellegrini, E. Ubiali et al., "The EEG assessment of low-grade hepatic encephalopathy: comparison of an artificial neural network-expert system (ANNES) based evaluation with visual EEG readings and EEG spectral analysis," *Clinical Neurophysiology*, vol. 117, no. 10, pp. 2243–2251, 2006.

[19] S. Montagnese, C. Jackson, and M. Y. Morgan, "Spatio-temporal decomposition of the electroencephalogram in patients with cirrhosis," *Journal of Hepatology*, vol. 46, no. 3, pp. 447–458, 2007.

[20] C. A. Stewart, M. Reivich, M. R. Lucey, and G. J. Gores, "Neuroimaging in hepatic encephalopathy," *Clinical Gastroenterology and Hepatology*, vol. 3, no. 3, pp. 197–207, 2005.

[21] A. Watanabe, "Cerebral changes in hepatic encephalopathy," *Journal of Gastroenterology and Hepatology*, vol. 13, no. 7, pp. 752–760, 1998.

[22] W. J. Cash, P. McConville, E. McDermott, P. A. McCormick, M. E. Callender, and N. I. McDougall, "Current concepts in the assessment and treatment of Hepatic encephalopathy," *QJM*, vol. 103, no. 1, Article ID hcp152, pp. 9–16, 2009.

[23] A. Mas, "Hepatic encephalopathy: from pathophysiology to treatment," *Digestion*, vol. 73, no. 1, pp. 86–93, 2006.

[24] M. Y. Morgan, A. Blei, K. Grüngreiff et al., "The treatment of hepatic encephalopathy," *Metabolic Brain Disease*, vol. 22, no. 3-4, pp. 389–405, 2007.

[25] T. D. Schiano, "Treatment options for hepatic encephalopathy," *Pharmacotherapy*, vol. 30, no. 5, pp. 16S–21S, 2010.

[26] G. T. Toris, C. N. Bikis, G. S. Tsouroufl is, and S. E. Theocharis, "Hepatic encephalopathy: an updated approach from pathogenesis to treatment," *Medical Science Monitor*, vol. 17, no. 2, pp. RA53–RA63, 2011.

[27] R. Chadalavada, Raja Shekhar Sappati Biyyani, J. Maxwell, and K. Mullen, "Nutrition in hepatic encephalopathy," *Nutrition in Clinical Practice*, vol. 25, no. 3, pp. 257–264, 2010.

[28] K. D. Mullen and F. L. Weber, "Role of nutrition in hepatic encephalopathy," *Seminars in Liver Disease*, vol. 11, no. 4, pp. 292–304, 1991.

[29] G. J. Schulz, A. C. L. Campos, and J. C. U. Coelho, "The role of nutrition in hepatic encephalopathy," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 11, no. 3, pp. 275–280, 2008.

[30] F. Simmons, H. Goldstein, and J. D. Boyle, "A controlled clinical trial of lactulose in hepatic encephalopathy," *Gastroenterology*, vol. 59, no. 6, pp. 827–832, 1970.

[31] P. L. Lanthier and M. Y. Morgan, "Lactitol in the treatment of chronic hepatic encephalopathy: an open comparison with lactulose," *Gut*, vol. 26, no. 4, pp. 415–420, 1985.

[32] M. Y. Morgan, M. Alonso, and L. C. Stanger, "Lactitol and lactulose for the treatment of subclinical hepatic encephalopathy in cirrhotic patients. A randomised, cross-over study," *Journal of Hepatology*, vol. 8, no. 2, pp. 208–217, 1989.

[33] F. Orlandi, U. Freddara, and M. T. Candelaresi, "Comparison between neomycin and lactulose in 173 patients with hepatic encephalopathy. A randomized clinical study," *Digestive Diseases and Sciences*, vol. 26, no. 6, pp. 498–506, 1981.

[34] E. Strauss, R. Tramote, E. P. S. Silva et al., "Double-blind randomized clinical trial comparing neomycin and placebo in the treatment of exogenous hepatic encephalopathy," *Hepato-Gastroenterology*, vol. 39, no. 6, pp. 542–545, 1992.

[35] G. Kircheis, R. Nilius, C. Held et al., "Therapeutic efficacy of L-ornithine-L-aspartate infusions in patients with cirrhosis and hepatic encephalopathy: results of a placebo-controlled, double-blind study," *Hepatology*, vol. 25, no. 6, pp. 1351–1360, 1997.

[36] P. S. Mantry and S. Munsaf, "Rifaximin for the treatment of hepatic encephalopathy," *Transplantation Proceedings*, vol. 42, no. 10, pp. 4543–4547, 2010.

[37] N. M. Bass, E. Bortey, and W. P. Forbes, "Rifaximin treatment in hepatic encephalopathy ," *The New England Journal of Medicine*, vol. 362, no. 25, pp. 2424–2425, 2010.

[38] P. Ferenci, A. Lockwood, K. Mullen, R. Tarter, K. Weissenborn, and A. T. Blei, "Hepatic encephalopathy—definition, nomenclature, diagnosis, and quantification: final report of the Working Party at the 11th World Congresses of Gastroenterology, Vienna, 1998," *Hepatology*, vol. 35, no. 3, pp. 716–721, 2002.

[39] Y. Muto, S. Sato, A. Watanabe et al., "Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis," *Clinical Gastroenterology and Hepatology*, vol. 3, no. 7, pp. 705–713, 2005.

[40] J. S. Bajaj, "Review article: the modern management of hepatic encephalopathy," *Alimentary Pharmacology and Therapeutics*, vol. 31, no. 5, pp. 537–547, 2010.

[41] B. Als-Nielsen, L. L. Gluud, and C. Gluud, "Dopaminergic agonists for hepatic encephalopathy," *Cochrane Database of Systematic Reviews*, no. 4, Article ID CD003047, 2004.

[42] S. Ahboucha, L. Coyne, R. Hirakawa, R. F. Butterworth, and R. F. Halliwell, "An interaction between benzodiazepines and neuroactive steroids at GABAA receptors in cultured hippocampal neurons," *Neurochemistry International*, vol. 48, no. 8, pp. 703–707, 2006.

[43] D. Mao, H. Qiu, and Y. Li, "Observation of curative effect on severe hepatitis with Jie Du Hua Yu Granule," *Guangxi Journal of TCM*, vol. 27, no. 4, pp. 4–6, 2004.

[44] S. Zheng, "Treatment of heavy hepatitis (45 Cases) by Qing ying liang xue Decoction with an gong niu huang pills to toxic heat flourishing type," *Zhejiang Journal of Traditional Chinese Medicine*, vol. 42, no. 7, pp. 378–379, 2007.

[45] A. Zhang, "Five ways in TCM dialectical therapy in treating heavy hepatitis concurrent hepatic encephalopathy," *Chinese Journal of Practical Nervous Diseases*, vol. 10, no. 9, p. 60, 2007.

[46] F. Wang, "Hepatic encephalopathy(12 cases) of TCM treatment based on syndrome differentiation," *Medical Journal of West China*, vol. 17, no. 3, p. 270, 2005.

[47] X. Li and J. Zhou, "Treatment on hepatic encephalopathy companion eyperglycemia (12 cases) by integrated TCM-WM," *Integrated Traditional Chinese and Western Medicine In Practice of Critical Care Medicine*, vol. 8, no. 3, p. 184, 2001.

[48] J. Li, J. Yao, and F. Liu, "Oral chinese medicine with retention enema in treating heavy hepatitis(30 cases)," *Zhejiang Journal of Integrated Traditional Chinese and Western Medicine*, vol. 6, pp. 383–384, 2006.

[49] Y. Cao, "Experienced treatment on hepatic encephalopathy (3 cases) with Yinchenwuling Powder," *Chinese Journal of Current Traditional And Western Medicine*, vol. 2, no. 10, pp. 67–68, 2004.

[50] T. Liu, D. Huo, and Y. Zhang, "Clinical observation of deficiency of liver-yin and kidney-yin sthenia-syndrome in hepatic encephalopathy with Du xiao Granule," in *Proceedings of the The 1st Infectious Diseases National Conference of Integrated TCM-WM*, 2006.

[51] H. Xu, *Curative Effect Research of Nourishing Yin and Alleviating Water with Putting Ascites and Inputting Albumen Through Vein in Curing Refractory Ascites of Liver and Kidney Yin Asthenia*, Guangzhou university of TCM, 2007.

[52] X. Zhou, W. Ma, and X. Sun, "Effects of plasma endotoxin levels and cell factor to liver failure patients with sheng mai powder," *Journal of Traditional Chinese Medicine*, vol. 1, pp. 119–120, 2009.

[53] H. Qiu, D. Mao, and B. Huang, "A clinical trial to evaluate the effects of detoxification and dissipation blood stasis granule on the prognosis of patient with chronic severe hepatitis patients," *Chinese Journal of Integrated Traditional and Western Medicine on Liver Diseases*, vol. 17, no. 5, pp. 259–260, 2007.

[54] F. Chen, "Treatment on severe Liver disease intestinal endotoxemia 30 cases with Chinese medicin enema therapy," *Shaanxi Journal of Traditional Chinese Medicine*, vol. 20, no. 4, pp. 157–158, 1999.

[55] H. Chen and X. Wang, "Clinical observation and nursing care of 57 cases early hepatic encephalopathy," *Shaanxi Medical Journal*, vol. 37, no. 9, p. 1268, 2008.

[56] D. Wang, "Clinical analysis of 8 cases cirrhosis concurrent chronic hepatic encephalopathy Degeneration," *Shaanxi Medical Journal*, vol. 26, no. 7, p. 406, 1997.

[57] Y. Li and R. Ma, "Treatment on hepatic encephalopathy(30 cases) and nursing care with rhubarb enema therapy," *Shaanxi Journal of Traditional Chinese Medicine*, vol. 31, no. 2, p. 178, 2010.

[58] W. Lv and X. Li, "Clinical observation of 33 cases hepatic encephalopathy in enema therapy with Tong fu xie re Mixture," *Zhejiang Journal of Traditional Chinese Medicine*, vol. 41, no. 5, p. 277, 2006.

[59] Z. Huang, "Curative effect observation of improving hepatic encephalopathy patients' consciousness by naloxone hydrochloride injection combined with rhubarb enema therapy," *Chinese Journal of Ethnomedicine and Ethnopharmacy*, vol. 5, p. 109, 2009.

[60] Z. Wang, D. Mao, and G. Huang, "Effects of heavy hepatitis concurrent hepatic encephalopathy in rhubarb enema therapy," *Journal of Guangxi TCM University*, vol. 9, no. 4, pp. 7–8, 2006.

[61] B. Jiménez, C. Montoliu, D. A. MacIntyre et al., "Serum metabolic signature of minimal hepatic encephalopathy by 1H-nuclear magnetic resonance," *Journal of Proteome Research*, vol. 9, no. 10, pp. 5180–5187, 2010.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 102641, 8 pages
doi:10.1155/2012/102641

Research Article

The Protective Effect of Yi Shen Juan Bi Pill in Arthritic Rats with Castration-Induced Kidney Deficiency

Hongyan Zhao,[1] Jian Li,[2] Xiaojuan He,[3] Cheng Lu,[3] Cheng Xiao,[4] Xuyan Niu,[3] Ning Zhao,[3] Dahong Ju,[3] and Aiping Lu[3]

[1] *Institute of Basic Theory of Chinese Medicine, China Academy of Chinese Medical Sciences, Beijing 100700, China*
[2] *Preclinical College, Beijing University of Chinese Medicine, Beijing 100029, China*
[3] *Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Science, Beijing 100700, China*
[4] *China-Japan Friendship Hospital, Beijing 100029, China*

Correspondence should be addressed to Aiping Lu, lap64067611@126.com

Received 25 September 2011; Revised 28 November 2011; Accepted 9 December 2011

Academic Editor: Wei Jia

Androgens have been linked to the onset, severity, and progression of rheumatoid arthritis (RA). In traditional Chinese medicine (TCM), the most common pattern in RA is kidney deficiency, which partly corresponds to a low sex hormone state. In this study, TCM kidney deficiency was induced in male Sprague-Dawley rats with castration surgery, and a TCM preparation, Yi Shen Juan Bi Pill (YJB), was used to treat collagen induced arthritis (CIA) rats with castration. Metabolomic technique was used to evaluate the pharmacological mechanism in castrated CIA rats treated by YJB. The results showed that castration significantly increased the severity of the arthritis in rats but was ameliorated by YJB. Its pharmacological mechanism was partially associated with lipid metabolites involving free fatty acid (FFA) and lysophosphatidylcholine (LPC). *In conclusion*, the experimental results demonstrate the protective effect of YJB on the TCM kidney deficiency pattern induced by androgen deficiency in CIA rats and support that YJB should be used for the clinical treatment of RA with TCM kidney deficiency pattern.

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease that primarily presents as chronic symmetric polyarthritis associated with inflammation and cartilage destruction. Epidemiological data suggest that approximately 1% of the world's population is afflicted with RA [1]. It is hypothesized that hormonal factors play a pathogenic role in RA onset [2–4].

In traditional Chinese medicine (TCM), RA is categorized as *Bi Zheng* (Bi syndrome or blockage syndrome). The TCM kidney deficiency pattern is the most common pattern addressed to manage RA; thereby reinforcing the TCM kidney is an important therapeutic target for RA [5, 6]. The Chinese patent drug Yi Shen Juan Bi pill (YJB) was approved (no. Z10890004) and has been marketed in pill form in China since 1987. The formula was prepared by the National TCM master professor Zhu Liangchun, and YJB has been proven as an effective treatment for RA with TCM kidney deficiency pattern [7]. YJB has been shown to ameliorate RA symptoms and to decrease the erythrocyte sedimentation rate (ESR), as well as C-reactive protein (CRP) and rheumatoid factor (RF) levels [8]. Recent studies have elucidated the mechanisms used by YJB; specifically, it significantly decreased prostaglandin E (PGE) and upregulated the pro-apoptotic family member Bax in rat synovium and decreased the production of peritoneal macrophage-derived tumor necrosis factor-alpha, interleukin 1 and nitric oxide [9–11]. Interestingly, a clinical trial suggested that YJB combined with methotrexate was effective in treating elderly onset RA, which was characterized by low plasma testosterone [12]. However, the pharmacological activity and mechanisms of YJB in the treatment of RA with kidney deficiency pattern are not clear.

TCM kidney deficiency was reported to be induced in castrated rats [13]. It has also been hypothesized that low

levels of sex hormones partially correspond to TCM kidney deficiency pattern [14]. Therefore, castrated rats are a suitable animal model for TCM kidney deficiency [13, 15].

The formulation of the YJB consists of complex components; therefore, it is challenging to understand therapeutic mechanisms with conventional methods. Recently metabolomic approaches have been utilized to understand pharmacological mechanisms of related compound Chinese herbs [16, 17]. In the present study, we established collagen-induced arthritis (CIA) in rats to evaluate the effect of YJB treatment on inflammatory responses in normal CIA rats and CIA rats with castration-induced TCM kidney deficiency pattern. We also obtained metabolic profiles of plasma from CIA rats with TCM kidney deficiency with or without YJB treatment to supply further evidence for the clinical application of YJP in the treatment of RA with TCM kidney deficiency.

2. Materials and Methods

2.1. Materials. YJB was obtained from GMP-approved Jiangsu Zhengda Qingjiang Pharmaceutical Co., Ltd. HPLC-grade acetonitrile and formic acid was purchased from Merck (USA). Freund's incomplete adjuvant and bovine type II collagen were purchased from Sigma-Aldrich (MO, USA). IL-6 (BMS625) and IL-10 (BMS629) assay kits were purchased from Bender (USA). Ultrapure water was from a Milli-Q50 SP Reagent Water System (Millipore Corporation, USA).

2.2. Animal Handling Procedure. Male Sprague-Dawley (SD) rats (150 ± 10 g) were purchased from the Institute of Experimental Animals in the Chinese Academy of Medical Science (rodent license no. SYXK 11-00-0039). The rats were housed under standard laboratory conditions, and food and tap water were provided ad libitum. Experimental procedures were reviewed and approved by the Animal Care and Use Committee in the China Academy of Chinese Medical Sciences before the animal experiments were carried out.

Castration was performed according to standard surgical procedures under pentobarbital anesthesia. In brief, a single incision was made in the scrotal skin, and the testicles were squeezed out with gentle pressure. The spermatic cord was ligated with chromic catgut, and scrotal incisions were treated as open wounds.

Arthritis was induced as previously described, 4 weeks after castration [18, 19]. Briefly, rats were intradermally injected at the base of tail with 100 μg of bovine type II collagen in 0.05 M acetic acid emulsified with an equal amount of incomplete Freund's adjuvant. The rats were given a booster with the same preparation 7 days after the primary immunization.

2.3. Experimental Groups and Drug Treatment. The experimental groups ($n = 10$) were as follows: (1) normal control (NC), (2) collagen-induced arthritis (CIA), (3) castration-induced kidney deficiency arthritis, and (4) castrated CIA rats with YJB treatment. On day 15 after the primary immunization, one group began receiving daily YJB (2.4 g/kg body weight) by intragastric administration for 14 days. The dose was based on the clinical application dosage of 24 g per day per 60 kg body weight. Other groups were treated with an equal volume of distilled water as a vehicle control.

2.4. Arthritis Assessment. CIA rats were assessed for disease severity every 2 days after the booster immunization. Arthritis severity was expressed as mean arthritic index on a 0 to 4 scale according to the conventional method [20]. In addition, joint tissue histopathology was assessed with H & E staining. Inflammation, pannus, cartilage damage, and bone damage were scored on scales from 0 to 3 (0: absent; 1: weak; 2: moderate; 3: severe) [21].

2.5. Measurement of IL-6 and IL-10. Serum levels of IL-6 and IL-10 were measured with ELISA according to the manufacturer's instructions. Briefly, blood serum was harvested after the rats were sacrificed and diluted 1 : 10. The absorbance was read at 450 nm using a microplate reader. Samples and standards were analyzed in triplicate.

2.6. Anti-Col II Antibody Measurement. The serum level of anti-Col II antibody was measured by ELISA. Briefly, collagen was dissolved to a final concentration of 10 μg/mL in acetic acid (0.1 moL/L), and the resulting solution was applied to 96-well flat-bottomed microtiter plates at 4°C overnight. Next, the wells were incubated with 0.5% ovalbumin at room temperature for 1 h to reduce nonspecific binding. After washing with phosphate-buffered saline containing Tween, diluted test serum and standards were added to the Col II-coated wells. Next, the biotin-conjugated goat affinity-purified antibody to rat IgG and sequentially streptavidin-HRP were added. The reaction was terminated by the addition of stop buffer, and absorbance was measured at 450 nm.

2.7. HPLC-Q-TOF-MS Conditions. HPLC-Q-TOF-MS analysis was performed on a Water-Q-TOF Micro MS system coupled with an electrospray ionization (ESI) source (Water Technologies, UK). Samples were separated on an Eclipse plus C18 column with the column temperature set at 35°C. Data were collected in full scan mode from 100 to 1000 m/z from 0 to 30 min. The standard sample ran six times continuously to confirm the stability of the method. The mass detection was operated in both positive and negative ion modes (flow rate: 8 L/min, gas temperature: 250°C, pressure of nebulizer gas: 35 psig, Vcap: 3 kV, fragmentor: 160 V, skimmer: 65 V). Target MS analysis was used to identify potential biomarkers.

2.8. Metabolomic Data Analysis. The raw data were analyzed with MarkerLynx software (Waters, UK) for peak deconvolution and alignment. The parameters were as follows: mass tolerance was set at 0.05 Da, peak width was set at ≥1.5%, baseline noise elimination was set at level 4, and the mass window was set at 0.1 min. The data were combined into a single matrix by aligning peaks with the same mass/retention time (0.3–12 min) from each data file in the

dataset, along with their associated normalized intensities. Principal component analysis (PCA) was performed with SIMCA-P software (Version 12.0, UMETRICS AB, Box 7960, SE 90719, Umea, Sweden) to visualize general clustering for further identification of differentially expressed metabolites that might account for the separation between YJB-treated rats and other groups.

2.9. Statistical Analysis. All of the quantitative data analyses were performed using SPSS 11.5 software package for Windows. Significance was determined with one-way analyses of variance (ANOVAs) followed by Student's t-tests. Results were expressed mean ± SD. P values less than 0.05 were considered significant.

3. Results

3.1. Change on Sex Hormone and Arthritic Evaluation. Castration-induced TCM kidney deficiency can significantly reduce dihydrotestosterone, testosterone, and estradiol serum levels in rats. No significant differences in these hormone levels were detected in the YJB-treated group compared to castrated arthritic control group (Supplementary Data S1 available online at doi:10.1155/2012/102641). Arthritis was reproducibly induced in both normal rats and castration-induced kidney deficiency rats that were given collagen combined with ICFA (data not show). The results showed significantly increased paw edema in castrated arthritic rats, which was suppressed by YJB administration (Figure 1). Histological evaluation of joint tissue demonstrated that, compared with CIA rats, castrated CIA rats showed extensive cartilage erosion, fibroplasia, and synovial membrane thickening (Figure 2(c)). Clinical evaluations and histological studies demonstrated that the severity score was higher in castrated CIA rats compared to normal CIA rats ($P < 0.01$, Figure 2(e)); YJB treatment reduced degradation and resulted in a significantly lower severity score compared to castrated CIA rats ($P < 0.01$, Figures 2(d) and 2(e)).

3.2. Serum Levels of Anti-Collagen Type II, IL-6, and IL-10. Castrated CIA rats developed a significantly higher immune response in terms of antibody generation against type II collagen compared to the CIA control ($P < 0.01$). The antibody titers of castrated CIA rats were significantly attenuated by YJB administration ($P < 0.05$, Figure 3). Arthritis induction caused increased serum levels of IL-6. While castrated CIA rats exhibited an on-going IL-6 increase ($P < 0.05$), this increase was significantly suppressed by YJB treatment ($P < 0.05$, Figure 4). However, the opposite was true for IL-10. Castrated CIA rats treated with YJB had significantly higher levels of IL-10 level compared to castrated CIA rats ($P < 0.01$, Figure 5).

3.3. Metabolite Identification. In order to understand the role of the castration-induced kidney deficiency in arthritis onset, we performed serum metabolic profiling of normal control rats, CIA control rats, and castrated arthritic rats. Figure 6(a) shows a clear separation trend of metabolites between normal rats, CIA rats, and castrated CIA rats using unsupervised analysis of PCA. This result suggests that castration-induced kidney deficiency perturbed the metabolic profile of castrated arthritic rats.

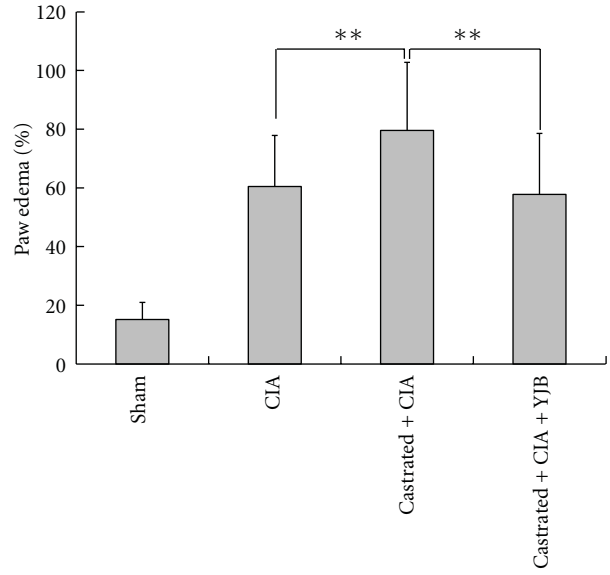


Figure 1: Paw edema by treatment ($n = 10$/group). YJB treatment suppresses paw swelling in castrated arthritic rats. Data are expressed as means ± SD (**$P < 0.01$).

To ascertain the effect of YJB on serum metabolite perturbation in castrated arthritic rats, we examined the metabolic profiles of serum in normal rats, castrated CIA rats, and castrated CIA rats treated with YJB. Figure 6(b) shows a clear separation of scores between normal control rats and castrated arthritic rats, and a clear separating trend between castrated CIA rats and castrated CIA rats treated with YJB. This finding suggests that YJB could ameliorate the pathological state induced by arthritis and castration. These results support the hypothesis that YJB has a therapeutic effect on arthritic rats with castration-induced TCM kidney deficiency pattern.

Over 300 peaks were obtained using LC-TOF-MS analytical protocols coupled with a software-based peak deconvolution procedure. Student's t-tests were performed on all metabolites. The variables selected were those with statistically significant differences ($P < 0.05$) between normal control rats, castrated CIA rats, and YJB-treated rats. A total of 20 individual metabolites were significantly different. Compound identification was performed with commercially available authentication standards. Among these perturbed variables, 14 (7 upregulated and 7 downregulated) were predicted by comparing the accurate MS and MS-MS fragments with metabolites found in databases (http://metlin.scripps.edu/; http://www.hmdb.ca/) that were later confirmed with commercial standards. Most of the metabolites were lipids, such as LPC and FFA (Supplementary Data S2). The statistical results demonstrated that YJB may downregulate LPC (Figure 7(a)) and upregulate FFA in the serum of castrated CIA rats (Figure 7(b)).

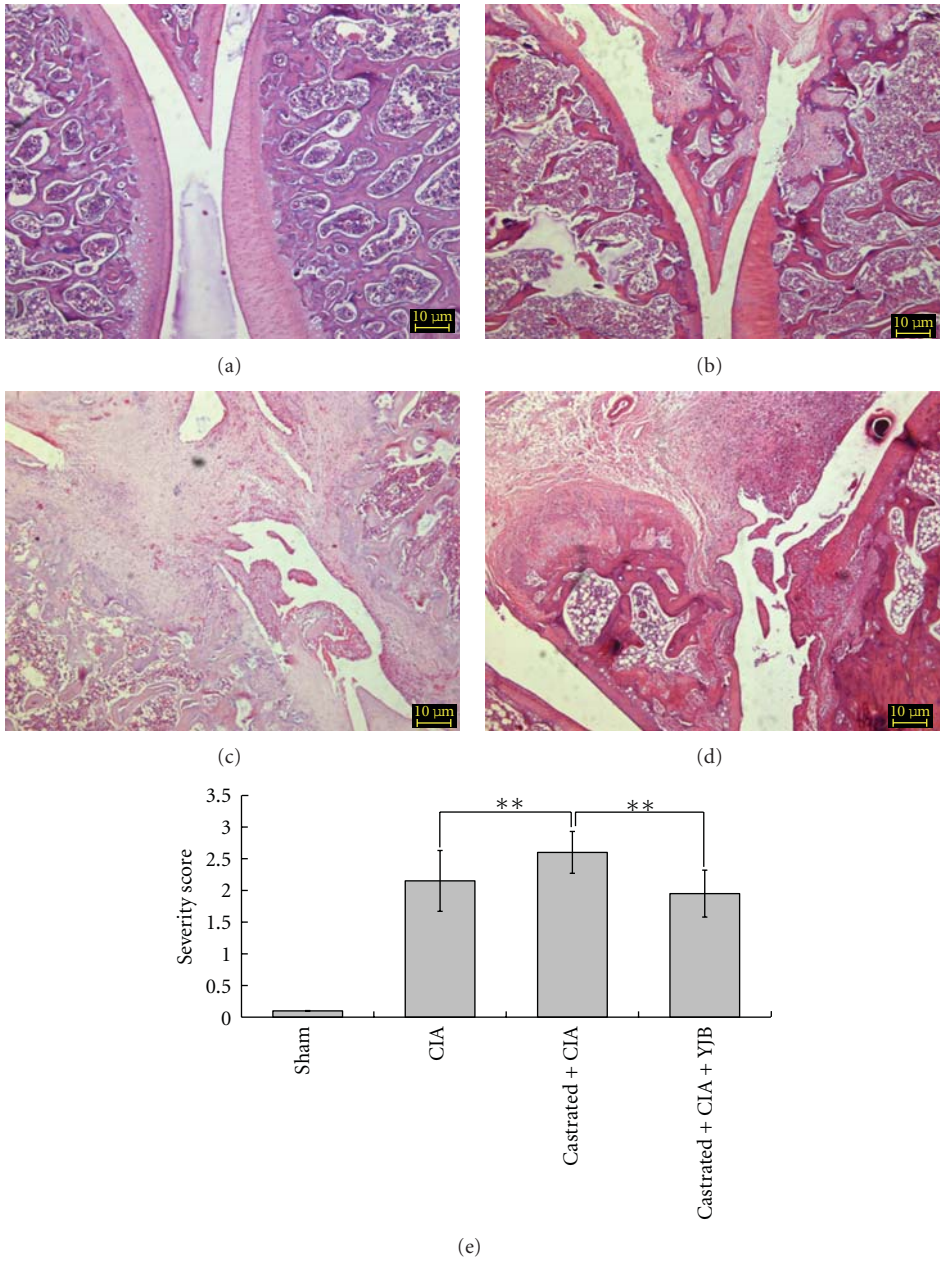


Figure 2: Histological evaluation of the joint tissue. Paraffin sections of knee joints were stained with H & E. Scale = 100 μm. (a) Sham group treated with distilled water; (b) CIA rats treated with distilled water; (c) castrated arthritic rats treated with distilled water; (d) castrated arthritic rats treated with YJB; (e) pathological severity score. Pathological changes were scored on a 1 to 3 scale. Data are expressed as means ± SD ($^{**}P < 0.01$).

4. Discussion

A major finding of this study is that CIA rats with castration-induced TCM kidney deficiency can develop severe arthritis, and YJB could have a therapeutic effect in castrated CIA rats. This result supports the clinical application of YJB in the treatment of RA patients with TCM kidney deficiency pattern.

It is well known that sex hormones, which are important factors in TCM kidney deficiency [5–7], might have a pathogenic role in RA onset. It has been reported that RA occurs 3-4 times more frequently in women than in men [2]. Furthermore, men with RA have lower serum testosterone levels than healthy men [3], and male gender has been found to be a major predictor of remission in early RA [4]. In our results, castrated male rats developed severe arthritis after collagen immunization.

YJB is a TCM compound that is hypothesized to reinforce kidney function. YJB consists of 20 medicinal materials, including *Fructus Xanthii, Herba Cistanchis, Radix*

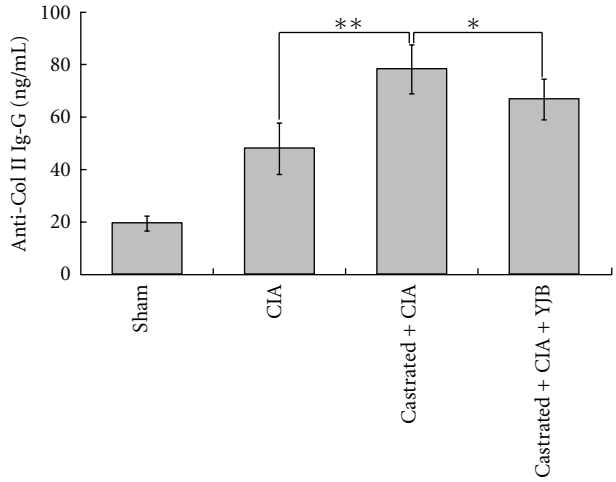


Figure 3: Changes of anti-Col II IgG in serum ($n = 10$). The level of anti-Col II was measured by ELISA. Data are expressed as means ± SD ($^{**}P < 0.01$, $^{*}P < 0.05$).

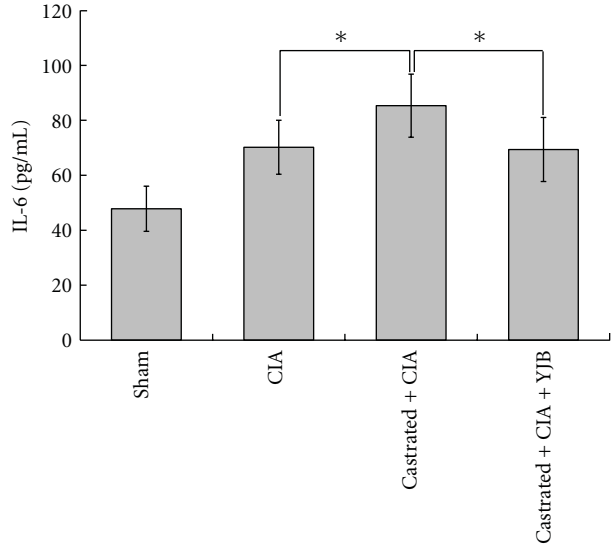


Figure 4: Changes of IL-6 in serum ($n = 10$). The level of IL-6 was measured by ELISA. Data are expressed as means ± SD ($^{*}P < 0.05$).

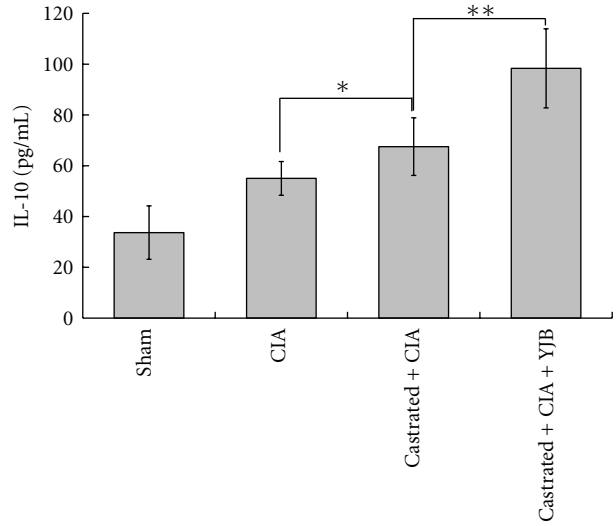


Figure 5: Changes of IL-10 in serum ($n = 10$). The level of IL-10 was measured by ELISA. Data are expressed as means ± SD ($^{**}P < 0.01$, $^{*}P < 0.05$).

angelica, Radix Rehmanniae, Pheretima, Radix Glycyrrhizae, Rhizoma Drynariae, Polygoni Cuspidati, Caulis Spatholobi, Bombyx Batryticatus, Herba Erodii, Herba Pyrolae, Allomyrina dichotoma, Scorpio, Radix Rehmanniae Preparata, Eupolyphaga Seu Steleophaga, Scolopendra, Zaocys (stir-fried with wine), Cynanchi Paniculati, Herba Aristolochiae, Rhizoma Corydalis, Herba Epimedii, Nidus Vespae (stir-baking), and Nidus Vespae. One study reported that YJB effectively treated arthritis in rats [11], and our results showing that YJB affects the balance of proinflammatory cytokines IL-6/IL-10 support its anti-CIA activity, further. IL-10 and IL-6 are important in the development of RA [22]. The exogenous addition of IL-10 in vivo has been shown to affect the immunopathological processes involved in RA, although the outcome of clinical studies using IL-10 was disappointing [23]. IL-6 is closely associated with the pathological process of RA. The evidence suggests that, in contrast to IL-6, IL-10 plays an active role in ameliorating arthritis caused by degeneration. In our study, YJB treatment increased IL-10 and decreased IL-6 in the serum of arthritic rats with castration-induced TCM kidney deficiency.

Another major finding in this study was the identification of 2 kinds of metabolites, including LPC and FFA, which are directly relevant to lipid metabolism. According to previously published data and biochemical databases (e.g., KEGG and METLIN), we demonstrated that FFA and LPC are critical intermediates of fatty acid metabolism [24]. As shown in Figure 7, decreased LPC levels were observed in the serum of YJB-treated rats. Researchers have demonstrated that LPC is involved in inflammatory disease pathogenesis, and LPC levels could increase in response to reactive oxygen species (ROS) and inflammatory conditions, such as RA, lung infection, diabetes, and liver injury [25–27]. The decreased level of LPC in response to YJB treatment could disturb choline and cholesterol metabolism, which might be the pharmacological mechanism of the compound. Along with decreased LPC, the increased level of FFA suggests increased acetyl-CoA, an important substrate in the TCA cycle, which is critical for energy production. The metabolic profile implies that YJB may influence lipid metabolism regulation in CIA rats with castration-induced TCM kidney deficiency.

Androgens are involved in the pathogenesis of RA to a surprising degree. Their modulation of the activity of cells involved in the immune inflammatory response is dependent on the androgen/estrogen ratio and concentration [28]. Clinical studies have revealed the effects of androgens on the treatment of autoimmune and chronic inflammatory diseases, such as RA, SLE, and tumors [29]. Unfortunately, conclusions from studies of sex hormone therapy were often paradoxical [30, 31]. The biological significance of

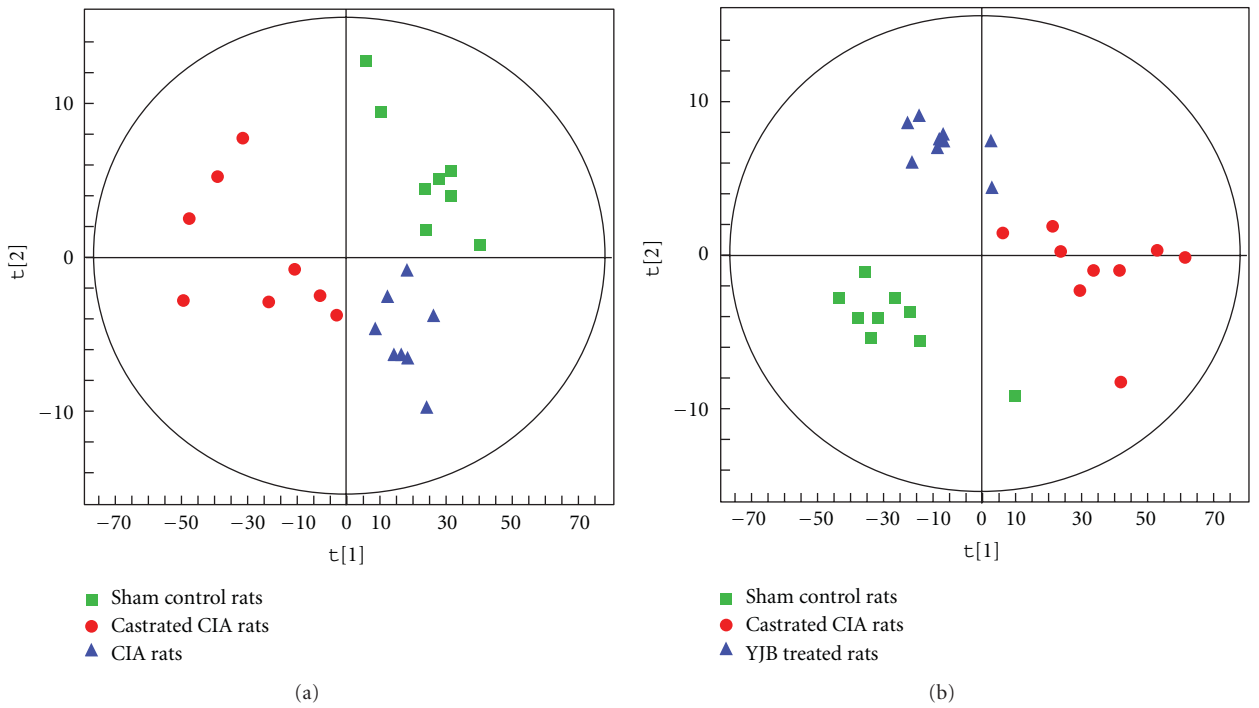


Figure 6: (a) Principal component analysis (PCA) scores (component 1 versus component 2) of serum metabolites derived from sham rats ( ), CIA rats ( ), and castrated CIA rats (•). The 3 groups were clearly separated. (b) Principal component analysis (PCA) scores (component 1 versus component 2) of serum metabolites derived from sham rats ( ), castrated CIA rats (•), and castrated CIA rats treated with YJB ( ). A clear separation of the score spot was observed in the three groups.

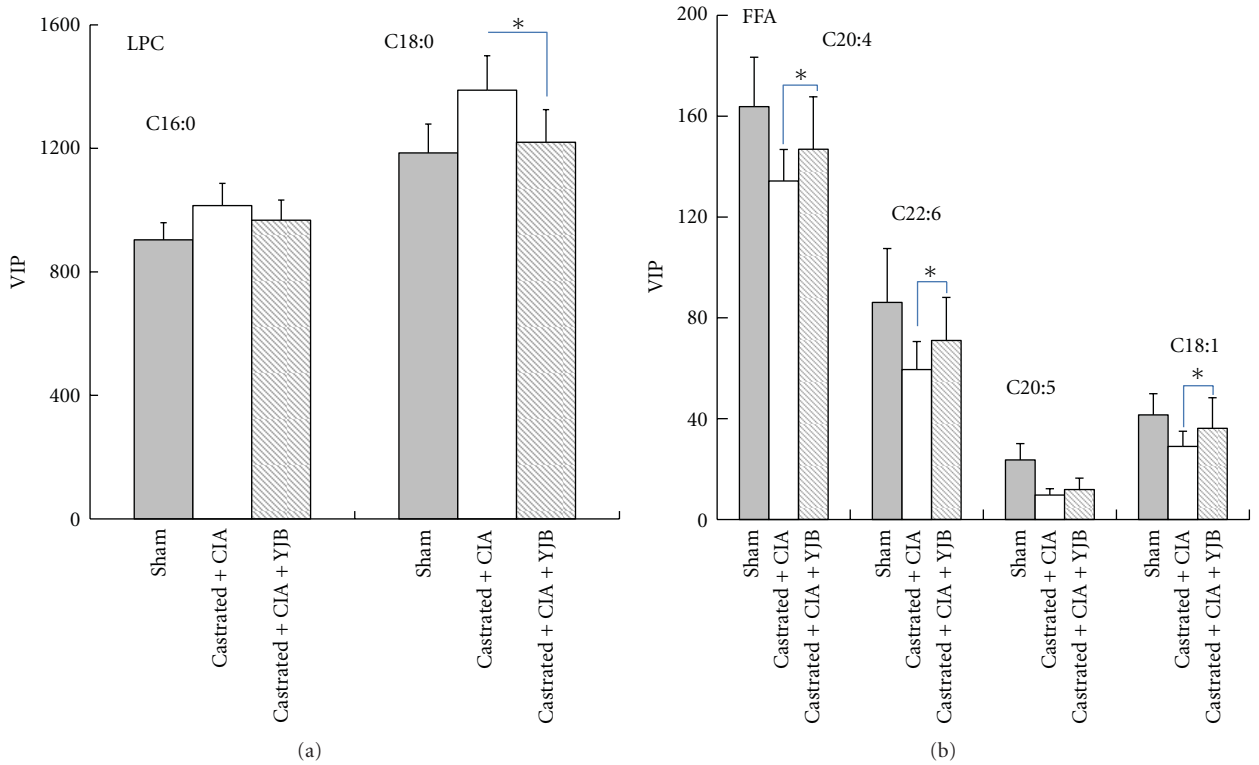


Figure 7: The value of variable important parameters (VIPs) for (a) LPC and (b) FFA by groups $^*P < 0.05$ versus the castrated CIA group (one-way ANOVA, followed by Student's t-test).

the association between androgens and RA remains unclear. In this study, we limit the conclusion to the effect of YJB, which protected against castration-induced androgen deficiency in arthritic male rats by downregulating IL-6 (a proinflammatory cytokine), upregulating IL-10 (an anti-inflammatory cytokine), and regulating lipid metabolism. Honestly, the major limitation of this study is that the metabolomic data were not fully collected and analyzed. Because lipid metabolism is a likely mechanism of YJB in the treatment of CIA rats, a more thorough metabolomic study is necessary.

5. Conclusion

In conclusion, castration-induced TCM kidney deficiency significantly increased the severity of arthritis in rats. YJB had protective effects on CIA rats with castration-induced TCM kidney deficiency, and its pharmacological mechanism likely involved lipid metabolites, including FFA and LPC. These results suggest that YJP should be used for the treatment of RA with TCM kidney deficiency pattern.

Acknowledgments

This study is supported in part by the projects from Ministry of Sciences and Technology of China (No. 2009ZX09502-019, 2006BAI08B01-01), National Science Foundation of China (No. 30825047, 30902000, 30901941, 30701067 and 81001676).

References

[1] S. E. Sweeney and G. S. Firestein, "Rheumatoid arthritis: regulation of synovial inflammation," *International Journal of Biochemistry and Cell Biology*, vol. 36, no. 3, pp. 372–378, 2004.

[2] D. P. M. Symmons, E. M. Barrett, C. R. Bankhead, D. G. I. Scott, and A. J. Silman, "The incidence of rheumatoid arthritis in the United Kingdom: results from the Norfolk Arthritis Register," *British Journal of Rheumatology*, vol. 33, no. 8, pp. 735–739, 1994.

[3] T. D. Spector, L. A. Perry, G. Tubb, A. J. Silman, and E. C. Huskisson, "Low free testosterone levels in rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 47, no. 1, pp. 65–68, 1988.

[4] K. Forslind, I. Hafström, M. Ahlmén, and B. Svensson, "Sex: a major predictor of remission in early rheumatoid arthritis?" *Annals of the Rheumatic Diseases*, vol. 66, no. 1, pp. 46–52, 2007.

[5] Y. Wang, H. Y. Zhao, D. H. Ju et al., "Effect of kidney deficiency on the occurrence and development of Bi syndrome (rheumatoid arthritis)," *Chinese Journal of Basic Medicine in Traditional Chinese Medicine*, vol. 15, no. 12, pp. 892–895, 2009 (Chinese).

[6] L. C. Zhu, "Discussion on the medicine experience from three main symptoms of Bi syndrome," *Beijing Journal of Traditional Chinese Medicine*, vol. 5, pp. 5–6, 1992 (Chinese).

[7] D. H. Zhou, Z. Q. Zhou, and W. H. Zhu, "Zhu Liangchun's experience prescription in 38 cases treatment of rheumatoid arthritis," *Journal of New Chinese Medicine*, vol. 39, no. 9, pp. 71–72, 2007 (Chinese).

[8] S. Li, X. Hu, and Z. Jing, "Therapeutic effect of Yi Shen Juan Bi pill on rheumatoid arthritis," *Zhejiang Clinical Medicine Journal*, vol. 10, pp. 628–629, 2008 (Chinese).

[9] P. K. Perera, Y. Li, C. Peng, W. Fang, and C. Han, "Immunomodulatory activity of a Chinese herbal drug Yi Shen Juan Bi in adjuvant arthritis," *Indian Journal of Pharmacology*, vol. 42, no. 2, pp. 65–69, 2010.

[10] P. K. Perera, C. Peng, X. Lv, Y. M. Li, W. R. Fang, and C. F. Han, "Effects of Yishen Juanbi (YJB) pill on experimental rheumatoid arthritis," *Chinese Journal of Natural Medicines*, vol. 8, no. 1, pp. 57–61, 2010.

[11] P. K. Perera, C. Peng, L. Xue, Y. Li, and C. Han, "Ex vivo and in vivo effect of Chinese herbal pill Yi Shen Juan Bi (YJB) on experimental arthritis," *Journal of Ethnopharmacology*, vol. 134, no. 1, pp. 171–175, 2011.

[12] T. Zhou, "Study of Yishen Juanbi Pill combined with methotrexate on elderly onset rheumatoid arthritis," *Journal of Clinical Rational Drug Use*, vol. 2, no. 8, pp. 13–15, 2009 (Chinese).

[13] J. L. Ai, A. P. Lu, S. J. Xu et al., "Experimental study on mechanism of gonadal dysfunction of Bi syndrome wth kidney deficiency," *Chinese Journal of Basic Medicine in Traditional Chinese Medicine*, vol. 1, no. 1, pp. 42–44, 1995 (Chinese).

[14] A. K. Kuang, J. L. Chen, Y. R. Lu et al., "Changes of sex hormone in female type II diabetics, coronary heart disease, essential hypertension and its relations with kidney deficiency," *Journal of Integrated Traditional and Western Medicine*, vol. 9, no. 6, pp. 331–334, 1989 (Chinese).

[15] S. Xu, A. P. Lu, A. M. Wang et al., "Effect of kidney deficiency caused by ovariectomy on serum osteocalcium level and tumor necrosis factor in mice with collagen induced arthritis," *Chinese Journal of Integrated Traditional and Western Medicine*, vol. 19, no. 1, pp. 34–36, 1999 (Chinese).

[16] J. K. Nicholson, "Global systems biology, personalized medicine and molecular epidemiology," *Molecular Systems Biology*, vol. 2, article 52, 2006.

[17] J. Li, Y. Lu, C. Xiao et al., "Comparison of toxic reaction of Tripterygium wilfordii multiglycoside in normal and adjuvant arthritic rats," *Journal of Ethnopharmacology*, vol. 135, no. 2, pp. 270–277, 2011.

[18] M. Hegen, J. C. Keith, M. Collins, and C. L. Nickerson-Nutter, "Utility of animal models for identification of potential therapeutics for rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 67, no. 11, pp. 1505–1515, 2008.

[19] Y. G. Cho, M. L. Cho, S. Y. Min, and H. Y. Kim, "Type II collagen autoimmunity in a mouse model of human rheumatoid arthritis," *Autoimmunity Reviews*, vol. 7, no. 1, pp. 65–70, 2007.

[20] P. Larsson, S. Kleinau, R. Holmdahl, and L. Klareskog, "Homologous type II collagen-induced arthritis in rats. Characterization of the disease and demonstration of clinically distinct forms of arthritis in two strains of rats after immunization with the same collagen preparation," *Arthritis and Rheumatism*, vol. 33, no. 5, pp. 693–701, 1990.

[21] K. Imada, N. Lin, C. Liu et al., "Nobiletin, a citrus polymethoxy flavonoid, suppresses gene expression and production of aggrecanases-1 and -2 in collagen-induced arthritic mice," *Biochemical and Biophysical Research Communications*, vol. 373, no. 2, pp. 181–185, 2008.

[22] M. Feldmann, F. M. Brennan, and R. N. Maini, "Role of cytokines in rheumatoid arthritis," *Annual Review of Immunology*, vol. 14, pp. 397–440, 1996.

[23] J. A. G. Van Roon, S. Wijngaarden, F. P. J. G. Lafeber, C. Damen, J. G. J. Van De Winkel, and J. W. J. Bijlsma, "Interleukin 10 treatment of patients with rheumatoid arthritis enhances Fcγ receptor expression on monocytes and responsiveness to immune complex stimulation," *Journal of Rheumatology*, vol. 30, no. 4, pp. 648–651, 2003.

[24] R. Stroh, T. A. Christopher, B. L. Lopez, Y. P. Guo, M. Amico-Roxas, and X. L. Ma, "L-propionyl carnitine, an endogenous ester in fatty acid metabolism, exerts anti-shock and endothelial protective effects in rat splanchnic ischemia-reperfusion injury," *Shock*, vol. 9, no. 3, pp. 216–222, 1998.

[25] T. Matsumoto, T. Kobayashi, and K. Kamata, "Role of lysophosphatidylcholine (LPC) in atherosclerosis," *Current Medicinal Chemistry*, vol. 14, no. 30, pp. 3209–3220, 2007.

[26] C. Schober, J. Schiller, F. Pinker, J. G. Hengstler, and B. Fuchs, "Lysophosphatidylethanolamine is—in contrast to—choline 10.1016/j.bioorg.2009.09.002generated under in vivo conditions exclusively by phospholipase A_2 but not by hypochlorous acid," *Bioorganic Chemistry*, vol. 37, no. 6, pp. 202–210, 2009.

[27] B. Fuchs, J. Schiller, U. Wagner, H. Häntzschel, and K. Arnold, "The phosphatidylcholine/lysophosphatidylcholine ratio in human plasma is an indicator of the severity of rheumatoid arthritis: investigations by ^{31}P NMR and MALDI-TOF MS," *Clinical Biochemistry*, vol. 38, no. 10, pp. 925–933, 2005.

[28] C. C. G. Chen and C. R. Parker, "Adrenal androgens and the immune system," *Seminars in Reproductive Medicine*, vol. 22, no. 4, pp. 369–377, 2004.

[29] M. Cutolo, B. Seriolo, B. Villaggio, C. Pizzorni, C. Craviotto, and A. Sulli, "Androgens and estrogens modulate the immune and inflammatory responses in rheumatoid arthritis," *Annals of the New York Academy of Sciences*, vol. 966, pp. 131–142, 2002.

[30] E. W. Karlson, L. B. Chibnik, M. McGrath et al., "A prospective study of androgen levels, hormone-related genes and risk of rheumatoid arthritis," *Arthritis Research and Therapy*, vol. 11, no. 3, article R97, 2009.

[31] M. Cutolo, "Androgens in rheumatoid arthritis: when are they effectors?" *Arthritis Research & Therapy*, vol. 11, no. 5, p. 126, 2009.