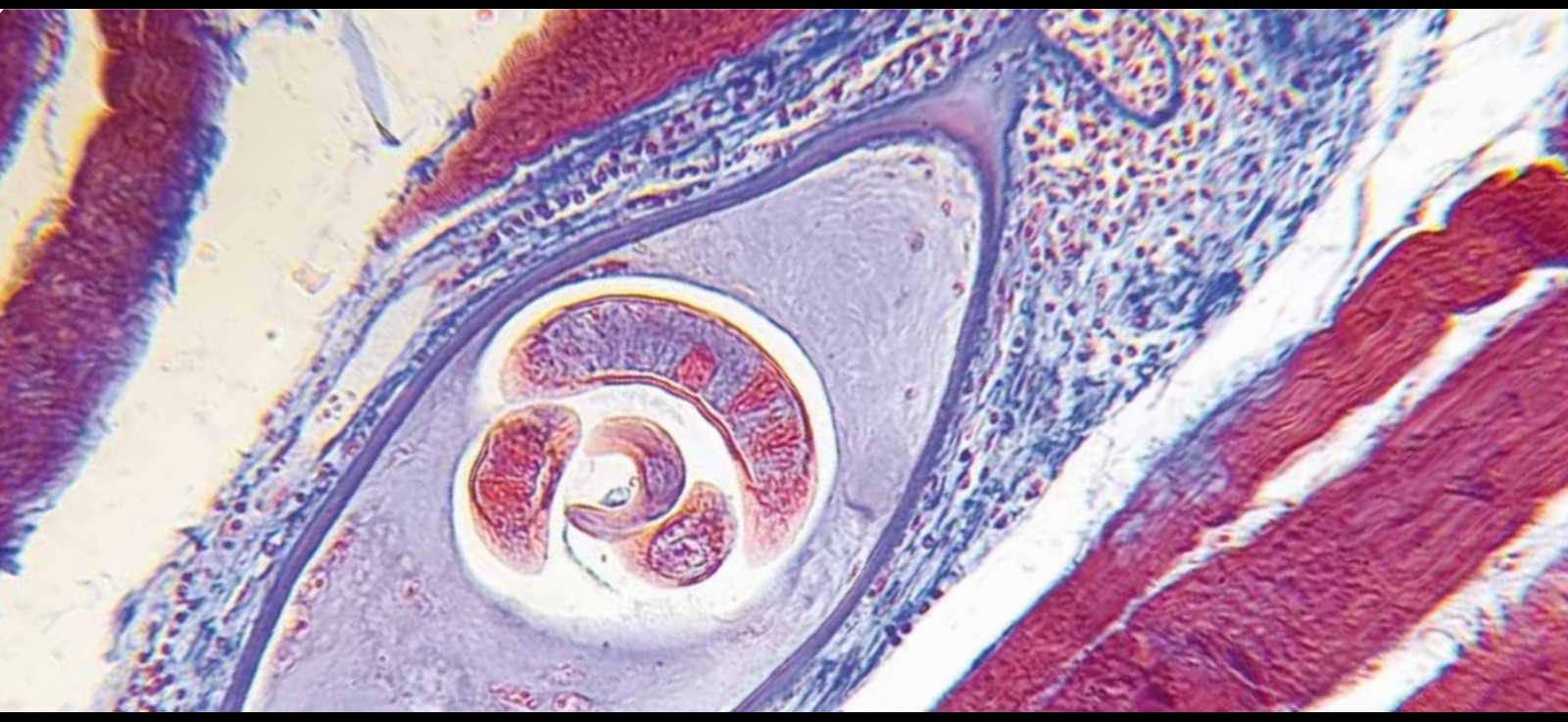
Gastroenterology Research and Practice

Gastroesophageal Reflux Disease

Guest Editors: Gaurav V. Kulkarni, Fernando A. M. Herbella, and P. Marco Fisichella



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Editorial Board

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Editorial

Gastroesophageal Reflux Disease

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In United States, gastroesophageal reflux disease (GERD) affects almost 20% of the population, and its incidence seems to be rising in relation to the widespread epidemic of obesity. The incidence of GERD, however, is different in other areas of the globe, and there have been multiple recent studies from other developed countries suggesting a correlation between the western lifestyle and emergence of this disease. Moreover, in recent years, diagnostic modalities of more complex manifestations of GERD, such as extraesophageal reflux, have been subjected to refinements. This paper discusses recent research regarding the changing spectrum of GERD in areas of the European Union and Asia subjected to urbanization in recent times and discusses recent and clinically important developments in the diagnostic modalities of extraesophageal reflux.

First Dr. L. Çela et al. from Albania present how westernization is taking its toll with respect to lifestyle changes occurring in regions of Albania where the traditional practices are being replaced by the urban lifestyle and the hazards that come in its wake. Traditionally, Albania has been mentioned as one of the countries, which resisted and refrained from adopting an unhealthy lifestyle due to various regional and economic factors. However, this recent study challenges this notion, as it found that the overall prevalence of GERD was 11.9%. The authors also found no significant sex differences but a higher prevalence of GERD among the older participants, as well as a positive relationship with smoking, physical inactivity, fried food consumption, and obesity.

A paper by Dr. P. Wu et al. from China similarly showed that foods implicated in the increased risk of GERD are all a major part of the "fast-food" diet of the modern civilization and seem to be making their way into the diet of this ethnic population and causing similar problems as they have in the western world. Specifically, the authors have shown that high intake of meat, oils, salt, and calcium is associated with an increased risk for Reflux Esophagitis (RE) while high intake of protein, carbohydrate, calories from protein, vitamin C, grains and potatoes, fruits, and eggs correlates with a reduced risk for RE. Though this study had some language limitations with the ethnic population, the authors have attempted to standardize the responses of participants for broader comparisons.

Dr. Y. Chen et al. from Tongji University in Shanghai describe the dual use of SF-36 questionnaire and the rabeprazole test for diagnosis of GERD. A rise to 65 points on the questionnaire in a week from starting 20 mg of rabeprazole per day in the affected and the control groups seemed to be a very reliable, sensitive, and cost efficient method for diagnosing GERD. Independent use of the two methods was associated with a low diagnostic value.

Dr. K. Zelenik et al. from the Czech Republic have evaluated extraesophageal reflux (EER) using reflux area index, number of reflux, and acid exposure times to assess for response to proton pump inhibitors (PPIs). They found a direct positive correlation between the response and higher incidence of the reflux parameters, which was more

pronounced when acid exposure times or reflux area indices were used for diagnosing EER rather than the number of reflux episodes. This study is clinically important since it indicates the relief response that PPIs provide in management of EER that has been proven by standardized testing.

Dr. Ö. I. Emilsson et al. address the issue of different biomarkers for GERD in respiratory diseases by comparing the efficacy of available tests assessing biochemical profiles of patients. It appears that lipid laden macrophage indices and quantification of pepsin do not appear to be sensitive for drawing conclusions regarding the occurrence or severity of GERD due to confounding factors present in pulmonary milieu. Moreover, the success of using bronchoalveolar lavage samples in transplant patients for assessing reflux-induced inflammation cannot be reproduced in other healthier patient populations in view of the invasiveness of the procedure. Recognizing a set of biomarkers rather than any one specific element from the samples obtained by exhaled breath condensates and studying particles in exhaled air have shown promise in initial studies and hold the key to further research.

Finally, Dr. R. Illig et al. from Austria have proposed that the probability to find one single specific biomarker providing all diagnostic, predictive, and prognostic significance in GERD, Barrett's esophagus, and/or esophageal adenocarcinoma is rather utopian. A panel of more sensitive and specific biomarkers is upcoming and is based on developments in technologies such as RNA and DNA microarrays, methylation profiling, epigenetics, and proteomics in association with bioinformatics. These technologies are promising in providing future insights in the complex GERD-Barrett's esophagus-adenocarcinoma sequence.

In summary, the collection of papers presented in this special issue aims to provide an ample overview of the recent epidemiologic research regarding the geographically changing spectrum of GERD and to illustrate developments in the diagnostic modalities of extraesophageal reflux, with the goal to present the reader with an updated overview of important research in the field of GERD.

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Research Article

Dietary Intake and Risk for Reflux Esophagitis: A Case-Control Study

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Background. Specific dietary components have been associated with gastroesophageal reflux disease (GERD) in Europe and the United States. However, the relationship between dietary components and GERD in Chinese still remains unclear. *Methods*. A total of 268 patients who were newly diagnosed as reflux esophagitis (RE) in Outpatient Endoscopy Center of Tongji Hospital were recruited. In addition, 269 sex- and age-matched subjects were also recruited as controls. The body measurements were determined, and the dietary intake during the previous year was evaluated using food frequency questionnaire (FFQ). Stepwise multiple logistic regression analysis was performed to examine the association between nutrients and RE. *Results*. After adjustment for WC, WHR, total energy intake, and demographics, there were a positive dose-response relationship between RE and calcium, meat, oils, and salt and a negative dose-response relationship between RE and protein, carbohydrate, calories from protein (%), vitamin C, grains and potatoes, fruits, and eggs. *Conclusion*. High intake of meat, oils, salt, and calcium is associated with an increased risk for RE while high intake of protein, carbohydrate, calories from protein (%), vitamin C, grains and potatoes, fruits, and eggs correlates with a reduced risk for RE.

1. Background

Gastroesophageal reflux disease (GERD) is a chronic disease usually caused by the reflux of acidic gastric and duodenal contents into the distal esophagus. The major symptoms of GERD include heartburn, acid regurgitation, and non-cardiac chest pain. GERD is a common digestive disease with the direct medical costs estimated around $9.3 billion annually [1], and with the symptoms portending a low quality of life [2]. Reflux esophagitis (RE) is one of the most common phenotypes of GERD [3]. In Western countries, GERD has a high prevalence. Especially in USA, about 44% of Americans suffer from GERD symptoms at least once monthly, 17% once weekly, and 7% once daily [4, 5]. Traditionally, GERD is less common in Asians [6]. However, it is reported that the prevalence of GERD in Asians is increasing [7]. The overall prevalence of RE in adult Japanese population is about 16% [8]. In Taiwan, the prevalence of RE is about 15% in patients evaluated for upper gastrointestinal tract symptoms [9] and about 10.5% in Korea healthy subjects [7]. In Chinese, few epidemiological data on GERD are available currently. In 1999, a Chinese study reported that the prevalence of GERD was 5.77% in Beijing and Shanghai, two biggest cities in China [10]. Although GERD is thought to be less prevalent in China than in Western countries and other Asian countries, recent studies reveal the incidence of GERD is on a rise in China [11].

Most of the factors involved in the pathogenesis of gastroesophageal reflux disease (GERD), previously described in European, Australian, and American studies, are present in Chinese patients with GERD, but at a lower scale. A low-fat diet probably contributes to a more favorable gastric distribution [12]. Another study reported GERD is highly prevalent in adult in Urumqi, especially in Uygur. Male, civil

servant, smoking, strong tea, alcohol drinking, meat diet, and BMI are risk factors correlated to GERD [13]. In Europe and the United States, some investigators have shown that dietary fat, cholesterol, saturated fatty acid (SFA), dietary fiber, and other nutrients are associated with GERD. However, this association is absent in other studies. An epidemiologic survey showed that there was a link between high fat intake and GERD [14], and in clinical studies, esophageal pH provided direct evidence on the association between dietary fat and acid reflux. In contrast, a number of studies reported that a high-fat diet had no influence on the transient lower esophageal sphincter relaxation (TLESR) or esophageal acid exposure [15, 16]. Moreover, dietary fiber, especially cereal fiber, has been found to decrease the risk for esophageal and gastric adenocarcinoma [17], for which GERD is well-known risk factor. The mechanism may be that dietary fiber decreases the intake of gastric nitrites, which have been implicated in promoting reflux by relaxing the low esophageal sphincter (LES) [18]. Similar effects were also seen in a recent study by El-Serag et al. [14]. In this cross-sectional study, EL-serag and his colleagues postulated that high-fiber diet played a protective role. However, Bouin et al. [19] suggested that dietary fiber decreased the number of gastrooesophageal reflux, but increased their duration and had no significant effect on gastric emptying and gastric acid secretion. Another independent risk factor for GERD-related symptoms is alcohol [20], but some studies fail to identify such relationship [21, 22].

In general, the effects of diet on GERD are not well understood, and the currently available data in Western countries do not support a strong relationship between GERD and dietary fat, fiber, alcohol, and other nutrients. Although there are conflicting data regarding the role of dietary nutrients in GERD, there is no direct evidence that some nutrients promote or protect against GERD. Due to the difference in dietary nutrients between Chinese and Westerns, and few studies reporting the association between dietary nutrients and GERD in China, we employed food frequency questionnaire (FFQ) to evaluate the relationship between dietary components and RE in a Chinese population in the present study aiming to clarify whether the diet habits affect the prevalence of RE.

2. Materials and Methods

2.1. Patients. A total of 537 Han Chinese were recruited from the Endoscopy Center of Tongji Hospital between May 2010 and May 2011 in Shanghai. Because the diet habits vary in different peoples and Han Chinese account for 91.51% of population in China, the Han Chinese were recruited in order to maximally ensure the accuracy of data. Among these subjects, the age of 268 patients who were newly diagnosed as RE based on the Los Angles (LA) classification [23] ranged from 20 to 82 years, and 269 controls aged 19–80 years. The controls received routine health examinations including annual upper endoscopy, and all the controls were normal on upper gastrointestinal endoscopy and had no reflux symptoms. RE patients and controls were randomly selected and matched in the gender and age. Subjects were excluded if they had peptic ulcer (active or quiescent), endoscopic gastrointestinal tumors, history of upper gastrointestinal surgery, and over-the-counter medication (histamine-2-receptor antagonists, proton pump inhibitors, etc.) or were unable to complete the questionnaire and physical examination.

2.2. Ethical Considerations. The whole protocol was approved by the Ethics Committee of Tongji Hospital. All subjects gave written informed consent before study.

2.2.1. Dietary Questionnaire. All subjects were trained to complete a detailed FFQ. Before survey, all subjects were required to complete a Reflux Diagnostic Questionnaire (RDQ), including "any symptoms including heartburn, acid regurgitation, and noncardiac chest pain," and "often changing dietary habits and avoiding certain foods." Controls with RDQ score of >12 were also excluded although the normal findings were present in the endoscopic examination. In order to avoid the influence of symptoms on the dietary intake, these subjects were asked to record the dietary intake before the onset of reflux symptoms. FFQ based on the Chinese Dietary Pagoda [24] was adapted for the Chinese population to enable completion within 40–50 min. A total of 120 kinds of food were included in the questionnaire based on the foods with high intake frequency in Chinese Nutrition Survey in 2002 and the new foods emerging in recent years. The food categories included grains, potatoes, meat, fish and shrimps, eggs, dark-colored vegetables, light-colored vegetables, fruits, nuts, beans and bean products, milk and dairy products, desserts, condiments, soft drinks, alcohol, western-style fast food, and animal oils. Participants were asked to report the foods (≥120) consumed in the past year. The intake of major foods was estimated according to the food moulds. The frequency of food intake in the FFQ ranged from "never or less than once monthly" to "twice daily." Each question included three options for portion size. Using these data, the total frequency of intake of each food was calculated in a fixed period. The intake of each nutrient was calculated using the following formula: (reported intake frequency daily) × (portion size in grams) × (nutrient content per 100 grams)/100. The intake of plant oils, salt, and sugar was surveyed and converted according to the monthly consumption in each family and the number of family members.

2.2.2. Anthropometric Measurements. The height, weight, waist circumference (WC), and hip circumference (HC) were measured under fasting conditions followed by endoscopy. Height was measured to the nearest 0.5 cm using a stadiometer, and weight to the nearest 0.25 kg in light clothing and without shoes using standard digital scales. BMI (kg/m^2) was calculated as a ratio of weight (kg) to the square of height (m^2). WC and HC were measured to the nearest 0.1 cm and the mean of three measurements was obtained. Waist-hip ratio (WHR) was calculated as a ratio of WC (cm) to HC (cm).

2.3. Quality Control and Methods. All investigators received professional training to collect and analyze data with stringent quality control standards. The investigators who collected anthropometric and dietary data were blind to

the findings in endoscopy. An investigator supervised and checked all data. A nutrient calculator software designed by the Department of Clinical Nutrition of Tongji Hospital on the basis of China Food Composition Tables [25] was used to calculate the daily intakes of calories and nutrients.

2.4. Statistical Analysis. Statistical analysis was performed using SPSS version 14.0 for Windows (Chicago, IL, USA). All data were expressed as mean ± standard deviation (SD). χ^2 test and Kruskal-Wallis H test were used to compare the categorical variables, and t test to compare the parametric continuous variables. Stepwise multiple logistic regression analysis was employed to examine variables. The main predictors in the model were the dietary variables serving as continuous variables. The model was adjusted for the frequency matched variables: WC, WHR, total energy per day, age, sex, and education level. Odds ratios (OR) were calculated on the basis of interquartile range for each nutrient and thus show risk comparing the 75th centile of intake for each nutrient with the 25th centile. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of Participants. Table 1 provides detailed characteristics of 537 subjects. RE patients were different from the controls in terms of education level ($P < 0.05$). RE patients had a higher WC and WHR than controls ($P < 0.05$) and there were no differences in the height, weight, BMI, and HC ($P > 0.05$) between them. Table 2 displays the anthropometric measurements of two groups. The extent of oesophageal mucosal damage was assessed using the LA grading system [23]. Of the 268 patients with RE, 213 had grade A, 45 had grade B, 9 had grade C, and 1 had grade D oesophageal mucosal damage. Patients with mild RE accounted for 96.3% (Grade A and B).

3.2. Mean Daily Intake of Nutrients and Food. Data on nutrient and food intake obtained from the FFQ are shown in Table 3.

The daily intake of total energy, protein, fat, carbohydrate, total SFA, dietary fiber, selenium, milk and dairy products, beans, and nuts was significantly higher in the RE group than in the control group ($P < 0.05$). The calories from protein (%), calcium, β-carotene, vitamin C, and vegetables were markedly lower in the RE group than in the control group ($P < 0.05$).

There were no significant differences in the intake of calories from fats and carbohydrates (%), cholesterol, zinc, ferrum, vitamin E, grains and potatoes, fruits, meat, fish and shrimps, eggs, alcohol, oils, and salt ($P > 0.05$).

3.3. Relationship between RE and Intake of Various Nutrients and Food. After adjustment for WC, WHR, total energy intake, and demographics (sex, age and education level), there was a positive dose-response relationship between RE and calcium (OR 1.63, 95% CI 1.26–2.11), meat (OR 1.39, 95% CI 1.07–1.79), oils (OR 1.56, 95% CI 1.18–2.06), and salt (OR 9.93, 95% CI 5.33–18.49), and there was an inverse dose-response relationship between RE and protein (OR 0.68, 95% CI 0.47–0.98), carbohydrate (OR 0.66, 95% CI 0.45–0.97), calories from protein (%) (OR 0.64, 95% CI 0.48–0.84), vitamin C (OR 0.51, 95% CI 0.39–0.66), grains and potatoes (OR 0.58, 95% CI 0.39–0.85), fruits (OR 0.65, 95% CI 0.51–0.83), and eggs (OR 0.69, 95% CI 0.53–0.91).

After adjustment for WC, WHR, total energy intake, and demographics (sex, age, and education level), there was no correlation of RE with fat, total SFA, alcohol, cholesterol, calories from fat (%), calories from carbohydrate (%), dietary fiber, vitamin E, selenium, ferrum, zinc, β-carotene, vegetables, fish and shrimps, milk and dairy products, soy, and nuts. The relationship between RE and different nutrients and food is shown in Table 4.

Table 1: Comparison of the RE group and control group.

Variables	RE group (%) ($n = 268$)	Control group (%) ($n = 269$)	P
Age (years) mean (SD)	50.9 ± 0.9	48.5 ± 0.8	0.055
20–29	21 (7.8)	27 (10.0)	0.181
30–39	41 (15.3)	47 (17.5)	
40–49	54 (20.2)	68 (25.3)	
50–59	81 (30.2)	60 (22.3)	
≥60	71 (26.5)	67 (24.9)	
Sex			
Men	137 (51.1)	131 (48.7)	0.575
Women	131 (48.9)	138 (51.3)	
Education level			
Illiteracy	21 (7.8)	16 (6.0)	<0.001
Primary school graduate	43 (16.0)	27 (10.0)	
Junior high school	79 (29.5)	103 (38.3)	
High school graduate	74 (27.6)	73 (27.1)	
College graduate	21 (7.8)	14 (5.2)	
Undergraduate	25 (9.3)	32 (11.9)	
Master graduate	5 (2)	4 (1.5)	

Abbreviations: RE: reflux esophagitis.

Table 2: Anthropometric measurements of RE group and control group.

Variables	RE group (%) ($n = 268$)	Control group (%) ($n = 269$)	P
Height	164.9 ± 0.5	164.4 ± 0.5	0.422
Weight	62.6 ± 0.7	62.8 ± 0.7	0.783
BMI	23.0 ± 0.2	23.2 ± 0.2	0.350
WC	82.8 ± 0.6	81.0 ± 0.6	0.031
HC	94.8 ± 0.4	94.2 ± 0.4	0.279
WHR	0.87 ± 0.0	0.86 ± 0.0	0.020

Abbreviations: RE: reflux esophagitis; BMI: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist-hip ratio.

Table 3: Mean daily intake of nutrients and food in two groups.

Variables/day	RE group (n = 268)	Control group (n = 269)	P
Total energy (kcal)	2438.6 ± 53.7	2148.2 ± 38.4	<0.001
Macronutrients			
Protein (g)	78.2 ± 1.9	72.5 ± 1.4	0.019
Fat (g)	88.0 ± 3.2	71.6 ± 1.6	<0.001
Carbohydrate (g)	327.0 ± 7.6	297.7 ± 6.2	0.003
Total SFA (g)	21.4 ± 0.3	20.6 ± 0.2	0.035
Cholesterol (mg)	309.0 ± 10.0	297.5 ± 8.3	0.375
Calories from protein (%)	12.9 ± 0.2	13.7 ± 0.2	<0.001
Calories from fat (%)	31.6 ± 0.5	30.6 ± 0.4	0.103
Calories from carbohydrate (%)	54.8 ± 0.5	55.0 ± 0.5	0.705
Dietary fiber (g)	9.0 ± 0.3	8.2 ± 0.2	0.024
Micronutrients			
Zinc (mg)	11.9 ± 0.3	11.5 ± 0.2	0.281
Ferrum (mg)	17.6 ± 0.3	17.3 ± 0.3	0.467
Calcium (mg)	376.2 ± 7.8	426.1 ± 8.3	<0.001
Selenium (μg)	52.9 ± 1.6	47.8 ± 1.1	0.010
β-carotene (μg)	3322.2 ± 75	3676.2 ± 95.4	0.004
Vitamin E (mg)	50.6 ± 0.7	49.0 ± 0.6	0.076
Vitamin C (mg)	108.5 ± 4.5	136.0 ± 2.6	<0.001
Food			
Grains and potatoes (g)	371.6 ± 9.6	355.4 ± 8.3	0.202
Fruits (g)	102.8 ± 5.9	103.6 ± 5.6	0.924
Vegetables (g)	276.8 ± 6.0	344.6 ± 12.0	<0.001
Meat (g)	114.0 ± 7.9	102.4 ± 5.0	0.212
Fish and shrimps (g)	47.9 ± 2.9	49.0 ± 3.4	0.805
Eggs (g)	26.1 ± 1.3	26.5 ± 1.1	0.815
Milk and dairy products (g)	86.0 ± 5.6	68.7 ± 5.7	0.031
Beans and nuts (g)	16.1 ± 2.5	9.3 ± 1.0	0.011
Alcohol (g)	12.5 ± 2.3	8.0 ± 1.8	0.122
Oils (g)	42.3 ± 0.7	44.6 ± 2.0	0.263
Salt (g)	10.9 ± 0.1	10.9 ± 0.2	0.863

Abbreviations: RE: reflux esophagitis; SFA: saturated fatty acid.

4. Discussion

This is the first study reporting an association between the risk for RE and dietary nutrients as well as food in a Chinese population. In this study, results showed that the RE was mild (Grade A and B) which was similar to previously reported [26], and RE patients had higher WC and WHR when compared with healthy controls. Several previous studies have shown that overweight and obesity (especially abdominal obesity) are important independent risk factors for RE [27–32]. However, in Western countries, studies reveal that increased fat consumption (especially cholesterol and SFA rather than just weight disorder) has a dose-dependent correlation with GERD symptoms [14, 33, 34]. Therefore, experts in Western countries believe that food consumption patterns may be associated with the increasing prevalence of GERD, with low-fat and high-fiber foods playing a protective role, and high-vitamin C foods reducing the risk for GERD [35, 36]. In some clinical studies, esophageal pH provides direct evidence on the association between dietary fat and acid reflux. Shapiro et al. [33] found that, of all the dietary ingredients, cholesterol was the most important risk factor for intraesophageal acid reflux episodes in patients with GERD. It has been established that high-fat or large meal decreases the lower esophageal sphincter pressure (LESP), increases the rate of TLESR, and delays the gastric emptying [37], which may lead to a greater incidence of reflux [38]. Thus, it would be expected to increase the esophageal acid exposure in GERD [39]. Moreover, the dietary fiber has been found to decrease the risk for GERD, which may be attributed to the LES relax by dietary fiber. El-Serag et al. [14] recently reported that the daily intake of total fat, saturated fat, cholesterol, energy from dietary fat, and average fat servings in patients with GERD symptoms increased significantly when compared with subjects without GERD symptoms, and intake of high-fiber food correlated with a reduced risk for GERD symptoms. However, our results failed to establish the significant relationship between RE and fat as well as dietary

Table 4: Risk for RE in patients with different intake of dietary nutrients and food groups.

Daily intake	OR	95% CI	*P*
Nutrients			
Protein (g/day)			
Unadjusted	1.01	0.79–1.28	0.97
Adjusted*	0.68	0.47–0.98	0.04
Fat (g/day)			
Unadjusted	1.32	1.04–1.68	0.02
Adjusted	1.24	0.93–1.85	0.14
Carbohydrate (g/day)			
Unadjusted	1.03	0.81–1.31	0.80
Adjusted	0.66	0.45–0.97	0.04
Total SFA (g/day)			
Unadjusted	1.06	0.83–1.35	0.65
Adjusted	1.05	0.81–1.37	0.70
Alcohol (g/day)			
Unadjusted	0.76	0.50–1.16	0.21
Adjusted	0.78	0.50–1.20	0.25
Cholesterol (mg/day)			
Unadjusted	1.05	0.83–1.34	0.67
Adjusted	0.96	0.74–1.25	0.78
Calories from protein (%)			
Unadjusted	0.64	0.50–0.81	<0.01
Adjusted	0.64	0.48–0.84	<0.01
Calories from fat (%)			
Unadjusted	0.94	0.74–1.19	0.58
Adjusted	1.04	0.79–1.37	0.80
Calories from carbohydrate (%)			
Unadjusted	0.95	0.74–1.20	0.64
Adjusted	0.95	0.74–1.23	0.70
Dietary fiber (g/day)			
Unadjusted	0.98	0.77–1.25	0.88
Adjusted	0.818	0.619–1.080	0.16
Vitamin C (mg/day)			
Unadjusted	0.52	0.40–0.66	<0.01
Adjusted	0.51	0.39–0.66	<0.01
Vitamin E (mg/day)			
Unadjusted	0.97	0.76–1.24	0.82
Adjusted	0.91	0.70–1.17	0.45
Selenium (μg/day)			
Unadjusted	1.01	0.82–1.25	0.90
Adjusted	1.04	0.84–1.30	0.70
Ferrum (mg/day)			
Unadjusted	0.96	0.75–1.22	0.71
Adjusted	0.94	0.73–1.20	0.59
Zinc (mg/day)			
Unadjusted	0.99	0.78–1.25	0.91
Adjusted	0.96	0.75–1.23	0.75

Table 4: Continued.

Daily intake	OR	95% CI	*P*
Calcium (mg/day)			
Unadjusted	1.63	1.27–2.09	<0.01
Adjusted	1.63	1.26–2.11	<0.01
Beta-carotene (mg/day)			
Unadjusted	1.28	1.01–1.63	0.04
Adjusted	1.29	0.99–1.67	0.06
Food			
Grains and potatoes (g/day)			
Unadjusted	1.04	0.82–1.32	0.76
Adjusted	0.58	0.39–0.85	0.01
Fruits (g/day)			
Unadjusted	0.64	0.51–0.80	<0.01
Adjusted	0.65	0.51–0.83	<0.01
Vegetables (g/day)			
Unadjusted	1.09	0.78–1.51	0.62
Adjusted	1.13	0.80–1.58	0.49
Meat (g/day)			
Unadjusted	1.35	1.06–1.73	0.02
Adjusted	1.39	1.07–1.79	0.01
Fish and shrimps (g/day)			
Unadjusted	0.97	0.74–1.29	0.85
Adjusted	0.96	0.71–1.30	0.77
Eggs (g/day)			
Unadjusted	0.73	0.57–0.94	0.02
Adjusted	0.69	0.53–0.91	0.01
Milk and dairy products (g/day)			
Unadjusted	1.34	1.09–1.65	0.01
Adjusted	1.20	1.00–1.44	0.06
Soy and nuts (g/day)			
Unadjusted	1.17	0.95–1.44	0.15
Adjusted	1.09	0.92–1.31	0.33
Oils (g/day)			
Unadjusted	1.65	1.26–2.15	<0.01
Adjusted	1.56	1.18–2.06	<0.01
Salt (g/day)			
Unadjusted	9.10	5.18–16.00	<0.01
Adjusted	9.93	5.33–18.49	<0.01

Data are expressed as odds ratio with 95% confidence intervals (95% CI).
*Adjusted for WC, WHR, energy, age, sex, and education level.
Abbreviations: OR: odds ratios; CI: confidence interval; WC: waist circumference; WHR: waist-hip ratio.

fiber after adjustment for WC, WHR, total energy intake and demographics. Our results provided evidence supporting a link between the high consumption of meat and oils and the increased risk for RE, and the consumption of protein, carbohydrate, calories from protein (%), vitamin C, grains and potatoes, fruits, and eggs was related to the prevention against RE.

Some studies have demonstrated that an increased prevalence of reflux symptoms in alcohol users, and alcohol is an independent risk factor for GERD-related symptoms, with alcohol consumption exacerbating GERD by increasing acid secretion through gastric stimulation, reducing LESP, increasing spontaneous LES relaxations, and impairing esophageal motility and gastric emptying [20, 40–42]. Modest alcohol intake has been shown to induce reflux symptoms and decrease the esophageal pH in healthy individuals without GERD symptoms despite the overall 24 h pH was normal. Wang et al. [43] reported reflux symptoms in 43% of heavy (210 g/wk) alcoholics when compared with 16% of nondrinkers. In our study, no dose-response relationship

was observed between RE and alcohol after adjustment for confounding variables, which was consistent with previously reported [21, 22]. The discrepancy may be due to the difference in the methodology. Pehl let al. showed that alcohol intake was correlated with obesity and suggested that patients with reflux symptoms should avoid intake of >300 mL of alcohol or beer [44].

Some previous studies found that an increase in salt consumption was associated with GERD [22, 45], which was attributed to the delayed gastric emptying and increased pancreaticobiliary secretion after high salt intake [46]. Similarly, our results also revealed a relationship between salt intake and RE. However, Aanen et al. [47] found that high dietary sodium did not increase the gastroesophageal reflux in healthy volunteers but reduced the LESP. Mizuta et al. also showed that slight increase in daily salt intake might be insufficient to affect the prevalence of RE [48]. Further investigations are needed to clarify this association.

Some experts proposed that high intake of vitamin C could exert a protective effect against GERD [35, 36]. Our findings revealed that excessive intake of animal products and less intake of vegetables may lead to vitamin C deficiency. Our results provided evidence supporting a relationship of high intake of vitamin C and fruits with prevention against RE. Our study suggests that RE patients should eat less energy-rich foods and more healthy foods such as vegetables and fruits, for health concerns.

Calcium is an important nutrient related to many diseases. However, to date, no studies have confirmed the relationship between calcium and GERD. To our knowledge, this is the first study to reveal the relationship between calcium intake and RE after adjustment for confounding variables. Nevertheless, the mechanism of such relationship is unknown. We speculated that calcium may stimulate the gastric acid secretion [49, 50], which may increase the esophageal acid exposure in GERD. Further studies are required to confirm the relationship between calcium and GERD.

The incidence of GERD is on the rise in China due to high intake of meat, oils, salt, and calcium, while high intake of protein, carbohydrate, calories from protein (%), vitamin C, grains and potatoes, fruits, and eggs correlates with a reduced risk for GERD, which is different from the findings in the study of El-Serag et al. The conflicting data may be attributed to differences in not only the race, geographic specificities, diet habit, and culture between Chinese and the Western, but the definition of GERD because studies based on GERD symptoms may be overinclusive, and our study based on GERD complications such as esophagitis is restrictive. Further studies are needed to clarify this association.

Our study has some limitations: first, in the present study, incomplete data on vitamin and calcium supplements were not included for analysis, which may affect the determination of vitamin and calcium intakes; second, the folate, lutein, and other micronutrients were not employed for analysis and discussion since they are not included in Chinese Food Composition Tables; third, FFQ is not a particularly accurate dietary assessment tool, and there is potential for measurement error. However, FFQ is one of the most well validated and commonly used food frequency questionnaires; forth, the recall bias and residual confounding might also influence the results.

5. Conclusions

Our results indicate that high intake of calcium, meat, oils, and salt is associated with an increased risk for RE while high intake of protein, carbohydrate, calories from protein (%), vitamin C, grains and potatoes, fruits, and eggs correlates with a reduced risk for RE in Han Chinese. Further studies are required to explore the relationships among diet, obesity, and RE comprehensively.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Ping Wu contributed to the study design, data analysis, paper drafting and participated in the survey; Shu-Chang Xu and Xiao-Hu Zhao contributed to the study design, participated in the survey, and revised the paper critically; Zi-Sheng Ai helped to design the study and analyze the data; Ying Chen contributed to the study design and participated in the survey; Hui-Hui Sun contributed to the study design and participated in the survey; Yuan-Xi Jiang and Yi-Li Tong participated in the survey. All authors read and approved the paper.

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Review Article

Biomarkers for Gastroesophageal Reflux in Respiratory Diseases

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Gastroesophageal reflux (GER) is commonly associated with respiratory symptoms, either through a vagal bronchoconstrictive reflex or through microaspiration of gastric contents. No diagnostic test is available, however, to diagnose when respiratory illnesses are caused by GER and when not, but research in this field has been moving forward. Various biomarkers in different types of biosamples have been studied in this context. The aim of this review is to summarize the present knowledge in this field. GER patients with respiratory diseases seem to have a different biochemical profile from similar patients without GER. Inflammatory biomarkers differ in asthmatics based on GER status, tachykinins are elevated in patients with GER-related cough, and bile acids are elevated in lung transplant patients with GER. However, studies on these biomarkers are often limited by their small size, methods of analysis, and case selections. The two pathogenesis mechanisms are associated with different respiratory illnesses and biochemical profiles. A reliable test to identify GER-induced respiratory disorders needs to be developed. Bronchoalveolar lavage is too invasive to be of use in most patients. Exhaled breath condensate samples need further evaluation and standardization. The newly developed particles in exhaled air measurements remain to be studied further.

1. Introduction

Gastroesophageal reflux (GER) is a growing health problem in the Western world [1]. It is now generally accepted that GER is a causative factor for inducing or worsening certain respiratory symptoms and diseases [2]. GER has also been shown to be associated with obstructive sleep apnea [3]. The respiratory diseases that have most frequently been studied with GER are asthma [4–15] and chronic cough [5, 13, 14, 16–23], but recently many studies have been published on GER and lung transplant (LTx) rejection [24–32].

Despite these evident associations, it is difficult to diagnose with certainty when respiratory diseases are caused by GER, or when they cooccur coincidentally. This increases the need for diagnostic methods to discriminate between patients with coincidental cooccurrences and actual causation associations [33].

Two different mechanisms are proposed to be responsible for the majority of GER-induced respiratory symptoms and diseases. One involves microaspiration of gastric fluids into the lungs causing irritation and inflammation, and the second bronchoconstriction caused by a vagal reflex from the distal esophagus, induced by acidic reflux to the distal esophagus (Figure 1) [34]. These two mechanisms probably both play a significant role, but to a different extent in different conditions.

Serum biomarkers such as gastrin, pepsinogen, and cleaved fragments of E-cadherin have been studied in patients suffering from GER. Cleaved fragments of E-cadherin were found to be significantly increased in serum from GER

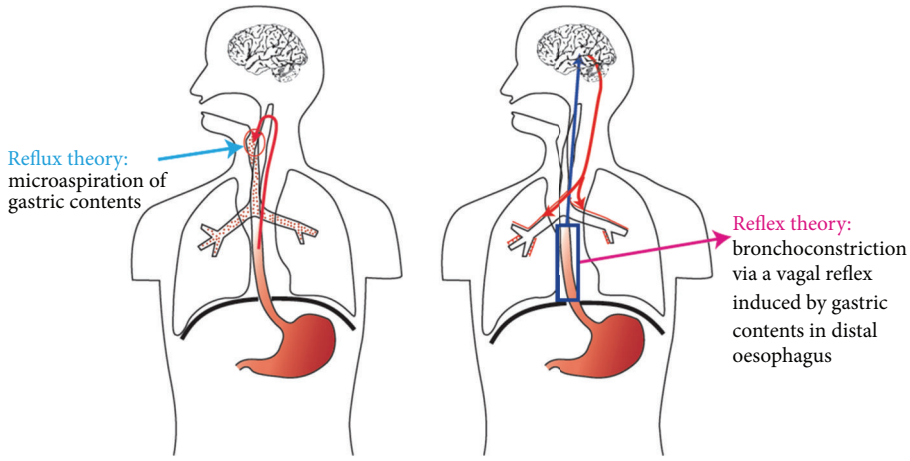

Figure 1: Two theories exist on how gastroesophageal reflux induces respiratory symptoms, called the reflux and reflex theories.

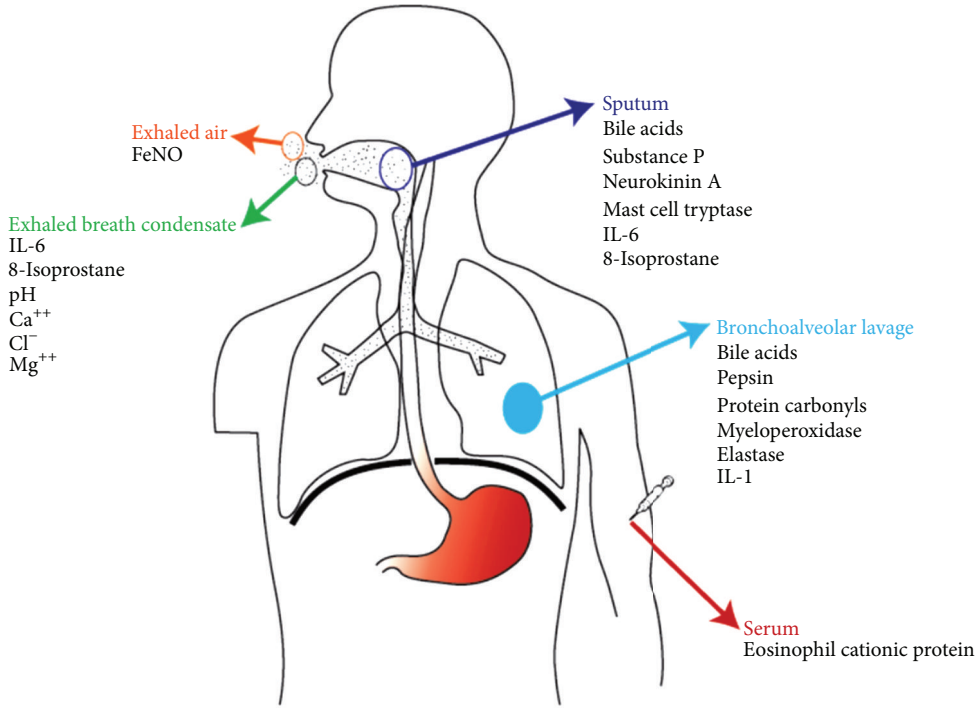

Figure 2: Summary of biomarkers shown to have an association with gastroesophageal reflux in respiratory illnesses.

patients. As E-cadherin is a junctional protein important in barrier function in esophageal epithelium, its cleavage likely explains the increase in junctional permeability in the esophageal epithelium of these patients [35, 36]. These studies, however, had no focus on respiratory symptoms.

Clinically it would be of great advantage to have a diagnostic test that could discriminate between respiratory symptoms and diseases caused by GER or other causes. To date, no such generally approved diagnostic test specific to this condition is available, but research in this field has been moving forward (Figure 2) [33]. The aim of the present paper is to summarize the findings of studies on various biomarkers in different biosamples, which have had the objective of distinguishing between respiratory diseases caused by GER- and other non-GER-related causes.

2. Methods

When preparing this paper we searched the MEDLINE database for relevant articles on biomarkers associated with GER and respiratory diseases, with special emphasis on biomarkers and biosamples from the distal airways, that is, bronchoalveolar lavage (BAL) and exhaled breath condensate (EBC). The MEDLINE database was searched in August–November 2012 for articles in English. The following search

Table 1: Biomarkers studied in gastroesophageal reflux with respiratory illnesses.

Biosample	Serum	Sputum		Bronchoalveolar lavage		Exhaled air	Exhaled breath condensate
Studies found	22	34		71		7	16
Studies reviewed	2	11		21		4	8
Biomarkers studied	Eosinophil cationic protein	Bile acids	Pepsin	Bile acids	Pepsin	FeNO	Pepsin
	Neurotrophin 3	LLMI	IL-4, IL-6	LLMI	IL-1, IL-8, IL-15		IL-4, IL-6
	BDNF	Substance P	Neurokinin A	IFN-gamma	Myeloperoxidase		8-Isoprostane
	Nerve growth factor	Nerve growth factor	Mast cell tryptase	Lactate dehydrogenase	Protein carbonyls		pH
		8-Isoprostane	Histamine	DPPC	SP-A, SP-D		Calcium
		Prostaglandin D2	Fibrinogen	Elastase	pH		Magnesium
		Eosinophil cationic protein					Chloride

phrases were used: "serum + biomarker + gastroesophageal + reflux" (22 articles), "sputum + respiratory + gastroesophageal + reflux" (34 articles), "bronchoalveolar + lavage + gastroesophageal + reflux" (71 articles), "exhaled + breath + condensate + gastroesophageal + reflux" (16 articles), "exhaled + nitric + oxide + gastroesophageal + reflux" (7 articles), and "particles + in + exhaled + air + gastroesophageal + reflux" (0 articles). Articles were excluded if they contained no abstract or were not relevant (i.e., not studying biomarkers in GER with respiratory symptoms). Review articles, case reports, and letters were also excluded. Animal studies were excluded. Cytopathological studies were mostly excluded, except for the exceptional case of the lipid-laden macrophage index (LLMI), usually in bronchoalveolar lavage, a marker commonly associated with pulmonary aspiration [37–39]. Studies on lung transplant (LTx) patients were included as GER has a special importance in these patients, causing inflammation and transplant rejections [40]. First, titles and abstracts were quickly evaluated with regard to the exclusion criteria, then a closer evaluation of the remaining articles was done. One study was moved from the EBC group to the BAL group [41]. After exclusions, the number of articles identified in each search was as follows: 1, 9, 21, 7, 4, and 0, respectively. No duplicate hits were found. A few studies used more than one biosample and were therefore included in more than one section in this paper (Table 1).

3. Serum Biomarkers

A specific and sensitive serum biomarker for the detection of respiratory disorders caused by or linked to GER has not been found at present.

In a study by Di Lorenzo et al. [6], patients with GER and asthma-like symptoms were significantly lower in serum eosinophil cationic protein (ECP) levels than patients with diagnosed mild asthma. In fact, those with GER and asthma-like symptoms had similar levels of ECP as healthy subjects, while asthmatic patients had three times higher values. Bronchial hyperresponsiveness was elevated in asthmatic patients, but was normal in those with GER and asthma-like symptoms. The authors hypothesize that this might reflect that those with asthma have mainly eosinophilic inflammation, whereas those with GER have mainly neutrophilic inflammation in the airways. However, this study was cross-sectional, and no follow-up studies have been published [6].

In a study by Chaudhuri et al. [17], serum levels of the neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor, and neurotrophin 3 were measured in 81 patients suffering from chronic cough and the levels compared to those in healthy controls. No significant association was found between GER-based chronic cough, defined by clinical presentation and treatment response, and these neurotrophins in serum [17].

4. Biomarkers in Sputum

samples contain many biomarkers of inflammation and infection that are relevant for lung diseases and have been proposed to be useful for the detection of respiratory disorders caused by or linked to GER. These markers include bile acids, pepsin, markers for neurogenic inflammation, and general inflammation.

In a cross-sectional study, bile acids were shown to be frequently present in induced sputum samples in patients suffering from cystic fibrosis. The levels of bile acids were also associated with the degree of lung function impairment [42]. In another study bile acid levels were shown to be significantly elevated in induced sputum from patients with GER and asthma-associated GER symptoms when compared to asthma patients and healthy controls. Patients with asthma had a moderate but statistically insignificant elevation of

bile acids in induced sputum samples, both those with and without GER [4]. No statistical difference was observed when bile acid levels in induced sputum from patients with GER-related chronic cough were compared with sputum samples from controls [18]. In vitro bile acids have been shown to induce fibroblast proliferation in airway epithelium, a finding of unknown importance in humans [4]. There is thus some evidence that bile acids in sputum indicate GER-induced respiratory disorder. However, the pathogenesis behind the association between cough and GER seems to be something other than aspiration of gastric fluids.

Two studies showed that pepsin concentration in sputum is not helpful in diagnosing GER-related chronic cough, and pepsin concentration is frequently detected in sputum from healthy children [18, 19, 43]. One reason why pepsin concentration in sputum might not be as useful as a marker of microaspiration, as originally thought, is that pepsinogen has been found to be produced in normal human lungs [44, 45]. Lipid laden macrophage index (LLMI), a semiquantitative evaluation of macrophage lipid content which is considered to be a biomarker of aspiration, has been studied in the sputum of GER patients with respiratory symptoms. In a small study of 22 patients and 15 controls, the LLMI in sputum was associated with the duration of GER symptoms, but the levels were not significantly different compared with controls [46].

In one cross-sectional study, the neurotrophin nerve growth factor (NGF) levels were measured in sputum from patients with chronic cough and compared with healthy controls, but no significant difference was observed. The same was true for the subgroup with chronic cough based on GER [17].

In another study patients with GER and chronic cough were shown to have 50–100 times more of the tachykinin substance P in their sputum when compared to GER patients without chronic cough or healthy subjects [16]. Similarly, a cross-sectional study of 32 subjects showed a positive association between GER and the tachykinins substance P and neurokinin A in induced sputum samples, both in asthmatics and nonasthmatics. A positive correlation between distal esophageal acid exposure time on 24-hour esophageal pH monitoring (24 h-pH-m) and tachykinin levels was found. The presence of these tachykinins suggests airway sensory nerve activation [5]. These findings support the theory that certain respiratory symptoms and diseases linked to GER are caused by a vagally mediated esophageal-tracheobronchial reflex.

The inflammatory biomarker mast cell tryptase has been found to be increased in the sputum of GER-associated chronic cough patients, compared to GER patients without chronic cough. However, other biomarkers of inflammation such as prostaglandin D2 and histamine were not significantly increased in these samples [16]. Another study on inflammatory markers in 20 GER patients with cough or mild asthma found no elevation in fibrinogen levels in induced sputum, and an elevation in ECP was more associated with asthma than with GER. The study was, however, limited by its size [13].

A study on interleukins (IL) and 8-isoprostane in the sputum of GER and asthma patients showed that IL-4 is similarly elevated in asthmatics, with or without GER. Conversely, IL-6 and 8-isoprostane were elevated in GER patients, irrespective of asthma status. Similar findings were found in BAL samples [7].

In summary, bile acid levels in sputum might be associated with GER-induced respiratory disorders. There seems to be a difference in the inflammatory pathways between asthmatics with or without GER. IL-6 and 8-isoprostane, as well as substance P and neurokinin A, in induced sputum seem to predict the presence of GER in subjects both with or without asthma. Substance P and mast cell tryptase seem also to predict GER in subjects with chronic cough. Further studies are needed to clarify these findings.

5. Bronchoalveolar Lavage Biomarkers

A bronchoalveolar lavage (BAL) sample is a biosample collected during a bronchoscopy by infusing saline into a small segment of the lung and then suctioning up this fluid again for analysis. The infusion-suction process is then repeated a few times until an adequate specimen has been obtained [47].

Measurements of bile acids in BAL samples consistently show that elevated levels of bile acids are a bad prognostic factor for rejection rates in LTx patients, development of bronchiolitis obliterans, and survival [26, 32]. There seems even to be a clear correlation between the time to onset of bronchiolitis obliterans and levels of bile acids in BAL. In a study by Blondeau et al., nocturnal GER was found to be a stronger risk factor for bile acid aspiration in LTx patients than GER in general, suggesting that nocturnal GER represents a worse form of GER [26, 28–32].

A study on 96 children with chronic cough, allergic asthma, and other chronic lung diseases showed no association between GER status, measured by 24 h-pH-m, and bile acids in BAL [48]. Also, a small study on Wegener's granulomatosis patients with subglottic stenosis showed no significant difference in BAL bile acids; however, since the study examined a very small number of patients, it may be a false negative finding [49].

The clinical use of pepsin as a biomarker in BAL samples has been studied extensively. Many of these studies were on LTx patients. Observations in these studies, however, were somewhat different from those on bile acids in BAL samples. In three of these studies, pepsin in BAL seemed not to be associated with a clinical decline in LTx patients. One recent study, however, showed that pepsin was present in lower quantities in LTx patients who underwent antireflux surgery than in those who did not and was undetectable in controls. Those who underwent antireflux surgery also had a better clinical outcome. This study did not measure bile acids [25, 28, 29, 31]. Another study on 8 LTx patients undergoing antireflux surgery showed a similar trend [24].

Two studies on pepsin in BAL samples from chronic cough patients showed conflicting results. The larger and more thorough one showed no increase in BAL pepsin concentration among chronic cough patients compared with

controls, even though they more often had GER, suggesting that aspiration is perhaps not the causative mechanism in GER-associated chronic cough [19, 21]. One study on children with chronic lung diseases showed that those with GER have a higher pepsin level in BAL than those without GER, but with low specificity [48].

Several studies on the clinical use of LLMI in BAL samples have been carried out. Three of them were on children with difficult-to-treat respiratory symptoms, often asthma-like, and one on infants with chronic respiratory diseases. These studies showed a clear association between GER status and LLMI [8, 50–52]. In a study on 34 LTx patients, elevated LLMI levels in BAL samples correlated significantly with abnormal 24 h-pH-m [27]. One study on 33 children with GER-related respiratory diseases found no increase in LLMI compared with controls [21]. A large study on 446 children with respiratory disorders thought to be GER-associated showed no associations between LLMI and various parameters in double channel 24 h-pH-m [53]. Therefore, LLMI in BAL seems to be of limited value in assessing GER-associated respiratory diseases, except perhaps in LTx patients.

Among 30 children with asthma-like symptoms, those with GER had higher levels of IL-8, myeloperoxidase, and elastase in BAL than those without GER [52]. Children with chronic lung diseases have been shown to have a positive correlation between IL-8 and protein carbonyl levels in BAL and proximal reflux events in 24 h-pH-m [48]. In LTx patients, IL-8 was found to be significantly elevated in those with elevated bile acids, but not IL-15 [31, 32]. Another study on 8 LTx patients which underwent antireflux surgery measured numerous inflammatory markers but found only that the level of IL-1-beta had decreased whereas the level of interferon-gamma had increased. However, these results were most likely confounded by the low number of participants and the high number of biomarkers studied [24].

Measurements of surfactant in BAL showed that dipalmitoylphosphatidylcholine did not differ between children with reflux esophagitis, cough, and healthy controls [20]. In another study, however, children with GER-associated chronic respiratory diseases were shown to have prominently reduced levels of surfactant-protein- (SP-) A and reduced levels of SP-D, compared with healthy controls [54]. Further studies are needed to evaluate the potential role of surfactant proteins as biomarkers to differentiate between chronic respiratory diseases with and without GER.

One study on bronchial aspirate in GER patients was found. Bronchial aspirate differs from BAL in that it does not introduce any foreign fluid into the lung but aspirates the pulmonary lining fluid directly. This study showed that GER patients have higher lactate dehydrogenase levels compared to healthy controls as well as a lower pH. Their lung function was also decreased compared to healthy controls [41].

To summarize, bile acids in bronchoalveolar lavage predict GER-induced transplant rejection in LTx patients. LLMI seems to be of limited value in assessing GER-associated respiratory diseases. GER can likely induce inflammation in the lungs and seems to have a different inflammatory profile than asthma.

6. Exhaled Breath Condensate Biomarkers

Exhaled breath condensate is a fluid biosample collected by guiding exhaled air into a condenser system, which cools the air and forms a condensate of the humidity in the air [55].

A recent study on pepsin levels in EBC samples from idiopathic pulmonary fibrosis patients did not show a significant elevation in pepsin, even though they had more GER symptoms on a questionnaire, compared to pulmonary fibrosis patients of a known cause [56]. The drawbacks of this study were, however, that it had few participants and used "homemade" equipment for EBC collection. Carpagnano et al. [7] showed elevated IL-4 in the EBC of asthmatics, irrespective of GER status. Conversely, IL-6 and 8-isoprostane were elevated in GER patients, irrespective of asthma status. These findings in EBC samples were similar to those in the sputum samples [7]. Another study found 8-isoprostane to be elevated in asthmatics, especially if they had comorbid GER, compared with healthy controls. This elevation was lowered significantly with proton pump inhibitor (PPI) treatment among the asthmatics with GER, but not among the asthmatics without GER [12].

Asthmatics with GER showed a lower pH in EBC than asthmatics without GER. PPI treatment seemed to elevate this low pH to a level similar to other non-GER asthmatics [10, 12]. In a 6-month prospective study on chronic obstructive pulmonary disease patients, a lower pH in EBC at baseline did not predict exacerbation frequency during followup. However, a lower pH in EBC was associated with GER status, and those with GER did have more exacerbations, suggesting this might be a false negative finding [57]. The EBC pH in chronic cough patients with GER was lower than in healthy controls [22].

Two studies from the same research group on calcium and magnesium in EBC showed conflicting results. The former study did not show a direct relationship between these electrolytes among 66 children with asthma, GER, or healthy children. The magnesium to calcium ratio, however, was lower in both children with asthma and those with GER. The later and larger study found calcium and magnesium to be elevated among children with GER, and inversely related to the EBC pH [9, 11]. Another study found levels of chloride to be lower in the EBC of 5 GER-induced chronic cough patients compared with 16 healthy controls [22]. As chloride and a higher pH have antitussive properties, the decrease in chloride and pH might contribute to the chronic cough in certain GER patients. How GER lowers chloride and even pH in the respiratory tract remains to be studied.

In summary, pulmonary inflammation in GER patients seems to be induced by different pathways than in asthma patients, as assessed by exhaled breath condensate. The pH value of EBC seems to be lowered in GER patients, and electrolyte disturbances have also been described.

7. Fractional Exhaled Nitric Oxide

Fractional exhaled nitric oxide (FeNO) has frequently been shown to be elevated in patients with classical asthma, and more recent evidence has accumulated that it is also a marker

of eosinophilic inflammation in patients with chronic cough (eosinophilic bronchitis). Subjects with chronic cough and GER seem to have significantly lower FeNO than those with asthma without GER [14]. A cross-sectional study of 20 GER subjects with cough or asthma, however, did not support this conclusion, as it was found that asthma rather than GER caused an elevation in FeNO levels [13].

The presence of GER has been shown to improve the specificity of FeNO for diagnosing eosinophilic airway inflammation. Indeed, FeNO seems only to be of use among chronic cough patients in diagnosing eosinophilic airway inflammation when GER is present [23]. In asthmatic children with GER, FeNO levels were lower than in non-GER asthmatic children, suggesting that inhalation of gastric contents may interfere with FeNO production in the airways [15].

8. Particles in Exhaled Air

Particles in exhaled air (PEx) are formed when the respiratory lining fluid in the small airways erupts as the airways expand, for example, during inhalation after a deep exhalation [58]. This breathing maneuver is used when PEx are sampled, using an instrument designed especially for this purpose. The formed particles follow the exhaled air, the number of PEx is calculated, and the particles are sampled on a teflon filter by impaction [59].

No studies on particles in exhaled air (PEx) in GER were found. The main constituents of PEx are phospholipids originating from the surfactant. A previous study indicated increased protonated (H^+) adduct formation of the major phospholipids among smokers, possibly related to alterations of the pH of the respiratory tract lining fluid (unpublished data). So far, there are no data on phospholipid alterations of the surfactant in GER in humans but gastric fluid aspiration is likely to influence the chemical composition and the pH of the respiratory tract lining fluid. Whether this also occurs in the distal airways, reflected by PEx, remains to be elucidated.

9. Conclusions

Numerous studies evaluating biomarkers in GER-related respiratory conditions have been carried out. This paper focused mostly on induced sputum, BAL, and EBC samples. Our conclusion is that GER patients with respiratory diseases seem to have a different biochemical profile compared to similar patients without GER. Inflammatory markers differ in asthmatics based on GER status, tachykinins are elevated in GER-related cough patients, and bile acids are elevated in LTx patients with GER. However, the studies on each biomarker in a specific biosample are often small and few in number, making definite conclusions on the importance of these problematic markers.

The studies reviewed here were both on children and adults. Although these studies seem to be similar in many ways, some differences can be found. For example, pepsin seems to be more common in induced sputum in the pediatric population than the adult population [19, 43]. Children with neurodisability have a high incidence of reflux aspiration and comprise a specific group of GER patients [60]. Therefore, it is important not to draw conclusions about the adult population from studies on children, and vice versa.

Studies on the lipid laden macrophage index (LLMI) in BAL samples showed conflicting results. LLMI has been found to be elevated in pulmonary diseases with no evidence of aspiration, which also makes it nonspecific [37, 61]. The usefulness of LLMI in BAL for diagnosing GER seems therefore to be minimal.

The presence of pepsin in biosamples from the respiratory tract can perhaps not be considered as pathognomonic for a GER-related pulmonary aspiration. Diagnostic methods for pepsin are different and recently it was shown that pepsinogen produced in the lungs could be a confounding factor. As quite a few studies only measure the presence or absence of pepsin, further studies should rather assess the exact magnitude of pepsin in these samples.

The pathogenesis behind the associations of GER with respiratory diseases seems to be different between different respiratory diseases. This is reflected in the different biochemical findings. In chronic cough, pepsin and bile acids are usually not elevated, but tachykinins such as substance P and neurokinin A are, indicating that a vagally-mediated bronchoconstrictive reflex is responsible. In contrast, LTx patients with GER have significantly elevated levels of pepsin and bile acids, indicating gastric fluid aspiration as a predominant causative factor. This difference in pathogenesis has to be thought of when planning studies on biomarkers in GER-associated respiratory diseases.

Reviewing respiratory biomarkers in GER leads to several perplexities. First and foremost is the wide definition of GER, which is basically the presence of bothersome symptoms caused by reflux of gastric contents [2]. GER is diagnosed based on widely different questionnaires, sometimes stressing the importance of sleep-related GER and sometimes not. Doing 24-hour esophageal pH monitoring (24 h-pH-m) is sometimes based on only one level of monitoring 5 cm above the lower esophageal sphincter, but sometimes higher (15 cm) as well [62]. It has also been pointed out that one negative 24 h-pH-m is not enough to eliminate the possibility of GER. As many as three nights might be needed. Also, in the case of EBC, these measurements have shown to have little reproducibility and are poorly standardized, making their usefulness currently limited. For the application of EBC to become more successful, collection methods and biomarker analyses in EBC samples need to become more standardized. This standardization would in turn make research collaborations easier, which is crucial for further development of this method [55, 63].

As the symptoms of GER-induced respiratory disorders often mimic other common respiratory disorders, a reliable test to identify GER-induced respiratory disorders needs to be developed. Such a test should ideally be noninvasive, with a high positive predictive value, low intraindividual variability, and change with effective treatment. In this context, the measurement of a biomarker or a set of biomarkers from the respiratory tract is of special interest. The BAL samples, which have been studied the most, are too invasive to be of

use in populations other than LTx patients. EBC samples are promising, but need further evaluation and standardization [55]. The newly developed PEx measurements remain to be studied further.

Abbreviations

24 h-pH-m: 24 hour esophageal pH monitoring
BAL: Bronchoalveolar lavage
EBC: Exhaled breath condensate
ECP: Eosinophil cationic protein
GER: Gastroesophageal reflux
IL: Interleukin
LLMI: Lipid laden macrophage index
LTx: Lung transplant
MII-pH: Multichannel intraluminal impedance pH monitoring
NGF: Nerve growth factor
PEx: Particles in exhaled air.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Review Article

GERD—Barrett—Adenocarcinoma: Do We Have Suitable Prognostic and Predictive Molecular Markers?

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Due to unfavorable lifestyle habits (unhealthy diet and tobacco abuse) the incidence of gastroesophageal reflux disease (GERD) in western countries is increasing. The GERD-Barrett-Adenocarcinoma sequence currently lacks well-defined diagnostic, progressive, predictive, and prognostic biomarkers (i) providing an appropriate screening method identifying the presence of the disease, (ii) estimating the risk of evolving cancer, that is, the progression from Barrett's esophagus (BE) to esophageal adenocarcinoma (EAC), (iii) predicting the response to therapy, and (iv) indicating an overall survival—prognosis for EAC patients. Based on histomorphological findings, detailed screening and therapeutic guidelines have been elaborated, although epidemiological studies could not support the postulated increasing progression rates of GERD to BE and EAC. Additionally, proposed predictive and prognostic markers are rather heterogeneous by nature, lack substantial proofs, and currently do not allow stratification of GERD patients for progression, outcome, and therapeutic effectiveness in clinical practice. The aim of this paper is to discuss the current knowledge regarding the GERD-BE-EAC sequence mainly focusing on the disputable and ambiguous status of proposed biomarkers to identify promising and reliable markers in order to provide more detailed insights into pathophysiological mechanisms and thus to improve prognostic and predictive therapeutic approaches.

1. Introduction

In western countries, the particular importance of gastroesophageal reflux disease (GERD) as a main risk factor for Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC) promoted by obesity, hiatus hernia, and tobacco use has increased constantly [1, 2]. Chronic injury of the gastroesophageal junction by gastric acid or bile juice induces and promotes initially reversible metaplastic changes of the squamous epithelia which is confirmed by endoscopic examination and histomorphology [3–7]. The classical GERD-BE-EAC sequence postulates a stepwise progression over different stages of dysplasia [8, 9]. However, the postulated consecutive sequence during cancerogenesis of BE has not been proven up to now [10]. Reid et al. characterized this issue as "the paradox of Barrett's esophagus," pointing out that the majority of EACs (95%) arise without prior diagnosis of BE or GERD which possibly indicates that steps of the proposed linear BE-EAC development are skipped. Nevertheless, consequent surveillance of patients with GERD and concomitant BE within well-defined time intervals with biopsy of suspicious lesions may prevent dysplasia—caused by epithelial injuries due to GERD—from developing into invasive cancer.

Although no increase of EAC incidence was postulated in epidemiologic studies, about 5% of patients with GERD and 0.5% with BE developed EAC [2, 11–13].

As dysplasia and adenocarcinoma are diagnosed by pathologists routineously (based on Haematoxylin-Eosin-stained biopsies), the question arises how the "risk progression" of GERD to BE and further to dysplasia and EAC can be evaluated and predicted by prognostic molecular markers and ideally may predict therapeutic success. In this paper, we try to refer to these FAQs and to provide a panel of diagnostic and predictive markers.

2. Definition of GERD, BE, EAC, and Types of Requested Prognostic and Predictive Markers

(i) GERD describes the chronic reflux of gastric acid or bile fluid to the esophagus resulting in metaplastic changes of the normal squamous esophageal tissue to columnar epithelium (BE) (for review, see [5]). The metaplastic changes—assessed by upper endoscopy and histological approval—comprise proximal columnar epithelia with intestinal type goblet cells, the junctional (cardial) subtype with mucous secreting glands and the gastric fundus subtype with parietal and chief cells [7, 14]. Up to now, a uniform definition of Barrett's esophagus (BE) remains controversial (e.g., which type of metaplastic changes qualifies the diagnosis BE?) leading to the striking statement "no goblets, no Barrett's" [4, 5], disregarding that nongoblet elements may also be involved in the malignant transformation of BE assessed by Sucrase-Isomaltase and dipeptidyl peptidase IV protein expression [15].

Whereas the detection of intestinal goblet cells in BE samples is already established by using histochemical staining like Alcian-PAS, the diagnosis of dysplasia in BE remains a great challenge due to inter- and intraobserver variation in histology grading (discussed later); therefore, the incidence of dysplasia inside BE varies from 5, to 10% according to national screening efforts and surveillance programs [16]. While diagnostic criteria of BE with dysplasia are relatively well defined by combining cytological and architectural changes, their prospective validation is still missing (for details, see [17–21]).

Moreover, diagnosis of the progression from BE with dysplasia to invasive EAC becomes sometimes impracticable when biopsies are small and criteria of invasiveness are mimicked by distorted rearrangement of glandular structures caused by ulceration and inflammation. At present, using the grade of dysplasia in BE represents the best biomarker in predicting the progression probability for nondysplastic BE (about 0.5%), low-grade dysplasia in BE (13%), and up to 40% in high-grade dysplasia in BE [22, 23]. Therefore, screening surveillance of BE and dysplasia remains still important to detect precursor lesions of EAC in order to avert the disastrous fate of progressive EAC which is characterized by an overall 5-year survival rate between 3.7% and 15.6% [24].

(ii) Complexity factor "diagnosis": Several issues in BE as well as in EAC detection are still unsolved. The majority of patients with BE remain undiagnosed [25–28], and/or patients with BE and dysplasia are often mis- or over-diagnosed due to inter- and intra-observer errors [10, 29, 30]. Based on the low progression rate of BE to EAC [11], endoscopic and bioptic surveillance studies could not convey a significant benefit for controlled patients [31]. Therefore, the demand for reliable biomarkers regarding prognosis and prediction of patients with BE without/with dysplasia as well as with EAC still remains indispensable.

(iii) Definition of predictive and prognostic factors (for reviews, see [32–34]): The widely used term "biomarker" represents a marker for physiological or pathological processes or therapeutic response. The clinical characteristics or endpoints (like patient performance status or disease-free period) which should be achieved by these biomarkers as well as methods applied (e.g., genome, transcriptome, proteome, or metabolome) are rather heterogeneous. The term "predictive factor" refers to the use as biomarker for prediction of the statistical probability of disease recurrence, metastasis, or tumor-related death as well as for prediction of specific therapeutic effectiveness.

As recommend by Pepe et al. [35] and McShane et al. [36], different and clearly defined "milestones" must be passed during biomarker development to evaluate their clinical prognostic and predictive potentials: starting with data obtained from experimental cell culture up to retrospective and prospective validation studies resulting in clinical applicability and significant decreasing mortality, and completed by increasing health and cost benefits.

3. "Classical" Genetic and Molecular Alterations in GERD, BE, and EAC

During carcinogenesis of BE to EAC, heterogeneous hallmarks of molecular changes are described in the literature [8, 37, 38].

(a) Genetic abnormalities of BE include loss of genetic information (especially loss of 9p21, 5q, 13q, 17p, and 18q), whereas for progressive disease, a more extensive imbalance including gain of genetic information (especially gain of 2p, 8q, and 20q) is observed. Finally, enhanced chromosomal instability could be found in the progressive lesions of EAC.

(b) These genetic abnormalities cause consecutive deregulations of their products like tumor suppressor genes (p53 (loss of 17p), p16 (loss of 9p21), fragile histidine triad protein (FHIT), adenomatous polyposis coli (APC) (loss of 5q), retinoblastoma (Rb) (loss of 13q)), cell cycle regulatory factors (cyclin D1 and MDM2 (mouse double minute 2 homolog)), growth factor receptors (EGFR (epidermal growth factor receptor), TGF-α (transforming growth factor)), c-erbB2 and cell adhesion molecules (E- and P-Cadherin and α- and β-Catenin), as well as proteases (uPA, urokinase-type plasminogen activator) according to the hallmarks of cancer [39]. Additionally, molecular alterations are associated with epigenetic changes such as the methylation and acetylation status as known for APC [40] and p16 [41].

(c) Distinct changes in expression pattern of various miRNAs (microRNA) have been demonstrated in BE or EAC. miRNAs are small regulative noncoding RNA molecules (18–22mer) which inhibit the expression of their target genes on posttranscriptional levels; about 30% of human genes are estimated to be regulated by miRNAs [42].

Using global miRNA expression profiling or *in situ* hybridization, several miRNAs (miR-143, -199a_3p, -199a_5p, -100, -99a [43], miR-16-2, -30E, and -200a [44]) have been identified whose expression was associated with reduced overall survival in EAC [43, 44]. A more detailed insight into the relevance of different miRNA expression has been provided recently by Leidner et al. [45] in $n = 20$ EAC samples; next generation sequencing and qRT-PCR identified a total of 26 miRNA that are deregulated in EAC more than

4-fold in >50% of cases compared to normal esophageal squamous tissue. After laser microdissection-based comparison between the steps of BE-EAC-sequence, two miRNAs (miR-31 and -31*) were downregulated in high-grade dysplasia and EAC cases, thus implicating an association with the transition from BE to HGD lesions. Another miRNA (-375) was exclusively down-regulated in EAC, whereas BE and HGD lesions showed normal expression. In a 5-year follow-up study, a different set of miRNAs (miR-192, -194, -196a, and -196b) could be identified in BE samples with progression to EAC compared to patients who did not progress to EAC [46]. The relevance of miRNA-196a as molecular markers associated with the progression from intestinal metaplasia to EAC has also been demonstrated earlier by Luzna et al. [47].

Recently, a link between EMT and miRNA expression in BE or EAC was established in both: Barrett's epithelia and EAC displayed a reduced expression of miRNA-200 family members [108]. These miRNAs take a central position in regulation of the initial step of metastasis by inhibiting the EMT effector transcription factors ZEB-1 and -2 [109].

Taken together, the relevance of miRNA for prognosis and progression of BE and EAC is being unveiled in current research. Final statements require additional studies using independent patient cohort—also with higher case-load—accompanied by functional verification [43, 110].

4. Predictive and Prognostic Factors for GERD, BE, and EAC?

Previous reviews already discussed the importance of biomarkers in this area and proclaimed further investigations thereof in gastroenterological oncology (for review, see Ong et al. [111], Fang et al. [112], and Huang and Hardie [113]). Usually, biomarkers are classified as markers for risk evaluation in patients with GERD to develop EAC or as biomarkers for predictive and prognostic evaluation in patients with diagnosed EAC. Hence, the presented data implicate—and pretend—that we have already reached "the end of the road" with available and significant biomarkers. However, detailed assessment and comparison with other cancers, such as breast, prostate, as well as colorectal [114], reveal them in a rather disillusioning light. Since endoscopic-bioptic surveillance studies yielded no significant benefit for BE patients [31], and prognosis of patients with EAC still is disastrous [24], further intensive experimental and clinical research of (molecular) pathological mechanisms are required urgently.

Based on studies regarding potential predictive and prognostic markers within the GERD-BE-EAC sequence, we classified them into four groups (Table 1 and Figure 1) and illustrated a patient-specific disease sequence (Figure 2; for details, see reviews [111–113, 115]): (A) diagnostic biomarkers—indicate the presence of disease, (B) progression biomarkers—indicate the risk of developing cancer, that is, progression from BE to EAC, (C) predictive biomarkers—predict response to therapy, and (D) prognostic biomarkers—indicate overall survival, that is, prognosis for EAC.

4.1. A = Diagnostic Biomarkers—Indicate the Presence of Disease. The conventional approach for detection and diagnosis is the histochemical analysis of endoscopically derived biopsies of the gastro-esophageal junction, albeit the proposed importance of histological subtypes, the gastric fundus, the cardiac subtype, and the metaplastic columnar epithelium with intestinal-type goblet cells remains unclear [116]. The relevance of these factors has been discussed for years, but prospective studies clarifying the prognostic ability of these histological subtypes are currently not available. Additionally, the trefoil factor 3 (TFF3) combined with a noninvasive diagnostic technique has been investigated intensively in otherwise asymptomatic BE patients [48, 49]. Their results are promising, possibly enabling a selective screening of patients; however, these findings require independent validation and assessment before further clinical application.

4.2. B = Progression Biomarkers—Indicate the Risk of Progression from BE to EAC. Similar to the situation for diagnostic biomarkers (A), the most frequently applied progression marker for clinicians and pathologists is the degree of dysplasia in obtained biopsies. Although the inter- and intra-observer error [10, 29, 30] is extremely unsatisfying, studies confirmed that high-grade dysplasia is associated with a 40% higher risk for progression of BE to EAC [22, 23]. Therefore, a primary goal should be the standardization of criteria for dysplasia based on conventional Haematoxylin-Eosin-stained specimens in order to avoid under- and overdiagnosis [10, 29, 30]. Several molecular markers are evaluated too (see Table 1)—the most promising ones according to their statistical robustness (based on OR and RR) are MCM2 expression pattern (highest OR of about 136, whereby the confidence interval is large, reducing the potency of this marker). Loss of heterozygosity on distinct gene loci, especially at 17p, indicates a high progression probability from BE to EAC. The expression pattern of P53 as well as the hypermethylation of p16 and APC suggests high potency, followed by the cell-cycle-associated proteins Cyclin A and D1. These markers were intensively evaluated within retrospective studies but did not succeed the direct transfer to clinical practice, especially due to cost- and time-intensive experimental work. In our experience, the immunohistochemical evaluation of P16 and P53 is well established in pathological diagnostics, whereby the quantification and standardization remains still an unsolved problem.

4.3. C = Predictive Biomarkers—Predict Response to Therapy. As displayed in Table 1, the number of potential predictive biomarkers is considerably lower than all other categories accompanied by mainly nonsignificant P values. Additionally, biomarkers of category A such as p53 and p16 are also listed in category C, indicating the overall impact of these biomarkers. In sum, the limited number of available and reliable C-markers must be considered as a starting point for inevitable research in the establishment of reliable predictive biomarkers.

4.4. D = Prognostic Biomarkers—Indicate Overall Survival–Prognostic in EAC. It is not surprising that the majority of

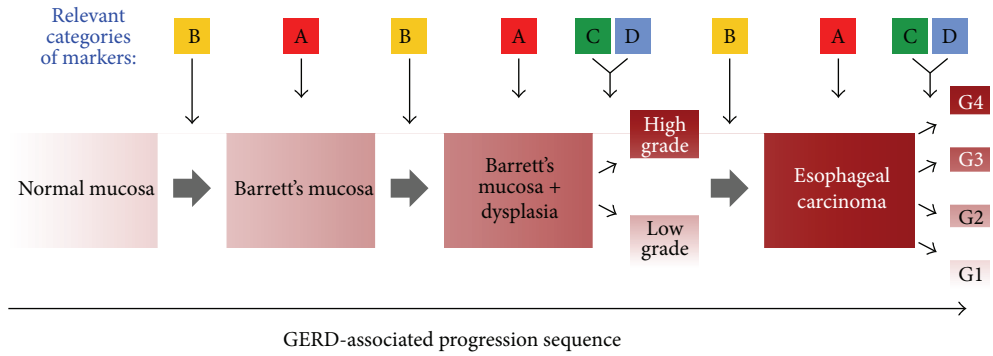

Figure 1: GERD-associated progression for Barrett's esophagus (BE) to esophageal adenocarcinoma (EAC). A–D refer to biomarkers which could be most relevant at the indicated stages of the disease progression (according to Table 1). Therefore A, B, C, and D stand for diagnostic, progressive, predictive, and prognostic biomarkers, respectively.

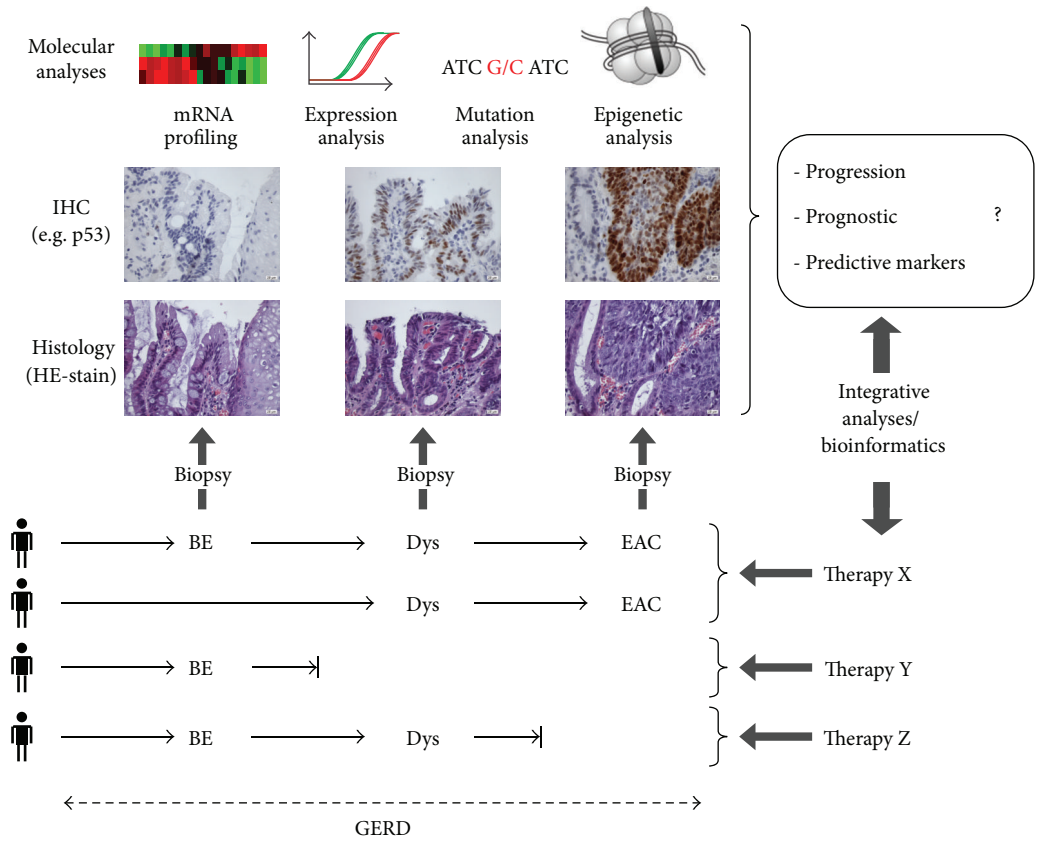

Figure 2: Proposed approach for identification of novel biomarkers for the GERD-BE-AEC sequence. Based on theheterogeneous and patient-specific progression sequence from BE to EAC, the figure indicates the disease stages and mandatory (histology, IHC) and supplementary potential methods for investigation of putative biomarkers for progression, prediction, and prognosis. These data possibly result in an evidence-based stratification of patients for various available therapies (X–Z) based on a rational selection and evaluation of specific biomarkers. Abbreviations. Esophageal adenocarcinoma: AEC; dysplasia: Dys; fluorescence *in-situ* hybridization: FISH; gastro-esophageal reflux disease: GERD; immunohistochemistry: IHC.

biomarkers are listed in the last category—displaying the typical survey of hallmarks of cancer [39] reaching from self-sufficiency in growth signals (Cyclin D1, EGFR, Ki-67, Her2/neu, TGF-α), insensitivity to growth inhibitory signals (TGF-β1, APC, P21), evasion of programmed cell death (Bcl-2, COX-2, NF-κB), limitless replicative potential (Telomerase), sustained angiogenesis (CD105, VEGF), invasion and metastasis (Cadherin, uPA, TIMP), tumor differentiation

Table 1: Summary of investigated and published biomarkers in the GERD-BE-EAC axis. The categorization is based on four groups according to their potential usage as A = Diagnostic Biomarker indicates the presence of disease, B = Progression Biomarker indicates the risk of developing cancer—progression in BE to EAC, C = Predictive Biomarker predicts response to therapy (CTX, RTX, photodynamic therapy), or D = Prognostic Biomarker indicates overall survival—prognostic in EAC (survival, recurrence).

	Biomarker	Method	Remarks/findings	OR/RR/*P* value	Refs
A = Diagnostic Biomarker	TFF3	IHC, esophageal cytosponge samples for BE combined with IHC for TFF3	novel nonendoscopic screening modality in a prospective cohort study	$P = 0.02$ (for maximal length of BE) $P = 0.009$ (for circumferential length of BE)	[48]
	TFF3		biomarker to screen asymptomatic patients for BE; TFF3 protein was expressed at the luminal surface of BE (not at normal esophageal or gastric mucosa)	$P < 0.0001$	[49]
	Chromosomes 7 and 17 (copy number changes)	ICDA & FISH	chromosomal gains in early stages of BE; valuable adjunct to conventional cytology to detect dysplasia or EAC	IND/LGD: 75% sensitivity, (76% specificity) HGD/EAC: 85% sensitivity, (84% specificity)	[50]
	8q24 (*C-MYC*), 17q12 (*HER2*), and 20q13 (copy number changes)	FISH	chromosomal gains in early stages of BE; represents a valuable adjunct to conventional cytology to detect dysplasia or EAC	LGD (50% sensitivity) HGD (82% sensitivity) EAC (100% sensitivity)	[51]
	17q11.2 (*ERBB2*)	Southern blotting, microarray analysis	amplified copies of the *ERBB2* gene in EAC	10-fold amplification in 3 of 25 (12%) tumors	[52]
	Serum proteomic pattern analysis	mass spectrometry	several limitations due to applied technology	identified 10 of 11 normal's; and 42 of 43 EAC's correctly	[53]
B = Progression Biomarkers	P53 positivity	IHC	limited efficacy as a single progression biomarker	OR 11.7 (95% CI: 1.93–71.4)	[54]
	P53 positivity	IHC	positive in 4/31 that regressed, 3/12 that persisted, and 3/5 that progressed to HGD or EAC	RR not available	[55]
	DNA content abnormalities	flow cytometry	higher relative risk for EAC in patients with tetraploidy (4N) or aneuploidy (>6%)	tetraploidy: RR 7.5 (95% CI: 4–14) ($P < 0.001$) aneuploidy: RR 5.0 (95% CI: 2.7–9.4) ($P < 0.001$)	[56]
	DNA content abnormalities	flow cytometry	4N fraction cut point of 6% for cancer risk aneuploid DNA contents of 2.7N were predictive of higher cancer risk presence of both 4N fraction of 6% and aneuploid DNA content of 2.7N is highly predictive for progression	RR 11.7 (95% CI: 6.2–22) RR 9.5 (95% CI: 4.9–18) RR 23 (95% CI: 10–50)	[57]
	LOH of 157p and 9p and DNA content abnormalities	flow cytometry, PCR	17p(p53) LOH associated with higher risk of progression to HGD + EAC	HGD: RR 3.6 ($P = 0.02$) EAC: RR 16 ($P < 0.001$)	[58]
		flow cytometry, PCR	combined LOH of 17p and 9p and DNA content abnormalities can best predict progression to EAC LOH of 17p alone LOH of 9p alone Aneuploidy alone Tetraploidy alone	RR 38.7 (95% CI: 10.8–138.5) not clinical applicable RR 10.6 (95% CI: 5.2–21.3) RR 2.6 (95% CI: 1.1–6.0) RR 8.5 (95% CI: 4.3–17.0) RR 8.8 (95% CI: 4.3–17.7)	[59]

Table 1: Continued.

	Biomarker	Method	Remarks/findings	OR/RR/*P* value	Refs
	mutations of *p16* and *p53* loci (clonal diversity measurements)	flow cytometry, PCR	significant predictors for EAC progression, not clinical applicable	$P = 0.001$	[60]
	EGFR	IHC	overexpression in HGD/EAC	35% of HGD/80% of EAC specimens	[61]
	MCM2	IHC	correlation between degree of dysplasia and level of ectopic luminal surface MCM2 expression	MCM2-positive staining in 42% (19/45) of BE samples	[62]
	Cyclin A	IHC	surface expression of cyclin A in BE samples correlates with the degree of dysplasia	OR 7.5 (95% CI: 1.8–30.7) ($P = 0.016$)	[63]
	Cyclin D1	IHC	association with increased risk of EAC	OR 6.85 (95% CI: 1.57–29.91)	[64]
	hypermethylation of *p16* (CDKI2A)	RT-PCR	association with increased risk of progression to HGD/EAC	OR 1.74 (95% CI: 1.33–2.2)	[41]
	hypermethylation of *RUNX3*		association with increased risk of progression to HGD/EAC	OR 1.80 (95% CI: 1.08–2.81)	
	hypermethylation of HPP1		association with increased risk of progression to HGD/EAC	OR 1.77 (95% CI: 1.06–2.81)	
	hypermethylation of *p16* and APC	PCR	predictor of progression to HGD/EAC	OR 14.97 (95% CI: 1.73–inf.)	[65]
	8 gene methylation panel	RT-PCR	age dependent; predicts 60.7% of progression to HGD/EAC within 2 yrs	RR not available (90% specificity)	[66]
	Gene expression profile	microarray analysis	64 genes up regulated 110 genes down regulated in EAC	$P = 0.05$	[67]
	Cathepsin D, AKR1B10, and AKR1C2 mRNA levels	Western blotting, qRT-PCR	dysregulation predicts progression to HGD/EAC	AKR1C2: ↑ levels in BE ($P < 0.05$) but ↓ levels in EA ($P < 0.05$)	[68]
	DNA abnormalities	ICDA	aneuploidy predicts progression to EAC	60% with LGD; 73% with HGD, and 100% with EAC (total number of samples = 56)	[69]
		ACIS	frequency and severity of aneuploidy predicts progression to EAC	unstable aneuploidy in 95% with EAC	[70]
		DICM	relationship between DICM status and progression to HGD/EAC	$P < 0.0001$	[71]
	SNP-based genotyping in BE/EAC specimens	flow cytometry, 33K SNP array	copy gains, losses, and LOH increased in frequency and size between early and late stage of disease	$P < 0.001$ (BE)	[72]
C = Predictive Biomarkers	*p16* allelic loss	FISH	decreased response to photodynamic therapy	OR 0.32 (95% CI: 0.10–0.96)	[73]
	DNA ploidy abnormalities	ICDA	DNA ploidy as a covariate value for recurrence	HR 6.3 (1.7–23.4) ($P < 0.0015$)	[74]
	HSP27	IHC	association between low HSP27 expression and no response to neoadjuvante chemotherapy	$P = 0.049$ and $P = 0.032$	[75]
	Ephrin B3 receptor	microarray	response prediction in EAC in patients with Ephrin B3 receptor positive versus Ephrin B3 receptor negative	Response rate <50%: 3 (15.8) versus 16 (84.2) ($P < 0.001$)	[76]
	Genetic polymorphisms	qRT-PCR	association between individual single nucleotide polymorphisms and clinical outcomes	comprehensive panel of genetic polymorphisms on clinical outcomes in 210 esophageal cancer patients	[77]

Table 1: Continued.

	Biomarker	Method	Remarks/findings	OR/RR/P value	Refs
	P21	IHC	alteration in expression correlated with better CTX-response	$P = 0.011$	[78]
	P53	IHC	alteration in expression correlated with better CTX-response	$P = 0.011$	[79]
	ERCC1	IHC	ERCC1-positivity predicts CTX-resistance and poor outcome	$P < 0.001$	[80]
D = Prognostic Biomarkers	DCK PAPSS2 SIRT2 TRIM44	RT-PCR, IHC	prognostic 4-gene signature in EAC predicts 5-year survival	0/4 genes dysregulated: 58% (95% CI: 36%–80%) 1-2/4 genes dysregulated: 26% (95% CI: 20%–32%) 3-4/4 genes dysregulated: 14% (95% CI: 4%–24%) ($P = 0.001$)	[81]
	p16 loss *C-MYC* gain	FISH	association between therapy response status and FISH positivity	$P = 0.04$	[82]
	ASS expression	microarrays	low expression correlates with lymph node metastasis	$P = 0.048$	[83]
	microRNA expression profiles	miRNA microarray, qRT-PCR	association with prognosis (e.g. low levels of mir-375 in EAC $\rightarrow$ worse prognosis)	HR = 0.31 (95% CI: 0.15–0.67) ($P < 0.005$)	[84]
	Genomic alterations	MLPA	reverse association between survival and DNA copy number alterations (>12 aberrations $\rightarrow$ low mean survival)	$P = 0.003$	[85]
	Cyclin D1	FISH, IHC	2 of 3 genotypes confers to $\downarrow$ survival	$P = 0.0003$	[86]
	EGFR	IHC	expression = $\downarrow$ survival	$P = 0.07$	[87]
		IHC	$\downarrow$ expression = $\downarrow$ survival	$P = 0.034$	[88]
	Ki-67	IHC	low levels of staining (<10%) = $\downarrow$ survival	$P = 0.02$	[89]
	Her2/neu	FISH	amplification = $\downarrow$ survival	$P = 0.03$	[90, 91]
	TGF-α	IHC	low levels = $\downarrow$ survival	$P = 0.03$	[92]
		IHC, ISH	high levels = tumor progression and lymph node metastasis	$P = 0.025$ and $P < 0.05$	[93]
	TGF-β1	qRT-PCR	overexpression = $\downarrow$ survival	$P = 0.0255$	[94]
		ELISA	high plasma levels = $\downarrow$ survival	$P = 0.0317$	[95]
	APC	RT-PCR	high plasma levels of methylation = $\downarrow$ survival	$P = 0.016$	[96]
	Bcl-2	IHC	expression = $\downarrow$ survival	$P = 0.03$	[97]
		IHC, RT-PCR	$\uparrow$ expression = $\downarrow$ survival, $\uparrow$ TN-stage, and recurrence	$P < 0.001$, $P = 0.008/0.049$, and $P = 0.01$	[98]
	COX-2	IHC	strong staining = $\downarrow$ survival	$P = 0.03$	[99]
		IHC	strong staining = $\downarrow$ survival, distant metastasis, and recurrence	$P = 0.002$, $P = 0.02$, and $P = 0.05$	[100]
	NF-κB	IHC	activated NF-κB = $\downarrow$ survival, and $\downarrow$ disease free survival	$P = 0.015$ and $P = 0.010$	[101]
	Telomerase	Southern blot analysis, RT-PCR	higher telomere-length ratio = $\downarrow$ survival	RR of death: 3.4 (CI: 1.3–8.9) ($P < 0.02$)	[102]

Table 1: Continued.

Biomarker	Method	Remarks/findings	OR/RR/P value	Refs
CD105	IHC	expression = ↓ survival,	$P < 0.01$	[103]
		angiolymphatic invasion	$P < 0.05$	
		↑ lymph node metastasis	$P < 0.01$	
		↑ T-stage	$P < 0.001$	
		↑ distant metastasis	$P < 0.01$	
VEGF		↑ expression = ↓ survival,	$P < 0.01$	
		angiolymphatic invasion	$P < 0.05$	
		↑ lymph node metastasis	$P < 0.01$	
		↑ T-stage	$P < 0.01$	
		↑ distant metastasis	$P < 0.01$	
Cadherin	IHC	↓ level = ↓ survival	$P = 0.05$	[89]
uPA	ELISA	↑ uPA = ↓ survival	$P = 0.0002$	[104]
TIMP	IHC, RT-PCR	↓ expression = ↓ survival, and ↑ disease stage	$P = 0.007$ and $P = 0.046$	[105]
Promoter hypermethylation of multiple genes	IHC, methylation specific PCR	if >50% of gene profile methylated = ↓ survival, and earlier recurrence	$P = 0.05$ and $P = 0.04$	[106]
MGMT hypermethylation	IHC, methylation specific PCR	correlation with higher tumor differentiation	$P = 0.0079$	[107]

ACIS: automated cellular imaging system; ASS: argininosuccinate synthase; APC: adenomatous polyposis coli; BE: barrett's esophagus; COX: cyclooxygenase; DCK: deoxycytidine kinase; DICM: digital image cytometry; EAC: esophageal adenocarcinoma; EGFR: epidermal growth factor receptor; ELISA: enzyme-linked immunosorbent assay; FISH: fluorescence *in-situ*-hybridization; ICDA: image cytometric DNA analysis; HSP27: Heat-shock protein 27; IHC: immunohistochemistry; LOH: loss of heterozygosity; PAPSS2: 3′-phosphoadenosine 5′-phosphosulfate synthase 2; PCR: polymerase chain reaction; qRT: quantitative reverse transcriptase; MLPA: multiplex ligation dependent probe amplification; NF-κB: nuclear factor kappa B; SIRT2: Sirtuin 2; SNP: single nucleotide polymorphism; TFF3: Trefoil factor 3; TGF: transforming growth factor; TIMP: tissue inhibitors of metalloproteinases; TRIM44: Tripartite motif-containing 44; uPA: urokinase-type plasminogen activator; VEGF: vascular endothelial growth factor.

Table 2: Synopsis of biomarkers in the GERD-BE-EAC axis. According to Table 1, most promising biomarkers are summarized indicating that only dysplasia is involved in all four categories. Dysplasia can be used as diagnostic biomarker as well as to assess the risk of progression to EAC or response to therapy and is associated with poor survival (↓ survival).

	Dysplasia	P53	P16	P21	Growth factors	Cell cycle
A = Diagnostic Biomarker	✓					
B = Progression Biomarker	✓	✓	✓	✓	✓	✓
C = Predictive Biomarker	✓	✓	✓	✓		
D = Prognostic Biomarker	↓ survival		✓		↓ survival	↓ survival

(MGMT), and cancer-related inflammation (NF-κB, COX-2) (see Table 1). Beside their functional heterogeneity, their applicability for prognosis is uncertain. How to use which markers and when? Should we use a panel of markers? The primary and secondary literature currently gives no further advice to solve this problem. Although high levels of significance could be achieved using these biomarkers ($P < 0.001$), the practicability and efficiency in daily routine is unknown. This observation is supplemented by the fact that the most applicable approach for prognostic stratification in EAC is based on the TNM system using conventional basic clinical and pathological findings of tumor extension as well as local and distant metastasis in lymph nodes and organs [117]. Therefore, intensive statistical analysis of comprehensive sets of EAC samples accompanied by selected biomarkers must be performed using factor or hierarchical cluster analysis to evaluate the best prognostic combination of biomarkers.

To assemble the sometimes confusing data on possible biomarkers (as listed in Table 1) in one point, the histological confirmation of "dysplasia" seems to be unique indicating the "limitation or limited outcome" of our biomarker repertoire (see Table 2). Nevertheless, we should keep in mind that BE is frequently under- and over-diagnosed resulting in huge inter- and intra-observer errors [10, 29, 30], thus demanding for detailed and decisive morphological criteria. From the set of molecular markers, "only" p53, p16, and p21 currently represent applicable biomarkers, especially for progression. Interestingly, growth factors and cell cycle associated factors are relevant for prognosis, but it seems impossible to highlight one exclusively out of the "myriad" of biomarkers [118].

Finally, two major questions arise and are still unsolved: (i) why are proposed biomarkers not (yet) really embedded in clinical routine, and (ii) what impairs the identification of more reliable and significant biomarkers?

First of all, two major limitations are the technical and financial aspects. Special molecular biological techniques require fresh frozen samples; DNA-, RNA-extraction, and nucleic acid amplification as well as subsequent hybridization or sequencing are time-consuming and need special facilities which are, again, cost intensive. Additionally, validation of specific methods to detect genetic and epigenetic alterations is still not completed. In conclusion, costs and practicability of these biomarkers are the limiting factors until now [111].

Possible answers to the second question are that more relevant entities like inflammation or epithelial-mesenchymal-transition (EMT), which have yet not been completely considered, should be integrated in the evaluation-process of biomarkers for GERD, BE and EAC.

The potential role of the localized inflammation in disease prediction and prognosis is currently rather underestimated in experimental and clinical investigations. Generally, it has been shown that inflammation influences cancerogenesis by key mediators including reactive oxygen species (ROS), NF-κB, inflammatory cytokines, prostaglandins, and specific microRNAs (miRNAs) [119]. Poehlmann et al. comprehensively reviewed the role of inflammation on genetic and epigenetic changes in BE and EAC focusing on oxidative stress and the NF-κB-pathway [120]. Beside NF-κB and COX-2 (see Table 1), other transmitters of inflammation like chemokines or cytokines should be investigated as possible biomarkers.

Additionally, the process of EMT with its key players Snail, Twist, and ZEB and their repressed target protein E-Cadherin is essentially linked to development, regeneration, inflammation, and cancerogenesis [121]. Several ontogenic pathways (e.g., WNT-, Hedgehog-, or Notch-signaling) are involved in EMT regulation and have also been associated with pathogenesis of BE to EAC as reviewed by Chen et al. [38]. Furthermore, increased expression of SLUG is associated with progression of EAC by consecutive repression of E-Cadherin indicating a role of EMT in EAC. Therefore, subsequent clinical trials have to be set up to elucidate distinct mechanisms of EMT in the pathogenesis of or as specific biomarkers in BE or EAC [122].

5. Approach and Outlook

The probability to find one single specific biomarker providing all diagnostic, predictive, and prognostic significance in GERD, BE, and/or EAC is rather utopian, and a panel of biomarkers maybe will solve this problem [81, 123, 124]. Upcoming new technologies such as RNA and DNA microarrays, epigenetics, and proteomics in association with bioinformatics give hope to find novel and reliable biomarkers in gastrointestinal tumors and especially for prognosis and prediction of BE and EAC [114]. These technologies may provide insights in this rather complex sequence of GERD-BE-EAC; for instance, Kaz et al. [125] stratified BE and EAC by methylation signatures and molecular subclasses using DNA methylation profiling. Interestingly, the authors found an increase of methylation during disease progression—supporting the postulated GERD-BE-EAC sequence and promoting studies of biomarkers based on epigenetic mechanisms which are specific for particular steps in the pathogenetic sequence. Additionally, miRNA profiling by Ko et al. [126] discovered five miRNAs which are significantly expressed in patients with EAC with and without complete remission after therapeutic interventions, whereby the connection of these interesting data to other prognostic/predictive biomarkers in EAC has not been performed. As mentioned by Jankowski and Odze [114], the new technologies are associated with "specific" limitations; RNA and DNA array techniques are retrospective and are frequently lacking phenotype controls. Epigenetic experimental approaches often showed an overlap of methylation pattern between normal and precancerous tissues with no possibility of discrimination between them. Proteomics is time-consuming and not applicable for daily routine work. This seems also true for bioinformatics' techniques.

As depicted in Figure 2, every stage of disease demands intensive morphological, genetic, as well as epigenetic analysis and consequently an exorbitant research effort due to the heterogeneity within the GERD-BE-EAC sequence. However, only consistent generating of data from patients with GERD, GERD with BE, GERD with EAC, or GERD with BE and EAC will allow integrative analysis and research, even if this implies that patients with GERD will be under consecutive, perhaps lifelong surveillance. Therefore, consolidation and evaluation of our intensive but partial not coherent findings regarding the "puzzle" of GERD-BE-EAC represent the first steps to discover the best biomarkers for diagnosis, therapy, and prognosis.

Abbreviations

ACIS: Automated cellular imaging system
ASS: Argininosuccinate synthase
APC: Adenomatous polyposis coli
BE: Barret's esophagus
COX: Cyclooxygenase
DCK: Deoxycytidine kinase
DICM: Digital image cytometry
EAC: Esophageal adenocarcinoma
EGFR: Epidermal growth factor receptor
ELISA: Enzyme linked immunosorbent assay
EMT: Epithelial-mesenchymal-transition
FHIT: Fragile histidine triad protein
FISH: Fluorescence *in-situ*-hybridization
GERD: Gastro-esophageal reflux disease
ICDA: Image cytometric DNA analysis
HSP27: Heat-shock protein 27
IHC: Immunohistochemistry
LOH: Loss of heterozygosity
PAPSS2: 3′-phosphoadenosine 5′-phosphosulfate synthase 2

PCR: Polymerase chain reaction
qRT: Quantitative reverse transcriptase
MDM2: Mouse double minute 2 homolog
MLPA: Multiplex ligation dependent probe amplification
NF-κB: Nuclear factor kappa B
Rb: Retinoblastoma
SIRT2: Sirtuin 2
SNP: Single nucleotide polymorphism
TFF3: Trefoil factor 3,
TGF: Transforming growth factor
TIMP: Tissue inhibitors of metalloproteinases
TRIM44: Tripartite motif-containing 44
uPA: Urokinase-type plasminogen activator
VEGF: Vascular endothelial growth factor.

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Clinical Study

The Diagnostic Significance of Coapplying a Rabeprazole Test with the SF-36 for Gastroesophageal Reflux Disease

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Gastroesophageal reflux disease is a diversity disease that affects life quality of people in the world. Due to the complicated pathogenesis and variations in clinical manifestations, there is still no true gold standard for GERD diagnosis, and it is still difficult to diagnose this disease in some patients. The proton pump inhibitor's diagnostic test (the PPI test) is noninvasive, of low cost, tied to treatment, and widely accepted. Our aim is to evaluate the diagnostic significance of coapplying a rabeprazole test with the SF-36 for GERD in this study. Our study shows that the SF-36 in combination with the rabeprazole test can screen GERD patients and increase the sensitivity and specificity of GERD diagnosis through reference to the change in SF-36 score before and after the treatment (65 in the trial).

1. Introduction

Excessive gastroesophageal reflux can cause damage to the esophageal, throat, and even tracheal mucosa. Patients with gastroesophageal reflux disease (GERD) present with symptoms of heartburn, sour regurgitation, vomiting, onset retrosternal pain, and dysphagia. Bodily pain may influence mental status and is negatively associated with the patients' work, study, and social intercourse. GERD has become a critical digestive system disease that strongly influences the quality of life [1].

GERD diagnoses are primarily based on reflux symptoms, 24-hour esophageal pH monitoring, and endoscopy. However, these tests have limitations. NERD (nonerosive reflux disease) patients account for 60% to 70% of GERD patients [2] and have no signs of esophagitis in endoscopy. Therefore, negative endoscopic results cannot exclude GERD, which decreases the sensitivity of endoscopy [3]. Previously, 24-hour esophageal pH monitoring was used as the "gold standard" for GERD diagnosis. However, 25% of patients with typical reflux esophagitis (RE) have normal acid exposure [4, 5]. Therefore, the value of 24-hour pH monitoring for diagnosing GERD is decreased. Moreover, the invasiveness of this technique limits its application in clinical practice. In recent years, novel testing technologies and methods have been continuously developed, including the proton pump inhibitor (PPI) diagnostic test, the reflux questionnaire, and esophageal impedance monitoring, all of which have dramatically improved GERD diagnosis. However, due to the complicated pathogenesis and variations in clinical manifestations, there is still no true gold standard for GERD diagnosis, and it is still difficult to diagnose this disease in some patients.

The proton pump inhibitor's diagnostic test (the PPI test) is noninvasive, of low cost, tied to treatment, and widely accepted [6, 7]. It has been reported that the sensitivity of the PPI test is 70% to 80% and the specificity is 55% to 85% [8]. GERD's complicated pathogenesis, however, results in poor sensitivity in certain patients. Application of the PPI test for diagnosing these patients is limited, and other diagnostic methods are required.

Table 1: Characteristics of three groups.

	Age (years)	LESP (mmHg)	UESP (mmHg)	SI (%)
A group	43.13 ± 13.11	15.90 ± 7.24	42.19 ± 13.17	49.09 ± 35.13
B group	48.03 ± 11.41	17.77 ± 7.40	48.63 ± 19.97	46.86 ± 33.90
C group	41.23 ± 13.62	17.85 ± 6.87	44.47 ± 20.49	54.47 ± 35.75
*P*value	0.109	0.496	0.385	0.687

The reflux questionnaire is convenient and economical within a certain range of sensitivity and specificity [9, 10]. Some studies illustrate that coapplying symptom scoring alongside the PPI test could increase specificity to 91% [11]. However, current questionnaire surveys have different focuses and vary in diagnostic values. Strong subjectivity, overly brief symptomatic scores, and few scoring levels make questionnaire surveys unable to sensitively reflect changes in health status and reach clinical requirements.

The 36-item short-form health survey (SF-36) is the most common method in the standardized measurement of the quality of life and has been widely used in a series of studies because of its excellent reliability, validity, sensitivity, and feasibility. Recently, the quality of life for GERD patients has been studied more and more [12–17].

In this study, we utilized a combination of the PPI test and the SF-36 to offer an important basis for GERD diagnosis, particularly for NERD patients. This approach increases the diagnostic efficiency and the cost-efficacy ratio of GERD diagnoses.

2. Materials and Methods

2.1. Subjects. A total of 90 patients were enrolled from September 2008 to December 2009. The inclusion criteria were as follows: (1) the patient visited due to epigastric discomfort, including sour regurgitation, vomiting, heart burn, and retrosternal pain, and was suspected to have GERD; (2) the patient was male or female, aged 18 to 70 years; (3) the patient was educated at least as far as elementary school and could read and fill out the questionnaire independently; (4) the patient had not been treated with nonsteroidal antiinflammatory drugs, PPI, H^2-receptor antagonists, anticholinergic agents, antibiotics, or prokinetics in the four weeks prior to visiting; (5) the patient provided written informed consent; (6) an RDQ score ≥ 6 was taken as a basic criterion.

Exclusion criteria were as follows: (1) the patient could not tolerate endoscopy or 24-hour esophageal pH monitoring; (2) the patient had other digestive tract diseases and systemic diseases that could induce digestive discomfort, such as diabetes mellitus, systemic sclerosis, or nervous system diseases; (3) the patient had a history of gastroesophageal surgeries, esophageal stenosis, digestive ulcers, and esophageal or gastric tumors; (4) the patient was pregnant, nursing, or suffered from severe cardiac, hepatic, or renal insufficiency.

All the enrolled patients filled out the SF-36 questionnaire under the direction of trained gastroenterologists. They received endoscopy and 24-hour esophageal pH monitoring. Patients with esophageal erosion in the endoscopy were graded according to the Los Angeles (LA) classification. Patients with a positive result on one of the two measures (endoscopy or 24-hour esophageal pH monitoring) were considered to have GERD; otherwise, they were classified as non-GERD.

2.2. Methods. Ninety patients were randomly and double-blindly divided into Group A ($n = 30$, rabeprazole 20 mg b.i.d. for 2 weeks), Group B ($n = 30$, rabeprazole 10 mg b.i.d. for 2 weeks), and Group C ($n = 30$, placebo for the 1st week and rabeprazole 10 mg b.i.d. for the 2nd week). The drugs were taken orally twice a day, 15 to 30 minutes before meals. All drugs and placebos used in the study were provided by Xian-Janssen Pharmaceutical Ltd.

All the agents were delivered one week before the treatment. The SF-36 and RDQ measurement scales were administered before therapy, one week after therapy, and two weeks after therapy.

3. Results

3.1. General Information. The ninety included patients (mean age 44.13 ± 12.71 years) consisted of fifty-nine men and thirty-one women (the male-to-female ratio approached 2 : 1), of which thirty-three (36.7%) showed negative results in endoscopy and fifty-seven (63.3%) had RE. They were classified according to LA: 31 for REA, 23 for REB, 3 for REC, and 0 for RED. No significant differences were noted between the three groups in sex, age, disease severity, lower esophageal sphincter pressure (LESP), upper esophageal sphincter pressure (UESP), and symptom index (SI) ($P > 0.05$) (Table 1).

3.2. Comparisons of SF-36 Scores before and One and Two Weeks after the Treatment. There were no significant differences among three groups in the pretreatment, one week and two weeks after treatment ($P > 0.05$). And there were significant differences between pretreatment and two weeks after treatment in each group ($P < 0.05$). Further analysis found that SF-36 scores showed significant differences between GERD and non-GERD patients in group A two weeks after treatment, but not between GERD and non-GERD patients before one and two weeks after the treatment in Groups B and C ($P > 0.05$) (Table 2).

3.3. Comparison of Improving SF-36 Scores between GERD and Non-GERD Patients. The differences were significant in improving SF-36 scores between GERD and non-GERD patients in Groups A and B ($P < 0.05$) but not significant between GERD and non-GERD patients in Group C ($P = 0.085$) after one week of therapy. Significant differences were

Table 2: Comparisons of SF-36 scores before and one and two weeks after the treatment.

	Before treatment	One week after treatment	Two weeks after treatment	P value
A group	551.81 ± 102.90	645.87 ± 74.54	691.63 ± 66.23	<0.00
B group	480.49 ± 114.98	615.37 ± 84.75	650.98 ± 91.23	<0.00
C group	517.47 ± 100.14	623.82 ± 74.97	686.45 ± 53.46	<0.00
P value	0.058	0.302	0.064	

Table 3: Comparison of improving SF-36 scores between GERD and non-GERD patients after one-week and two-week therapy.

	One week after therapy		P value	Two weeks after therapy		P value
	GERD	Non-GERD		GERD	Non-GERD	
A group	129.76 ± 44.84	10.52 ± 20.19	$P < 0.00$	181.10 ± 79.09	43.26 ± 30.40	<0.00
B group	144.26 ± 60.37	73.88 ± 38.88	$P = 0.031$	144.26 ± 60.37	73.88 ± 38.88	0.038
C group	114.88 ± 49.77	72.22 ± 63.11	$P = 0.085$	185.59 ± 73.55	102.55 ± 98.78	0.028

noted between GERD and non-GERD patients in all three groups after two weeks of therapy ($P < 0.05$) (Table 3).

3.4. Comparison of Improvement Rate of SF-36 Scores between GERD and Non-GERD Patients after Treatment. There were significant differences in improvement rates for SF-36 scores between GERD and non-GERD patients in Group A ($P = 0.006$) but not significant differences between GERD and non-GERD patients in Groups B and C ($P > 0.05$) after one week of therapy; significant differences were noted between GERD and non-GERD patients in Group A ($P = 0.037$) but not in Groups B and C after two weeks of therapy ($P > 0.05$) (Table 4).

3.5. Value of the SF-36 in Diagnosing GERD Prior to Treatment. A receiver operating characteristic (ROC) curve was plotted on the basis of pretreatment SF-36 scores. The area under the ROC (Az) was 0.27, indicating poor diagnostic value. Therefore, it was not suitable for diagnosing GERD (Figure 1).

3.6. Effects of Rabeprazole Dose and Treatment Course on Coincident Rate of the Rabeprazole Test. The scores decreased in group A (40 mg/day) and group B (20 mg/day). However, the differences in sensitivity, specificity, and coincident rate were not significant from the perspective of diagnostic efficacy ($P = 0.095$, 0.117, resp.). Significant differences were also not noted in overall coincident rate between one-week and two-week treatment in Group A (40 mg/day) and Group B (20 mg/day) ($P = 0.688$, 0.774, resp.) (Tables 5 and 6).

3.7. Value of Coapplying the Rabeprazole Test and SF-36 in Diagnosing GERD. The 95% confidence intervals (CI) of Az after one- and two-week treatment overlapped, illustrating that the diagnostic value after one week and two weeks did not differ significantly (Figure 2). Results were judged using different cut-off values according to the decreasing score within 1 week. Lastly, a score of 65 was taken as the cut-off value in line with the maximal principle of the Youden index (Figure 3), within which the sensitivity and specificity were optimal for the GERD screening.

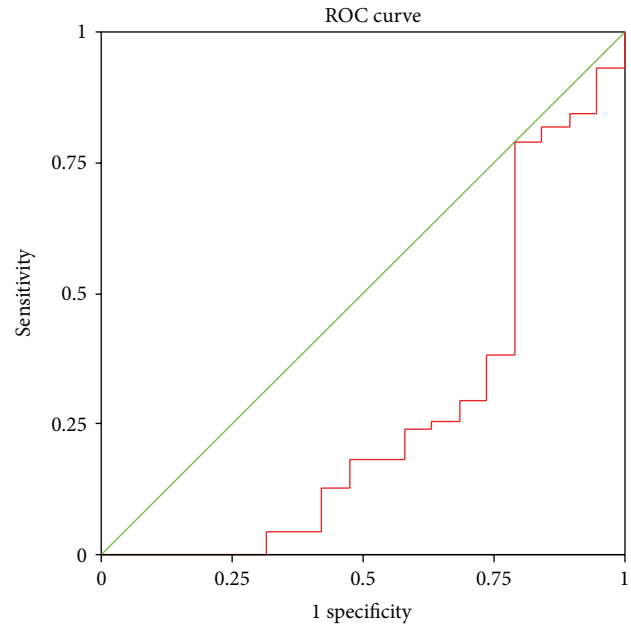

Figure 1: ROC of SF-36 scores prior to treatment.

3.8. Logistic Regression Analysis of Coapplying the Rabeprazole Test and SF-36. The area above the ROC was 0.884 (95% CI, 0.778–0.991, $P < 0.001$), illustrating that this score screening yields excellent reliability for integrative screening (Figure 4).

At $P = 0.606$, the Youden index was highest, and the sensitivity was negatively associated with the specificity. This value can be defined as the threshold for screening tests (Figure 5).

3.9. The Sensitivity and Specificity of the Rabeprazole Test Combined with SF-36 in Diagnosing GERD when $P = 0.606$. Based on the above results, rabeprazole 10 mg b.i.d. was applied in the PPI test. An increment of 65 score units from pretreatment to posttreatment was taken as the standard for a GERD diagnosis. Thus, of 71 patients with GERD, 67 were diagnosed with GERD and 4 were excluded from GERD; the false negative rate was 5.6%. Of 19 non-GERD patients, 4 were

Table 4: Comparison of improving SF-36 rate between GERD and non-GERD patients after one-week and two-week therapy.

	One week after therapy (%)		P value	Two weeks after therapy (%)		P value
	GERD	Non-GERD		GERD	Non-GERD	
A group	28.32 ± 25.78	2.26 ± 3.68	0.006	40.66 ± 43.87	8.24 ± 6.85	0.037
B group	36.22 ± 27.60	13.63 ± 6.38	>0.05	45.58 ± 35.25	16.76 ± 8.83	>0.05
C group	25.22 ± 17.43	16.13 ± 18.62	>0.05	42.35 ± 29.46	24.07 ± 31.62	>0.05

Table 5: Comparison of rabeprazole tests of different doses and treatment courses.

Administration duration	Dose	Sensitivity	Specificity	Coincident rate
One week	20 mg	85.7%	44.5%	73%
	40 mg	88.4%	63.4%	81%
Two weeks	20 mg	90.4%	33.3%	80%
	40 mg	92.3%	50.0%	83%

Table 6: Comparison of coincident rates of rabeprazole tests of different doses and treatment courses.

Dose	Treatment course		
	One week	Two weeks	P value
20 mg	73%	80%	0.688
40 mg	81%	83%	0.774
P value	0.095	0.117	—

diagnosed with GERD and 15 were excluded from GERD; the false positive rate was 21.1% (Table 7).

4. Discussion

A randomized, double-blind, and controlled design was adopted in this trial. The physicians who were responsible for endoscopy, 24-hour esophageal pH monitoring, and administering the questionnaire survey were relatively independent. After tests, another physician performed the statistical analysis in order to guarantee the objectivity and validity of tests.

Results showed that the 90 patients consisted of 59 males and 31 females, with the male-to-female ratio approaching 2 : 1. This ratio is similar to previous reports and may be due to histories of smoking, and drinking. Reportedly [17], drinking, smoking, obesity and overeating are major risk factors for GERD. No significant differences were noted in age, LESP, UESP, and SI among the three groups ($P > 0.05$), suggesting that patients in various groups were comparable after randomized and double-blinded grouping.

Group A (40 mg/day for two weeks), Group B (20 mg/day for two weeks), and Group C (placebo for the 1st week and rabeprazole 20 mg/d for the 2nd week) were designed to investigate the effects of PPI in different doses and treatment duration on test results. Results indicate that SF-36 scores had no significant differences between pretreatment and after one or two weeks of treatment, which may be attributed to non-GERD patients mingling between all the groups. Therefore,

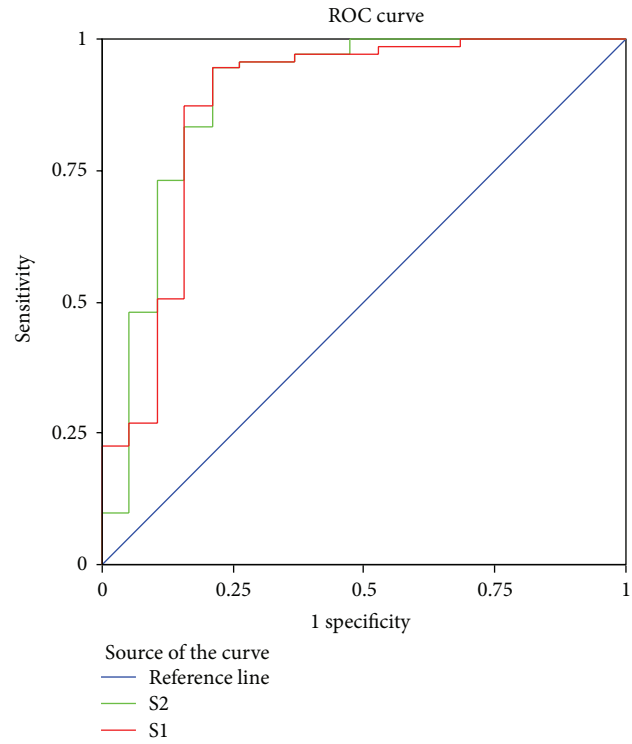

Figure 2: ROC of changes of SF-36 scores after one-week or two-week treatment.

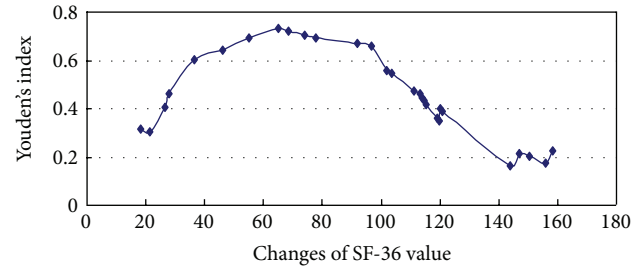

Figure 3: Changes of Youden's index in GERD patients.

Table 7: Sensitivity and specificity of the rabeprazole test combined with SF-36 in diagnosing GERD when $P = 0.606$.

Screening results	GERD		Non-GERD		Total	
	Number of patients	95% CI (%)	Number of patients	95% CI (%)	Number of patients	95% CI (%)
+	67	94.4 (89.1–99.7) Sensitivity	4	21.1 (2.7–39.4) False positive	71	94.3 (88.9–99.7) Positive predictive value
−	4	5.6 (0.2–10.9) False negative	15	78.9 (60.5–97.2) Specificity	19	67.8 (60.6–97.2) Negative predictive value
Total	71	100	19	100	90	91.1 (85.2–96.9) Coincident rate

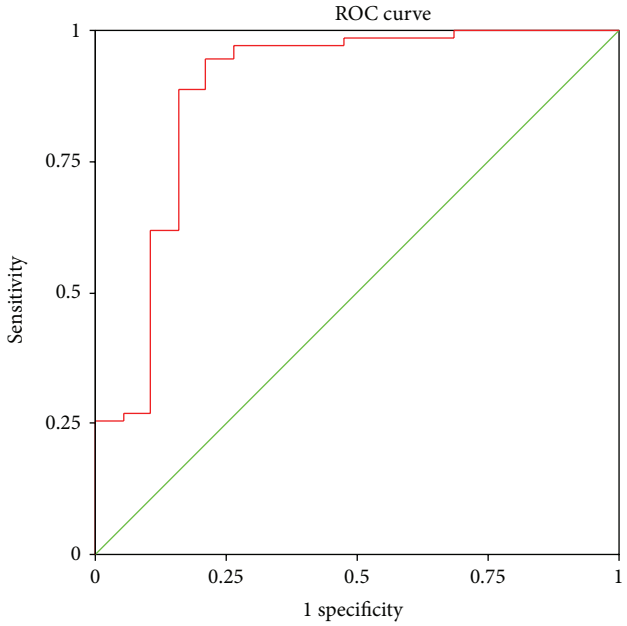

Figure 4: ROC curve for coapplying rabeprazole test and SF-36 in diagnosing GERD.

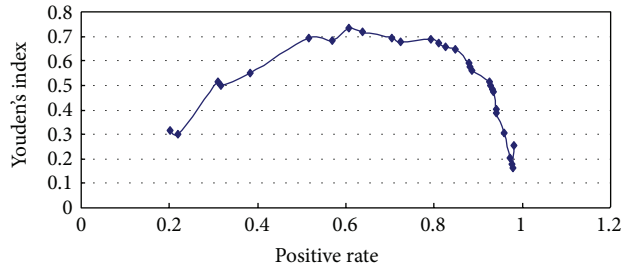

Figure 5: Youden's index for coapplying rabeprazole test and SF-36 in diagnosing GERD.

GERD and non-GERD patients should be analyzed separately. When patients were grouped according to GERD and non-GERD diagnosis, differences in SF-36 scores were only noted between GERD and non-GERD groups after two weeks of treatment for Group A. The differences were not significant between GERD and non-GERD groups before and after one and two weeks of treatment in other groups. It is believed that SF-36 scores, the common disease scale, are affected not only by GERD itself but also the occupation, material status, family and social relationships, education, household income, and social class of patients. The Az was 0.27 in the SF-36 score ROC prior to the treatment, suggesting that SF-36 alone is a poor tool for diagnosing GERD in a primary care setting and is not sufficient to establish a GERD diagnosis. Further analysis showed that the differences were significant in improved SF-36 scores between GERD and non-GERD patients in Groups A and B ($P < 0.05$) but not significant between GERD and non-GERD patients in Group C ($P = 0.085$) after 1-week therapy; significant differences were noted between GERD and non-GERD patients among the three groups after two-week therapy ($P < 0.05$). However, statistical differences in improvement rate were not noted between GERD and non-GERD patients in Groups B and C after two-week treatment (20 mg/d), as the improvement rate is correlated with improved scores and basic scores, and the basic SF-36 scores are related not only to GERD itself but also to many other factors mentioned above. In Group A (40 mg/d), statistical differences in improvement rate are noted between GERD and non-GERD patients, which may be strongly related to the increase of improved scores. Improved scores and basic scores can both influence the improvement rate. Therefore, improvement rate is not regarded as a criterion of the PPI test in improving scores. An improved score, the difference in life quality before and after the treatment, represents the degree of improvement in life quality and can be affected only by a few factors. Therefore, the improved score can be taken as a criterion for the PPI test. Our results show that according to the Youden value principle, an improved score to 65 is most efficient for a GERD diagnosis. At this level, the diagnostic sensitivity was 94.4% and the specificity was 78.9%. If the improved score is boosted, the specificity increases but the sensitivity decreases; therefore, an SF-36 score increase of 65 is taken as the criterion for a positive PPI test result.

In this trial, the diagnostic value of the rabeprazole test did not differ significantly according to duration; the diagnostic value can be considered approximately equivalent after one week of treatment and after two weeks of treatment. The diagnostic coincident rate is 73%, the sensitivity is 85.7%, and the specificity is 44.5% after a one-week administration of rabeprazole 10 mg twice per day. Significant differences in diagnostic sensitivity and specificity at the two-week mark are not noted compared to that at the one-week mark. Diagnostic efficacy is consistent and the expense increases

significantly. Therefore, diagnostic administration for two weeks is unnecessary for judging results. There are no significant differences in the sensitivity and specificity of the 40 mg/day and 20 mg/day groups. Therefore, rabeprazole given as 10 mg b.i.d. for one week is optimum for the PPI test and has the added benefit of being less costly. Results of diagnostic tests with rabeprazole, as reported by Schenk et al. [18], show that the sensitivity, specificity, and negative predictive value were, respectively, 68%, 63%, and 68% in the trial group and 20%, 95%, and 83% in the control group. Johnsson et al. [19] conducted a trial with omeprazole (20 mg b.i.d.) and results showed that the one-week sensitivity was 75% and the specificity was 55%. Cho et al. [20] reported that if lansoprazole 30 mg was given as b.i.d. for two weeks, the diagnostic sensitivity and specificity were 77% and 56%, respectively, illustrating that as the diagnostic sensitivity and specificity increased, the total coincident rate was similar, but the expense rose significantly. Therefore, the two-week administration did little to improve diagnosis. If patients with reflux symptoms are given rabeprazole 10 mg b.i.d. for one week and their SF-36 score increases 65 units following treatment, they can be diagnosed as GERD. Further logistic regression analysis suggests that the diagnostic sensitivity was 94.4%, specificity was 78.9%, the coincident rate was 91.1%, the false negative rate was 5.6%, and the false positive rate was 21.1%.

The SF-36 consists of 3 major parts: functional status, health satisfaction, and total evaluation. It includes eight fields: physical function, physical responsibility, body pain, activation, social function, and emotional responsibility. The eight fields are classified further into physical component scales and mental component scales. The SF-36, a common scale, not only measures its own items but also investigates several specific problems as affecting factors when determining the quality of life with GERD. It comprises more contents than the relatively limited RDQ. Some GERD patients present primarily with extraesophageal symptoms such as coughing and throat discomfort, which strongly influence the quality of life. After treatment, patients improved and their life quality increased. Therefore, rabeprazole in combination with the SF-36 can make a diagnosis through a comparison of pretreatment and posttreatment scores. A common scale may be more helpful than a GERD-specific scale to clarify the reason for decline in the quality of life.

The PPI test is a current diagnostic method for GERD. This study aims to determine the effectiveness of coapplying the PPI test and the SF-36 for GERD diagnosis. Rabeprazole is metabolized in nonenzymic fashion, with a longer half-life, more stable pharmacokinetics, and greater efficacy than the first generation of PPI. In clinical practice, administration of rabeprazole can improve reflux symptoms and the quality of life rapidly [21–23]. In this study, SF-36 scores increase significantly after administration of rabeprazole and differences are more significant than the pretreatment. These differences are induced by rabeprazole for an individual with the same specific problems and thus interference from specific problems can be excluded. Administration of the basic SF-36 seems to have no value for diagnosing GERD at the patient's initial visit. However, increase in the SF-36 score after rabeprazole treatment can be used for the cut-of f value in the rabeprazole test and thus provides the preliminary quantitative criteria for the PPI test. Large-sample, multicenter trials are required to confirm this result in clinical practice.

In conclusion, our study shows that the SF-36 in combination with the rabeprazole test can screen GERD patients and increase the sensitivity and specificity of GERD diagnosis through reference to the change in SF-36 score before and after the treatment (65 in the trial). This not only reduces the expense of clinical diagnosis but also reduces the pain that might be inflicted for gastroscopy and pH monitoring. With this method, diagnosis and treatment can be performed concurrently to shorten diagnosis duration.

Certainly, more exact diagnostic criteria require more large-sample, multicenter, randomized control, and double-blinded studies.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Research Article

Lifestyle Characteristics and Gastroesophageal Reflux Disease: A Population-Based Study in Albania

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Aim. We aimed to assess the prevalence and lifestyle correlates of gastroesophageal reflux disease (GERD) in the adult population of Albania, a Mediterranean country in Southeast Europe which has experienced major behavioral changes in the past two decades. *Methods*. A cross-sectional study, conducted in 2012, included a population-representative sample of 845 individuals (≥18 years) residing in Tirana (345 men, mean age: 51.3 ± 18.5; 500 women, mean age: 49.7 ± 18.8; response rate: 84.5%). Assessment of GERD was based on Montreal definition. Covariates included socioeconomic characteristics, lifestyle factors, and body mass index. Logistic regression was used to assess the association of socioeconomic characteristics and lifestyle factors with GERD. *Results*. The overall prevalence of GERD was 11.9%. There were no significant sex differences, but a higher prevalence among the older participants. In fully adjusted models, there was a positive relationship of GERD with smoking, physical inactivity, fried food consumption, and obesity, but not so for alcohol intake and meat consumption. *Conclusion*. We obtained important evidence on the prevalence and lifestyle correlates of GERD in a Western Balkans' country. Smoking, physical inactivity, and obesity were strong "predictors" of GERD in this population. Findings from this study should be replicated in prospective studies in Albania and other transitional settings.

1. Introduction

Gastroesophageal reflux disease (GERD) is a condition usually manifesting such symptoms as heartburn and acid regurgitation [1]. Nevertheless, symptoms may also include chest pain or evidence of extra-esophageal manifestations such as pulmonary, ear, nose, or throat symptoms. Most of the patients have no visible mucosal damage at the time of endoscopy (nonerosive GERD), whereas a few others may have esophagitis, peptic strictures, Barrett's esophagus, or esophageal adenocarcinoma [2].

GERD is a multifactorial process and one of the most common diseases. Causes of GERD are not clear, although it is recognized that increased transient lower esophageal sphincter relaxations and the presence of significant hiatal hernia contribute to development of the disease [2]. Typically, GERD begins in the middle age, suggesting that various environmental and lifestyle factors may contribute to its pathophysiology. Dietary factors such as shorter dinner-to-bed time, a high dietary fat intake, obesity, and smoking have been implicated in increasing the risk for GERD. Other lifestyle factors include stress, major negative life events, and alcoholism [2–5]. Furthermore, residents in rural areas and those with a positive family history are associated with a higher risk of GERD [6, 7]. Socioeconomic status and a "westernized" diet, suggested as potential risk factors, have not been confirmed yet.

Estimates of the actual prevalence of GERD are difficult to obtain, because individuals seeking health care probably represent only the tip of the iceberg. The epidemiology

of GERD is primarily based on population-based surveys conducted in the affluent western regions like the United States and Europe [6, 8]. In different studies, 10–20% of the adult western population has been reported to experience GERD symptoms (heartburn and/or regurgitation) at least once per week [9].

Population-based data on the magnitude and distribution of GERD in transitional countries of Southeast Europe including Albania are scarce. Traditionally, Albanian diet has been characterized by a low consumption of total energy, meat, and dairy products, but a high consumption of fruit, vegetables, and carbohydrates [10]. However, after the breakdown of the communist regime in early 1990s, Albania has undergone a rapid transition including dietary changes [11] with an emergent "western" behavior consisting of processed foods higher in salt and saturated fats.

In this context, we aimed to assess the prevalence and socioeconomic and lifestyle correlates of GERD in the adult population of Albania, a country which has experienced major dietary changes in the past decades in line with the socioeconomic and political transition towards an open-market system.

2. Material and Methods

A cross-sectional study was conducted in Tirana, the Albanian capital, in March–August, 2012.

2.1. Study Population. A simple random sample of 1000 adult individuals (≥18 years) was drawn based on family physicians' lists in Tirana municipality. Calculations of the sample size were made for a number of behavioral hypotheses including smoking, physical inactivity, and dietary patterns. The significance level (two-tailed) was taken as 5% and the power was set at 80%. Based on conservative calculations, the required minimal number of participants to be recruited was about 700. We decided to recruit 1000 individuals in order to increase the power of the study considering also the potential degree of non-response.

Of 1000 people in the sample, 845 individuals agreed to participate and were subsequently examined at primary health care centers in Tirana (345 men, mean age: 51.3 ± 18.5; 500 women, mean age: 49.7 ± 18.8; overall response rate: 845/1000 = 84.5%).

2.2. Data Collection. The Montreal instrument for assessment of GERD [1] was translated into the Albanian language by following the standard methods of cross-cultural adaptation of the questionnaires. Subsequently, the Albanian version of the tool was pretested in a small sample of individuals ($N = 30$) attending primary health care services in Tirana before conducting the current survey.

In line with the Montreal definition of GERD for population-based studies [1], individuals were classified into two groups based on the presence (or absence) of GERD. Participants were considered as having GERD if, during last year, they reported heartburn or regurgitation occurring at least once a week and having at least moderate problems from such symptoms. Participants reporting use of medications for heartburn or regurgitation at least once weekly were also included in the GERD group, irrespective of symptom severity. Heartburn was defined as a burning sensation in the retrosternal area (behind the breastbone). Regurgitation was defined as the perception of flow of refluxed gastric content into the mouth or hypopharynx. Conversely, individuals with no reflux symptoms and/or those with reflux symptoms that were not regarded troublesome were classified as non-GERD.

In addition, participants were asked about their smoking habits (categorized into: current smoker, former smoker, and never smoker), alcohol intake (dichotomized into: no/occasional intake versus moderate/heavy intake), physical exercise (low, moderate, and high), and dietary habits including frequency of meat consumption and frequency of fried food consumption (each trichotomized into: frequent consumption, moderate consumption, and rare/no consumption).

Demographic (age and sex) and socioeconomic data (educational attainment (years of formal schooling, trichotomized subsequently into: 0–8 years, 9–12 years, ≥13 years) and income level (low, middle, and high)) were also collected.

Physical examination included measurement of height and weight, based on which, body mass index (BMI: kg/m^2) was calculated for each participant.

2.3. Statistical Analysis. Binary logistic regression was used to assess the association of demographic and socioeconomic characteristics and behavioral/lifestyle factors with GERD. Initial models were unadjusted. Next models included adjustment for age. Subsequently, logistic models were additionally adjusted simultaneously for sex and socioeconomic factors (education and income level). Finally, lifestyle factors (smoking, alcohol intake, physical activity, frequency of meat consumption, and frequency of fried food consumption) and BMI were also introduced into the logistic models in a backward stepwise elimination procedure with a P value to exit set at $P < 0.10$. Unadjusted (crude), next age-adjusted, and finally multivariable-adjusted odds ratios (ORs) and their respective 95% confidence intervals (95% CIs) were calculated. Hosmer-Lemeshow goodness-of-fit test was used to assess the validity of the logistic models; all models satisfied the criterion. All the statistical analysis was conducted in SPSS (Statistical Package for Social Sciences, version 15.0).

3. Results

The overall prevalence of GERD in this study population was 101/845 = 11.9%. There were no significant sex differences, but a higher prevalence among the older participants (>40 years) compared with their younger counterparts (≤40 years) ($P = 0.015$, Table 1). Furthermore, there was evidence of an inverse linear relationship of GERD with age (overall $P = 0.018$), but no association with income level.

The prevalence of GERD was substantially and significantly higher among current smokers ($P < 0.001$), individuals who reported low levels of physical exercise ($P = 0.004$),

Table 1: Distribution of gastro esophageal reflux disease (GERD) by demographic and socioeconomic characteristics of a representative sample of Albanian adults.

Variable	No GERD	GERD	OR (95% CI)[c]	*P* value
Sex				
Men	298 (86.4)[a]	47 (13.6)	1.30 (0.86–1.98)	0.215
Women	446 (89.2)	54 (10.8)	1.00 (reference)	
Age (years)	52.0 (32.0)[b]	57.0 (25.0)	1.02 (1.01–1.03)	0.001
Age				
≤40 years	240 (91.6)	22 (8.4)	1.00 (reference)	0.015
>40 years	465 (85.5)	79 (14.5)	1.85 (1.13–3.05)	
Educational level (years)	12.0 (4.0)[b]	12.0 (6.75)	0.92 (0.87–0.98)	0.003
Educational level				**0.018 (2)**[d]
Low (0–8) years	111 (81.6)	25 (18.4)	2.23 (1.27–3.93)	0.005
Middle (9–12) years	273 (86.4)	43 (13.6)	1.56 (0.96–2.53)	0.072
High (≥13 years)	317 (90.8)	32 (9.2)	1.00 (reference)	—
Income level				**0.474 (2)**
Low	83 (84.7)	15 (15.3)	1.57 (0.75–3.27)	0.232
Middle	450 (87.0)	67 (13.0)	1.29 (0.74–2.24)	0.365
High	156 (89.7)	18 (10.3)	1.00 (reference)	—

[a]Numbers and row percentages (in parentheses). Discrepancies in the totals are due to missing covariate values.
[b]Median and interquartile range (in parentheses).
[c]Unadjusted (crude) odds ratios (ORs: GERD versus no GERD), 95% confidence intervals (95% CIs), and *P* values from binary logistic regression.
[d]Overall *P* value and degrees of freedom (in parentheses).

those with moderate-to-heavy alcohol intake ($P = 0.006$), participants who reported a high frequency of meat and/or fried food consumption ($P = 0.091$ and $P < 0.001$, resp.), and among obese individuals ($P < 0.001$) (Table 2).

In age-adjusted logistic regression models, smoking, alcohol intake, physical inactivity, frequent consumption of meat and/or fried food, and obesity were all positively and significantly associated with GERD (Table 3, model 1). Upon further adjustment for sex and income level, there was evidence of an inverse association of GERD with educational attainment (Table 3, model 2), whereas the strong relationships with each lifestyle characteristic and obesity persisted strongly after additional adjustment for sex and socioeconomic variables (Table 3, model 2). In fully-adjusted models with all covariates included in a backward stepwise elimination procedure, the positive relationship of GERD with smoking, physical inactivity, and fried food consumption persisted strongly and significantly, but not so for alcohol intake and meat consumption (Table 3, model 3). In addition, upon multivariable-adjustment, there was evidence of a positive relationship of GERD with age (Table 3, model 3).

4. Discussion

To the best of our knowledge, this is the first study assessing the prevalence and distribution of GERD in Albania: a Mediterranean country in Southeast Europe. Findings from this study indicate that the prevalence of GERD in Albania is about 12%, and that smoking, physical inactivity, a frequent consumption of fried food, and obesity were all positively and significantly associated with GERD.

The prevalence of GERD in Albania seems lower than that observed in other (mainly Western) populations [9]. It should be noted that the prevalence of GERD has been increasing in Western countries over the past 30 years [9]. It has been argued that the decreasing prevalence of Helicobacter pylori may play an important role to the increasing prevalence of GERD in these regions. Recent data suggests that many patients with Helicobacter pylori-induced gastritis have involvement of the antrum and corpus, decreasing parietal cell mass, reducing acid secretion, and elevating gastric pH. This may have a protective effect on the esophageal mucosa in patients susceptible to GERD [12]. Furthermore, in Asian populations, other possible reasons for the lower GERD prevalence include low dietary fat intake, low BMI, and a lower gastric acid output, possibly related to Helicobacter pylori infection [13]. Our findings are comparable with reports from countries with a high prevalence of Helicobacter pylori infection [13]. Therefore, the seemingly lower prevalence of GERD in our study population may be related to the high prevalence of Helicobacter pylori infection in the Albanian population [14].

On the other hand, the different age ranges examined in various studies might explain the differing results between studies involving assessment of GERD. In our study, there was evidence of a positive relationship of GERD with age. However, the relationship between GERD and age is controversial in that some studies have observed a positive relationship [15, 16], a few other studies have reported an inverse association [17, 18], whereas some further reports have pointed to a lack of association between age and GERD [19, 20]. In our study, in fully-adjusted models, the positive association of age with

Table 2: Distribution of lifestyle characteristics of participants with and without gastro esophageal reflux disease (GERD).

Variable	No GERD	GERD	OR (95% CI)[b]	*P* value
Smoking				**<0.001 (2)**[c]
Current smoker	129 (67.9)[a]	61 (32.1)	12.3 (7.17–21.15)	<0.001
Former smoker	75 (78.9)	20 (21.1)	6.95 (3.57–13.5)	<0.001
Never smoker	521 (96.3)	20 (3.7)	1.00 (reference)	—
Alcohol consumption				
No/Occasional intake	539 (89.4)	64 (10.6)	1.00 (reference)	0.006
Moderate/Heavy intake	152 (81.7)	34 (18.3)	1.88 (1.20–2.96)	
Physical activity				**<0.001 (2)**
Low	150 (78.9)	40 (21.1)	6.32 (2.75–14.54)	0.004
Moderate	370 (53.9)	51 (12.1)	3.27 (1.45–7.36)	<0.001
High	166 (96.0)	7 (4.0)	1.00 (reference)	—
Fried food frequency				**<0.001 (2)**
Frequent consumption	266 (81.8)	59 (18.2)	3.13 (1.86–5.91)	<0.001
Moderate consumption	224 (90.0)	25 (10.0)	1.67 (0.87–3.20)	0.125
Rare/no consumption	239 (93.7)	16 (6.3)	1.00 (reference)	—
Meat consumption frequency				**0.050 (2)**
Frequent consumption	548 (85.9)	90 (14.1)	3.45 (0.82–14.50)	0.091
Moderate consumption	99 (92.5)	8 (7.5)	1.70 (0.35–8.33)	0.515
Rare/no consumption	42 (95.5)	2 (4.5)	1.00 (reference)	—
BMI (kg/m^2)	26.2 (5.48)[d]	28.7 (5.91)	1.08 (1.04–1.13)	<0.001
BMI				**<0.001 (2)**
<25	265 (93.3)	19 (6.7)	1.00 (reference)	—
25–29.9	306 (87.9)	42 (12.1)	1.91 (1.09–3.37)	0.025
≥30	128 (76.2)	40 (23.8)	4.36 (2.43–7.83)	<0.001

[a]Numbers and row percentages (in parentheses). Discrepancies in the totals are due to missing covariate values.
[b]Unadjusted (crude) odds ratios (ORs: GERD versus no GERD), 95% confidence intervals (95% CIs), and *P* values from binary logistic regression.
[c]Overall *P* value and degrees of freedom (in parentheses).
[d]Median and interquartile range (in parentheses).

GERD was borderline significant, similar to a prior report from Locke et al. [21].

In our study, obesity was strongly and significantly associated with GERD, in line with the findings observed in other populations [21, 22], but in contrast with findings reported by Lagergren et al. [23]. A positive relationship between BMI and symptomatic GERD was previously observed in a sample of adult patients in Albania where, upon adjustment for socioeconomic characteristics and behavioral factors, the positive association of GERD with BMI persisted strongly [24]. A potential biological mechanism underlying an increased risk of reflux among obese persons has been suggested through increased extrinsic gastric compression by surrounding adipose tissue and anatomical disruption of the gastroesophageal junction [8, 25]. Our study assessed a wide range of behavioral/lifestyle factors associated with GERD in the Albanian adult population. Frequent consumption of meat and/or fried food, smoking, and physical inactivity were all positively associated with GERD. Consumption of meat was confirmed as a risk factor for GERD in this Albanian population (notwithstanding the lack of statistical significance in fully-adjusted logistic regression models due to multicollinearity with other covariates, mainly with fried food consumption), compatible with results from other studies conducted elsewhere [26]. Higher fat content of meat may be responsible for this increased risk, as fat delays gastric emptying and, therefore, meat is a well-accepted risk factor for GERD [26].

The relationship of GERD with cigarette smoking has been reported in different studies [6, 21]. Three cross sectional studies have indicated a significant positive association between GERD symptoms and smoking, in the same line with our findings [6, 21]. Furthermore, a few longitudinal studies have investigated the relationship with smoking. The UK-GP database study found that there were significantly more former smokers (OR 1.2, 95% CI = 1.1–1.4) and slightly more current smokers (OR 1.1, 95% CI = 1.0–1.2) among patients with a new diagnosis of GERD than in the control cohort [4].

In our study, upon multivariable adjustment, there was no significant relationship with alcohol intake. Previous studies provide inconsistent results with no association of GERD with alcohol consumption [4, 9] (consistent with our findings), whereas a study conducted in Germany showed alcohol to be a significant risk factor [26].

We observed a strong inverse relationship between physical activity and GERD which, on the face of it, is counterintuitive. However, previous studies have indicated conflicting results with regard to physical exercise [27, 28].

Table 3: Association of demographic, socioeconomic, and lifestyle factors with GERD; age-adjusted and multivariable-adjusted odds ratios (ORs) from binary logistic regression.

Variable	Model 1[a]		Model 2[b]		Model 3[c]	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Sex						
Men	1.27 (0.83–1.94)	0.270	1.36 (0.88–2.10)	0.168	0.32 (0.17–0.59)	<0.001
Women	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Age						
≤40 years	—	—	1.00 (reference)	0.099	1.00 (reference)	0.067
>40 years			1.56 (0.92–2.65)		1.89 (0.96–3.76)	
Educational level		**0.147 (2)**[d]		**0.063 (2)**		
Low (0–8) years	1.94 (1.08–3.51)	0.028	2.06 (1.12–3.77)	0.020		
Middle (9–12) years	1.42 (0.87–2.33)	0.162	1.45 (0.87–2.39)	0.151		
High (≥13 years)	1.00 (reference)	—	1.00 (reference)	—		
Income level		**0.655 (2)**		**0.854 (2)**		
Low	1.41 (0.67–2.96)	0.371	1.22 (0.57–2.59)	0.612		
Middle	1.22 (0.70–2.13)	0.483	1.15 (0.66–2.02)	0.622		
High	1.00 (reference)	—	1.00 (reference)	—		
BMI		**<0.001 (2)**		**<0.001 (2)**		**0.002 (2)**
<25	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
25–29.9	1.79 (1.00–3.21)	0.050	1.58 (0.87–2.85)	0.130	1.71 (0.86–3.38)	0.124
≥30	3.97 (2.12–7.41)	<0.001	3.74 (1.99–7.01)	<0.001	3.79 (1.79–8.03)	<0.001
Smoking		**<0.001 (2)**		**<0.001 (2)**		**<0.001 (2)**
Current smoker	13.36 (7.7–23.2)	<0.001	23.6 (12.3–45.3)	<0.001	29.3 (13.9–61.2)	<0.001
Former smoker	6.03 (3.06–11.8)	<0.001	12.68 (5.9–27.2)	<0.001	9.79 (4.22–22.7)	<0.001
Never smoker	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
Alcohol consumption						
No/Occasional intake	1.00 (reference)	0.013	1.00 (reference)	0.021		
Moderate/Heavy intake	1.79 (1.13–2.83)		1.83 (1.10–3.06)			
Physical activity		**<0.001(2)**		**<0.001 (2)**		**<0.001 (2)**
Low	5.79 (2.46–13.6)	<0.001	5.47 (2.32–12.9)	<0.001	7.03 (2.68–18.4)	<0.001
Moderate	3.10 (1.37–7.01)	0.007	3.01 (1.33–6.84)	0.008	2.75 (1.11–6.78)	0.028
High	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
Fried food frequency		**<0.001 (2)**		**<0.001 (2)**		**0.001 (2)**
Frequent	4.13 (2.28–7.48)	<0.001	3.57 (1.97–6.49)	<0.001	3.01 (1.52–6.20)	0.002
Moderate	1.91 (0.99–3.69)	0.055	1.69 (0.86–3.32)	0.128	1.21 (0.55–2.65)	0.634
Not frequent	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
Meat consumption frequency		**0.057 (2)**		**0.060 (2)**		
Frequent	3.52 (0.83–14.8)	0.087	3.93 (0.91–17.0)	0.068		
Moderate	1.79 (0.36–8.81)	0.476	2.09 (0.41–10.6)	0.372		
Not frequent	1.00 (reference)	—	1.00 (reference)	—		

[a]Model 1: adjusted for age.

[b]Model 2: simultaneously adjusted for age, sex, and socioeconomic variables (education and income level).

[c]Model 3: adjusted for age, sex, socioeconomic variables, and behavioral factors (smoking, alcohol intake, physical activity, frequency of meat consumption, frequency of fired food consumption, and body mass index). All variables were entered in a backward stepwise elimination procedure with a *P* value to exit set at <0.10. Empty cells refer to the variables excluded from the final model.

[d]Overall *P* value and degrees of freedom (in parentheses).

A fairly recent study concluded that intermediate frequency of physical activity might decrease the risk of GERD among obese individuals, while no influence of physical activity on GERD was found in non-obese participants [29].

Conversely, it has been argued that physical exercise may increase the risk of GERD possibly by increasing transient relaxation of the lower esophageal sphincter, or by decreasing the gastrointestinal blood flow and changing the esophageal and gastric motor function [30–32]. A plausible explanation for the strong positive relationship between physical inactivity and GERD in our study may be the avoidance of physical exercise among people with GERD symptoms.

A major strength of the current study conducted in Albania includes the population-based design involving a representative sample of adult men and women with a high participation rate (84.5%). In our study, all symptoms were measured with a well-validated instrument fulfilling the consensus criteria for GERD [1]. Nevertheless, we cannot fully dismiss the possibility of information bias. More importantly, findings from cross-sectional studies are not assumed to be causal. From this point of view, the issue of reverse causality, including lifestyle modification (e.g., changes in dietary patterns and/or physical exercise) after onset of GERD symptoms, remains unresolved from such cross-sectional designs.

5. Conclusions

We obtained important evidence on the prevalence and lifestyle correlates of GERD in the adult population of Albania, a transitional country in the Western Balkans which, in the past two decades, has experienced a major deviation from the traditional Mediterranean diet to a "western" behavioral prototype including intake of junk food and processed food. However, findings from this study should be replicated in prospective studies in Albania and other transitional settings.

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Clinical Study

Extraesophageal Reflux: What Is the Best Parameter for pH-Monitoring Data Analysis from the Perspective of Patient Response to Proton Pump Inhibitors?

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Objectives. To analyze the pH-monitoring records of patients with suspected extraesophageal reflux (EER) using three different parameters (number of refluxes (NOR), acid exposure time (AET), and reflux area index (RAI)), with a view to determining which type of analysis is best at selecting the patients who will respond to a proton pump inhibitor (PPI). *Methods*. Demographic data were obtained and the level of the complaint was assessed using the Visual Analogue Scale. A dual probe pH-monitoring study was conducted. NOR greater than six, AET more than 0.1%, and RAI higher than 6.3 mpH were taken to be the thresholds for EER. Subsequently the response to a 12-week PPI trial was analyzed. *Results*. A total of 81 patients were analyzed. The percentages of patients with substantial EER based on NOR, AET, and RAI were 36%, 28% and 26%, respectively. Statistically significant, often positive PPI trials were confirmed in the group identified as having substantial EER using all three types of analysis. When using AET and RAI, the significance was more pronounced ($P = 0.012$ and $P = 0.013$, resp.) in comparison with NOR ($P = 0.033$). *Conclusions*. Patients with EER diagnosed using AET or RAI will respond to PPI significantly often.

1. Introduction

Ambulatory 24-hour dual probe pH-monitoring remains a widely used diagnostic method for detecting extraesophageal reflux (EER). At present, there is a substantial consensus regarding the methodology for this procedure: the upper probe should be placed above the level of the upper esophageal sphincter (UES) [1]. However, there is still a lack of consensus regarding the interpretation of the data recorded, and physicians continue to argue about what constitutes "normal" and what constitutes pathological EER for most patients. Currently, there are three basic parameters being used for data analysis: number of refluxes (NOR), acid exposure time (AET), and reflux area index (RAI) (Figure 1) [2, 3]. NOR is the sum of all reflux episodes per 24 hours, regardless of their duration and the pH level reached. AET, also sometimes called fraction time, is the percentage of time during the study (usually 24 hours) when the pH is below 4.0. This parameter reflects the severity of EER more objectively. Reflux area (RA) is the sum of the area under the curve for all episodes of pH < 4.0 recorded during the study in units of Ph*minutes. The RAI (in units of mpH) is the RA corrected for the duration of the study. RAI takes into consideration not only the AET but also the level of pH decline and is currently considered the most accurate parameter for measuring the severity of EER (Figure 1) [2, 3].

Every physician who has evaluated recorded pH-monitoring data is familiar with the fact that results may vary with the parameter used for analysis. The question then becomes which parameter is the most precise and best correlates with the response to proton pump inhibitors (PPI). The aim of the present study was to analyze the pH-monitoring records of patients examined for suspected EER using these three different parameters, compare the results,

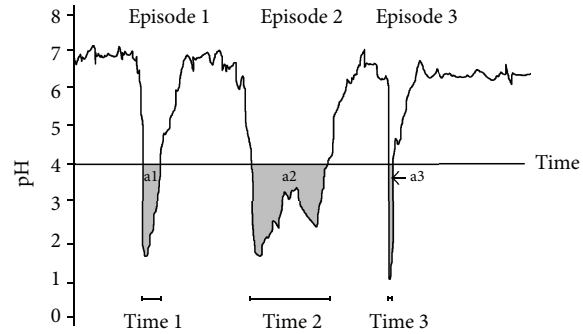

Figure 1: Three different parameters used for pH-monitoring data analysis are compared. There are 3 reflux episodes displayed (Episode 1, Episode 2, and Episode 3). Acid exposure time is the sum of Time 1, Time 2, and Time 3. Reflux area is the sum of calculated a1, a2, and a3 areas.

and determine which parameter was the best predictor of a positive response to PPI treatment. To our knowledge, this is the first study to compare all three of these parameters and thereby attempt to establish whether using different criteria has any clinical impact.

2. Materials and Methods

The prospective study was performed in accordance with the Declaration of Helsinki, the requirements of good clinical practice, and all applicable regulatory requirements and was approved by the Institutional Review Board. Written informed consent was obtained from all participants before initiating any procedure.

Outpatients aged 18 to 64 years with the complaints commonly attributed to EER (dysphonia, globus, cough, and throat cleaning) lasting more than three months were included in the study, conducted between January 2010 and June 2011. Both those patients who had and those who had not been treated for gastroesophageal reflux disease with a PPI were included in the study, since this fact has no bearing on the aim of the study. Patients with acute upper respiratory infection and oropharyngeal and laryngeal cancer and patients with other serious illnesses (e.g., cardiovascular and neurological complaints, diabetes, and other illnesses) were excluded from the study, because these conditions can significantly aggravate patient complaints. Epidemiologic data (age, sex, BMI, smoking history, bronchial asthma, and professional use of voice) were obtained via questionnaire, and assessment of the severity of the complaints commonly associated with EER (dysphonia, globus, cough, and throat cleaning) was done using the visual analogue scale (VAS). Reflux Finding Scores (RFS) were assessed using rigid video laryngoscopy to determine the level of the laryngeal signs of EER. Afterwards, an ambulatory 24-hour dual probe pH-monitoring study was conducted. A digitrapper pH400 device (Alpine Biomed, Denmark, 2007) with double probes with a fixed distance of 15 cm was used. The proximal sensor was placed immediately above the UES using flexible laryngoscopic guidance (Smit technique). The data recorded were analyzed using GastroTrac software (Alpine Biomed, Denmark, 2007). Upper probe events with pH < 4.0 were only accepted as EER events when Postma's criteria (the pH decreases to less than 4; the pharyngeal pH drops during or immediately after distal esophageal acid exposure; the pH drop does not occur during an episode of eating; the proximal sensor pH drop is rapid and sharp, not gradual) were met [1]. NOR, AET, and RAI were assessed. NOR greater than six, AET greater than 0.1%, and RAI higher than 6.3 mpH were considered the thresholds for substantial EER [3–6]. Subsequently, all patients were put on a PPI (30 mg lansoprazole) twice a day for twelve weeks and were assessed using VAS at the end of this period to ascertain whether their symptoms (dysphonia, globus, cough, and throat cleaning) had completely vanished, been relieved, or persisted. A drop of at least 3 points in the 10-point VAS, as compared to the VAS value assessed before the PPI trial, was taken to indicate a relief of symptoms, while a decrease of two or less was taken to indicate the persistence of symptoms. A two-sample Student's t-test and Fischer's exact test were used to assess differences in RFS and responses to the PPI trial between the groups analyzed. Stata software (version 10) was used for all statistical calculations.

Table 1: Characteristics of the study group.

Patients	$n = 81$
Mean age	50 years (SD ± 14)
Sex	31 male, 50 female
BMI	27,29 (SD = 5.33)
Smokers	$n = 14$ (17.3%)
Voice professionals	$n = 21$ (25.9%)
Bronchial asthma	$n = 15$ (18.5%)

3. Results

A total of 90 patients were recruited for the study, nine of whom were excluded from the statistical analysis (five did not tolerate a catheter and four did not come to the last session). A total of 81 patients (31 men and 50 women, mean age 50, SD ± 14) were analyzed (Table 1).

The percentages of patients with substantial EER based on NOR, AET, and RAI were 36% (29 patients), 28% (23 patients), and 26% (21 patients), respectively (Table 2).

Statistically significant higher RFS was confirmed in the group with substantial EER in comparison to the group determined not to have EER using all three types (NOR, AET, and RAI) of analysis ($P = 0.0166$, $P = 0.0071$, and $P = 0.0007$, resp.) (Table 3).

Statistically significant, often positive PPI trials in the group with substantial EER in comparison to the group without EER as determined using all types of analysis (NOR, AET, and RAI) was confirmed as well ($P = 0.033$, $P = 0.012$, and $P = 0.013$, resp.) (Table 4).

Table 2: Number (N) and percentage (%) of patients diagnosed with EER (EER^+) and without EER (EER^-) using three different parameters of pH monitoring analysis (NOR: number of refluxes, AET: acid exposure time, and RAI: reflux area index).

Parameter	EER^+ (N)	EER^+ (%)	EER^- (N)	EER^- (%)
NOR	29	35,80	52	64,20
AET	23	28,40	58	71,60
RAI	21	24,93	60	75,07

Table 3: Average reflux finding score (RFS) and its standard deviation (SD) in group of patients with extraesophageal reflux confirmed by pH monitoring (EER^+) and group of patients without EER (EER^-) using three different parameters (NOR: number of refluxes, AET: acid exposure time, and RAI: reflux area index). The two-sample Student's t-test was for statistical analysis of differences between the EER^+ and the EER^- group.

Parameter	EER^+	EER^-	P
NOR	8.00 ± 3.10	6.31 ± 2.67	0.0166
AET	7.93 ± 2.65	6.15 ± 2.83	0.0071
RAI	8.57 ± 3.00	6.16 ± 2.59	0.0007

Table 4: Number of patients with a positive therapeutic trial (TT^+) and a negative therapeutic trial (TT^-) in group of patients with extraesophageal reflux confirmed by pH-monitoring (EER^+) and without EER (EER^-) using three different parameters (NOR: number of refluxes, AET: acid exposure time, RAI: reflux area index). Fischer's exact test was used for statistical analysis of differences between the EER^+ and the EER^- group.

Parameter	EER^+		EER^-		P
	TT^+	TT^-	TT^+	TT^-	
NOR	25	4	30	22	0.033
AET	20	3	35	23	0.012
RAI	19	2	36	24	0.013

4. Discussion

Diagnosing EER and establishing its involvement in patient problems continue to be a challenging and controversial business. This has to do with the complicated pathophysiology of EER and the fact that EER symptoms are nonspecific and vary over time, and moreover with the fact that different patients evince different sensitivities to reflux [1]. The lack of diagnostic criteria for EER and inconsistency in the response to therapy is a source of frustration to many physicians. There is as yet no clear answer to that most important question: "which patients will respond to treatment?" Nevertheless, EER causes very real problems and affects hundreds of thousands of patients annually. It is estimated that up to 10%–15% of all visits to otolaryngology offices are prompted by manifestations of EER [7].

Ambulatory 24-hour dual probe pH monitoring for the detection of EER was introduced by Wiener et al. in 1989 [8]. The methodology involved was refined over the years, and over the last two decades the technique has come to be widely used for the diagnosis of EER. At present, there is a substantial consensus regarding the methodology for this procedure: the upper probe should be placed above the level of the UES. This can be achieved using direct laryngoscopy guidance (Smit technique), or else the position of the UES can be ascertained using manometry [1–3, 9].

The role of pH monitoring in the examination of patients with suspected EER continues to be a contentious issue. Authors who argue that pH testing should be preceded by a PPI trial make a point of stressing inconsistencies in interpretation criteria and unreliability in predicting the response to therapy [9]. On the opposing side, authors who advocate pH testing before a PPI trial point out the risk of PPI overuse: its adverse effects (hip fractures, enteritis, and anaphylactic reaction, among others), the rebound phenomenon when medication is stopped, and the economic impact [10, 11]. Moreover, meta-analysis involving over 790 extraesophageal pH reports in 16 studies over a period of 12 years confirmed that the aggregate number of reflux episodes and the percentage of AET were both significantly greater in persons with EER than in controls [12]. Thus, hypopharyngeal pH-monitoring does appear to be capable of distinguishing persons with EER from normal controls [11, 12].

The dispute over whether pH monitoring or a PPI trial should be used as a first intervention in patients with suspected EER is fuelled by differences in the definitions of physiological and pathological EER adopted by different authors. Some authors consider any pharyngeal reflux abnormal, while others report small amounts of pharyngeal reflux in healthy individuals and consider a small number of EER refluxes (most often three to six refluxes) a threshold for pathological EER [1, 4–6]. Moreover, NOR does not seem to be the best parameter for analysis, because the length and severity of individual EER episodes vary significantly. As a result, two other parameters are currently being used for the analysis of pH monitoring records: AET and RAI. RAI is currently considered the most accurate parameter as it takes into account the severity of the reflux episode, not just its duration (Figure 1) [3].

In the present study, the data recorded during pH-monitoring were analyzed using all three criteria (NOR, AET and RAI). We did not find any studies in the world literature that compared all three criteria. Our results indicate that AET, and RAI are similar parameters and that pathological EER is diagnosed in 28% and 26% of patients, respectively, using these methods. They are more specific and less sensitive in comparison to NOR. Using NOR, pathological EER was diagnosed in 36% of patients. The response to the PPI was

significantly higher in patients diagnosed with pathological EER using all three types of analysis. However, when AET and RAI were used, the significance was more pronounced ($P = 0.012$ and $P = 0.013$, resp.) than when NOR was used ($P = 0.033$). In practice this means that if we use more specific types of analysis (AET or RAI) we will diagnose fewer patients with pathological EER, but a higher proportion of diagnosed patients will respond to PPI treatment. This result supports the assertion that the response to a PPI can be predicted by the result of pH testing and that the stricter the criteria adopted for pathological EER, the greater the number of patients responding to PPI treatment.

Similar conclusions can be reached by examining the details of the study published by Hartman [13]. He analyzed five randomized placebo controlled trials which tracked the response to a PPI in patients with suspected EER [13]. In two of them, the effect of the PPI was significantly higher as compared to the placebo, and in one the PPI was reported as possibly having an effect [14–16]. In two other studies, the effect of PPI as compared to the placebo was not confirmed [17, 18]. When we look at these studies closely, a very important fact emerges. In all studies which showed a significant effect of PPI in comparison to the placebo, the diagnosis of EER was arrived at by pH-monitoring, and patients were assigned to the EER group accordingly [14–16]. And conversely, in studies which did not show a significant effect of PPI as compared to the placebo, patients were assigned to the EER group only according to their symptoms and/or signs [17, 18]. Therefore, it can be assumed that, in studies which assigned patients to EER groups without pH testing, more patients are believed to have EER suffered from non-EER laryngitis. This also explains why the effect of PPI in the EER group as compared with the non-EER group did not differ in these studies.

The same result was arrived at in our previous study of patients with globus pharyngeus. In the group of patients with globus pharyngeus and pathological EER as confirmed by pH monitoring, the response to the PPI was significantly higher than in the group of patients with globus pharyngeus but without EER [19].

Even if the use of more specific criteria for the diagnosis of EER improves the practical outcome of pH monitoring, one has to be aware of the limits of this technique [11]. Hence RFS designed by Belafsky is recommended as an important part of the examination of patients with suspected EER, to be used as an adjunct to pH testing [11]. RFS has displayed excellent inter- and intrarater reproducibility [20]. But RFS alone is also limited in specificity because inflammatory changes of the larynx can have many other causes (tobacco, environmental pollutants, infection, excessive voice use, and allergy). Thus, laryngoscopy alone cannot be relied upon to make a diagnosis of EER either, and the combination of laryngoscopy and dual-probe pH testing seems to be of much higher diagnostic sensitivity and specificity for EER [11]. Oelschlager et al. reported that 88% of persons with an abnormal RFS and an abnormal pharyngeal pH test improved with antireflux therapy, as compared with just 44% of persons with an abnormal pH test but normal RFS [21]. This result strongly indicates that the combination of both diagnostic tools offers the best opportunity to accurately secure the diagnosis of EER and reliably predict the response to antireflux therapy.

An additional result of our study was that the sound diagnostic value of RFS was confirmed. RFS was significantly higher in groups of patients with pathological EER diagnosed using all three types of analysis. Moreover, using AET and RAI, which were confirmed to be more specific criteria for the diagnosis of EER, the significance was more pronounced ($P = 0.0071$ and $P = 0.0007$, resp.) in comparison with NOR ($P = 0.0166$).

New devices for the detection of EER—Multichannel Intraluminal Impedance (MII) testing and oropharyngeal pH testing using a Restech device—have emerged recently. The main advantage of MII testing is the ability to detect weakly acidic and alkaline EER episodes. Over the past few years, the device has been used primarily for the examination of impedance below the UES. Normative data for pharyngeal probes have only recently been supplied, by Hoppo [22]. The authors conclude that EER episodes are very rare in asymptomatic populations [22]. The Restech device for the examination of oropharyngeal pH is very sensitive and the examination is well tolerated by patients. Normative data have been available from several recent studies [23–26]. It is very important to keep in mind that even if these new devices seem to be better in terms of their sensitivity to EER, they will raise exactly the same questions as dual probe pH testing has over the past two decades. Most of these have been discussed and summarized in this paper, along with some new perspectives afforded by the results of our study. The most important objective of all methods devised to measure oro- and hypopharyngeal pH is to verify given normative data for different groups of patients and to determine if the results of these tests can predict the response to antireflux therapy.

5. Conclusions

When using AET and RAI in the diagnosis of EER, the significance was more pronounced in comparison with NOR. Using these types of analysis (AET or RAI) we will be able to identify the patients who will respond to PPI treatment.

Conflict of Interests

The authors declare that there is no actual or potential conflict of interests in relation to this paper. No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this paper.

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