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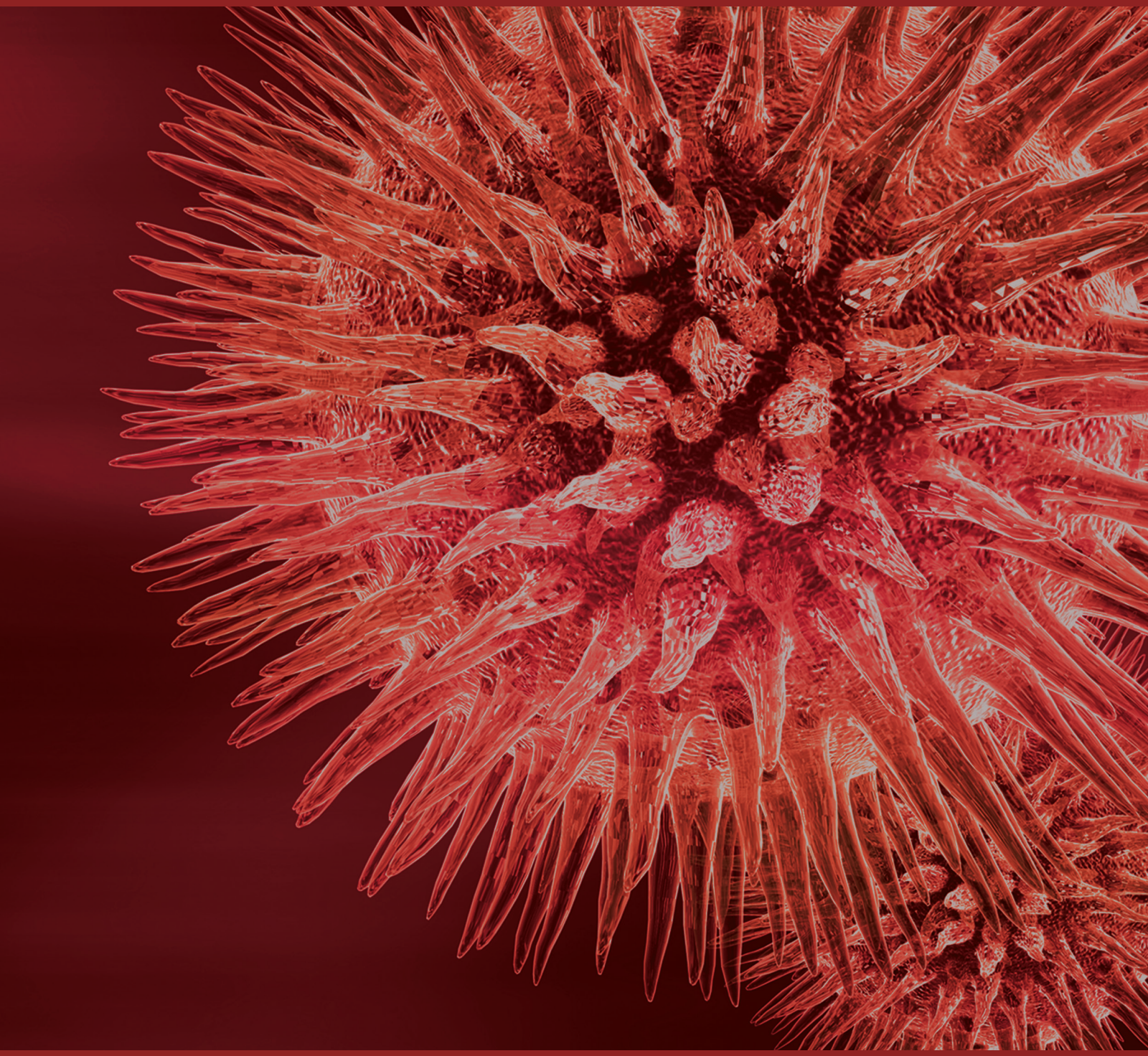
Spine and Rheumatic Diseases

Guest Editors: James Cheng-Chung Wei, Yi Liu, Hsi-Kai Tsou, and Irene Eva van der Horst-Bruinsma



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Editorial

Spine and Rheumatic Diseases

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Rheumatic diseases include a wide spectrum of diseases involving musculoskeletal and immune system. Spinal disorders, whether mechanical or inflammatory, are main manifestations in daily clinical practice of rheumatologists, neurologists, orthopaedics, and general physicians.

Spondyloarthritis (SpA), typically ankylosing spondylitis (AS), is one of the major rheumatic spinal disorders. The pathogenesis of SpA involved many interaction domains including genes, immunity, mechanical stress, infection, and other environmental factors [1]. Genetic backgrounds, such as HLA-B27, B60, ERAP-1, and IR23R, play major roles in the initiation and pathogenesis of SpA. These multifactorial gene-environmental interaction mechanisms lead to variable clinical phenotypes of this complex diseases spectrum, mainly arthritis in spine, sacroiliac joints, and peripheral joints, but also extra-articular manifestations, such as uveitis, psoriasis, and bowel inflammation (Figure 1).

The Assessment in Spondyloarthritis International Society (ASAS), an international task force, had generated new ASAS criteria for axial and peripheral SpA by predominant pattern of clinical manifestations in 2009 [2] and 2011 [3]. These classification criteria aimed to standardize definition of this diseases spectrum for clinical researches and emphasized early detection of SpA by usage of magnetic resonance imaging (MRI) in sacroiliac joints and human leukocyte antigen- (HLA-) B27 in diagnosis of SpA. A new disease term, nonradiographic axial SpA (nr-ax SpA), was thus created to describe patients who fulfilled the 2009 ASAS criteria for axial SpA but not the 1984 modified New York criteria for AS [4]. However, there are still many debates about the scope of this new term, nr-ax SpA, including the definition, disease natural course, clinical diagnosis, and management strategies.

Treatment goals for SpA are maintenance of physical function, disease activity, and prevention of radiographic progression to ankylosis. Current effective therapies to control SpA disease activity are available, including exercise and physical therapy [5], nonsteroidal anti-inflammatory drugs (NSAIDs), disease modifying antirheumatic drugs (DMARDs) such as sulfasalazine, and biological agents, mainly TNF blockers. Potential experimental therapies are promising, including IL17 blockers and IL23 blockers [6].

In this special issue, 16 articles were accepted, while the other 21 were rejected. The scope of this special issue covered clinical and basic studies on spinal or rheumatic diseases. A nationwide epidemiological study of hyperuricemia in Taiwan was published by C.-Y. Wei et al. Genetic studies including HLA-B27 subtype, MUTYH gene polymorphisms, SIRT1, and microRNAs were investigated by Y. Mou et al.,

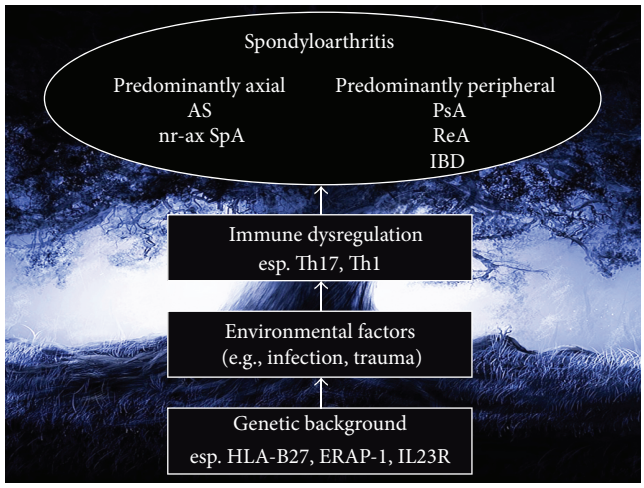

Figure 1: Development and pathogenesis of spondyloarthritis (SpA). Patients of SpA need a genetic background, such as HLA-B27, B60, ERAP-1, and IL23R. After being triggered by environmental factors, such as infection or mechanical trauma, the immune dysregulation and inflammation occurred in enthesis through Th17 and Th1 immune reaction. With different genetic backgrounds and environmental factors, patients may have different phenotype, such as ankylosing spondylitis, psoriatic arthritis, or inflammatory bowel diseases. AS: ankylosing spondylitis; nr-ax SpA: nonradiographic axial spondyloarthritis; PsA: psoriatic arthritis; ReA: reactive arthritis; IBD: inflammatory bowel diseases.

Y.-J. Kung et al., S. Zhou et al., and Q. Lv et al. Biomarkers studies including erythrocyte C4d-to-complement receptor 1, soluble CD30, and vitamin D and pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) were done by C.-H. Chen et al., R. Gao et al., and P. Zhang et al. Clinically, anthropometric measurement of Schober's test was examined by Y.-R. Yen et al. Many articles investigating therapies, including infliximab, a novel antirheumatic drug T-614, surgical interventions, home exercise, and whole-body cryotherapy with kinesiotherapy, were addressed by Z. Lin et al., Y. Wei et al., L.-F. Hsieh et al., and A. Stanek et al. We hope this special issue covered many important aspects in inflammatory joint diseases, which will surely provide us with a better understanding about the pathogenesis, diagnosis, and treatment of spine and rheumatic diseases.

James Cheng-Chung Wei
Yi Liu
Hsi-Kai Tsou
Irene Eva van der Horst-Bruinsma

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Research Article

The Anthropometric Measurement of Schober's Test in Normal Taiwanese Population

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The measurement of lower back mobility is essential in the assessment of lower back pain including ankylosing spondylitis. Original Schober's test (OST) and modified Schober's test (MST) are popularly conducted in daily rheumatology and orthopedics clinical practices. To our knowledge, this report is the only anthropometric reference study in a normal oriental population. The OST declined with age from 5.0 cm in the youngest (20–30 years old) to 3.1 cm in the aged (70–80 years old) male subjects and from 3.6 cm to 2.4 cm in the female subjects. The male OST was significantly more than the female OST. There was a good correlation between OST and MST in each of the three age groups of both sexes.

1. Introduction

The mobility of the lumbar spine is restricted in patients with ankylosing spondylitis (AS) [1]. AS is a chronic inflammatory autoimmune disease of the axial skeleton primarily involving spine and sacroiliac joints [2, 3]. The typical early symptoms are chronic low back pain and spinal stiffness causing difficulty in bending [4]. Eventually it results in complete fusion and rigidity of spinal joints [5]. The most simple and noninvasive screening method for lumbar mobility is Schober's test.

Schober's tests, including original Schober's test (OST) [6–8], modified Schober's test (MST) [7, 8], and modified-modified Schober's test (MMST) [7, 9], are not harmful for measuring flexibility of lumbar spine with the subject bending forward. These tests can be simply conducted in the clinic. The OST and MST are popularly used [7, 10–13]. The measurement of these tests is not only useful for screening the status of AS disease, but also useful for the determination of progression and therapeutic effects of AS [14] as well as other pathologic conditions associated with low back pain [7, 10, 13, 15].

Reports of anthropometric reference data are rare [11, 16], and none were reported in oriental populations. We measured the Schober indices in different ages of normal Taiwanese men and women who had no history of low back pain or any diseases involving the lower back. This study provides reference data for clinicians to judge the degree of low back motility and disease status [16]. We measured OST in normal subjects for lumbar mobility in relation to age and sex. The correlation between OST and MST was also studied.

2. Methods and Materials

Volunteer subjects were recruited from the clinic of AIR (allergy, immunology, and rheumatology), Chung Shan Medical University Hospital in central Taiwan. The exclusion criteria included any structural abnormality such as kyphosis, scoliosis, surgical history, lumbar deformity, and skin problems of the lumbar area and functional abnormality such as arthropathy, acute or chronic pain in the lumbar area, or neurological diseases involving the lumbar area.

There were 165 men and 122 women for OST measurements and 135 men and 84 women for both OST and MST measurements.

The OST and MST were measured according to the methods described [8]. Namely, for the measurement of OST,

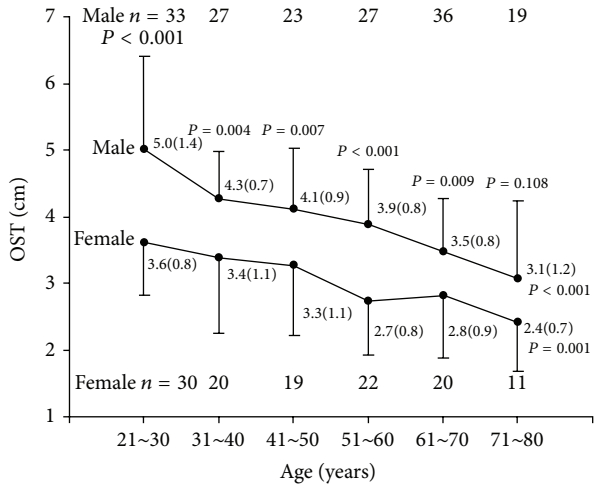

Figure 1: The relation between original Schober's test (OST) and age. OST data were presented as the mean (±SD).

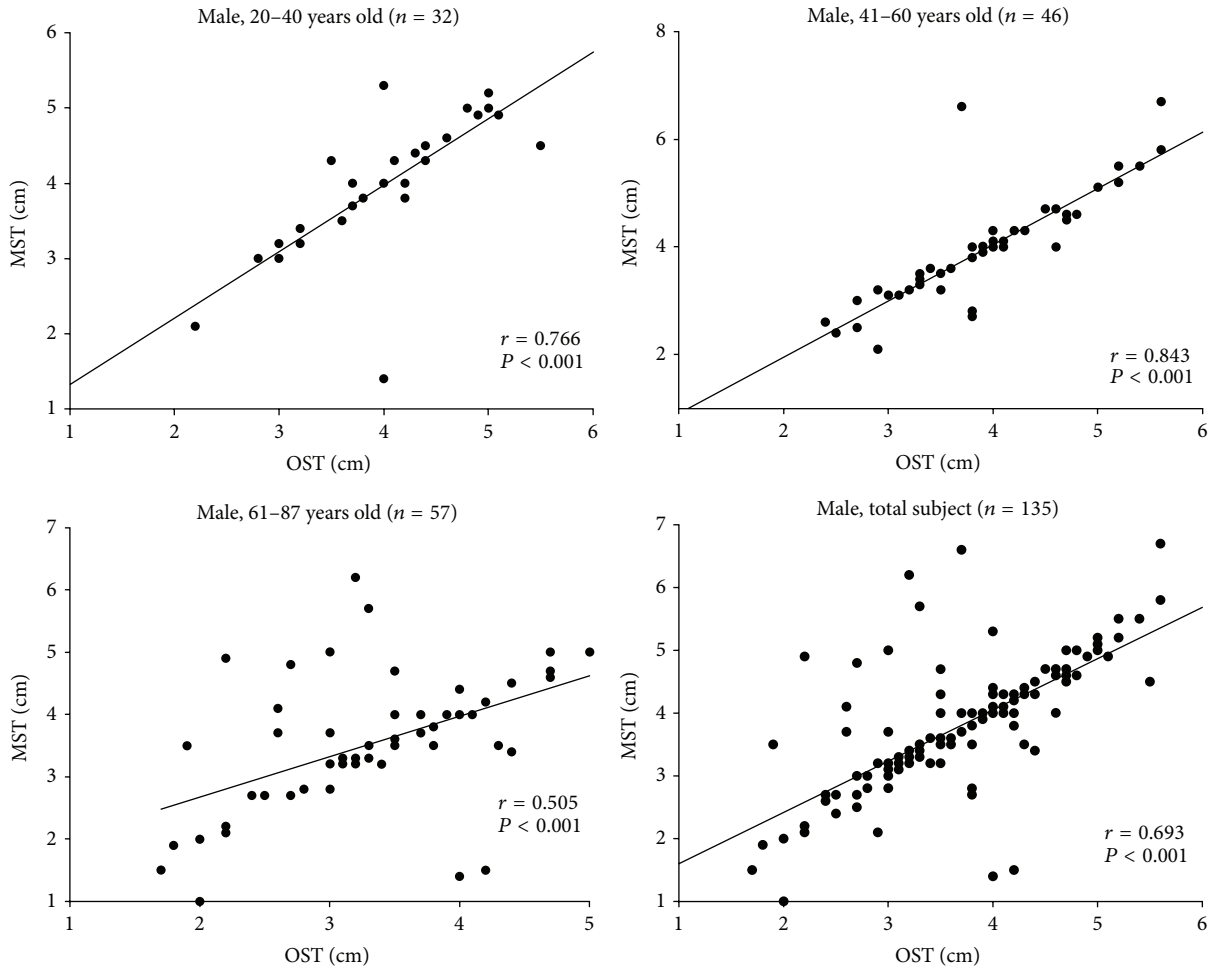

Figure 2: The correlation between original Schober's test (OST) and modified Schober's test (MST) in the male group.

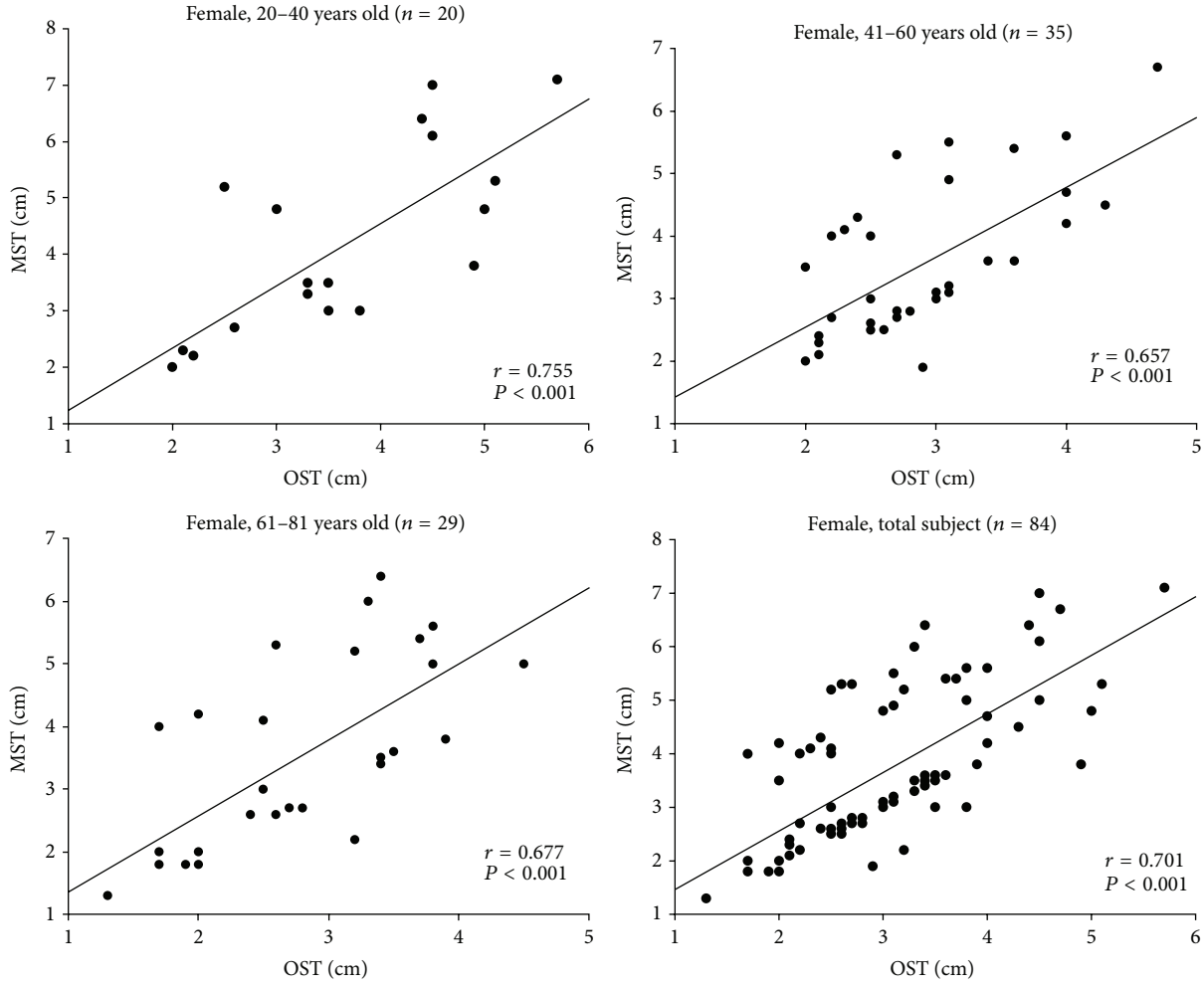

Figure 3: The correlation between original Schober's test (OST) and modified Schober's test (MST) in the female group.

the participant stood erect while the lumbosacral junction was marked as indicated by the dimples of Venus. A second mark was placed 10 cm above the junction. The participant was then asked to bend forward as far as possible, and the stretched distance was indicated as the OST in cm.

For measuring the MST, we put a mark 5 cm below and 10 cm above the junction. The participant was asked to bend forward as far as possible and the stretched distance of these two points was measured as the MST value.

Statistical analysis of the differences between different age and sex groups was carried out using the Statistical Package for the Social Sciences (SPSS), version 7.0 (Chicago, IL, USA). A value of $P < 0.05$ was considered significant.

The design of this study about the measurement of lower back flexibility conformed to the Declaration of Helsinki and this project was reviewed and approved by the Institutional Review Board of the Chung Medical University Hospital (CSMUH number 13211).

3. Results

3.1. The OST among Different Age Groups. For the measurement of OST, 165 men and 122 women were recruited. The OST measurements in different age groups and in both sexes are shown in Figure 1.

The OST declined with age as tested by ANOVA, $P < 0.001$ for men and $P = 0.001$ for women. For male subjects, OST decreased an average of 0.38 cm for each decade of age and 0.24 cm for female subjects. The male OST, by t-test, was significantly more than the female OST in each age group (P values were between 0.001 and 0.009) except for those older than 71 years of age. On average, the male OST was 0.95 cm more than the female OST.

3.2. The Relationship between OST and MST. In this study, all subjects (n = 219) were divided into three age groups, 20–40, 41–60, and above 60 years old. For males (n = 135) (Figure 2), there was a close correlation between OST and MST in all three age groups and total male subjects with $P < 0.001$ by a Pearson correlation test. In each panel, the coefficient of correlation, r, was between 0.505 and 0.843.

For females (n = 84) (Figure 3), there was a good correlation between OST and MST with $P < 0.001$ for all three age groups and total female subjects. In each panel, the r was between 0.657 and 0.755. These data seemed more scattered than in the male group.

4. Discussion

To our knowledge, this is the first report on anthropometric measurements in a normal oriental population.

Our data showed that both the OST and the MST declined with age, there being more lumbar mobility in the younger subjects than the aged (Figure 1). The degree of mobility declination of the OST over age among males was very significant from an average of 5.0 cm in the youngest group to 3.1 cm in the oldest group and 3.6 cm to 2.4 cm among females. It was difficult to recruit enough aged normal subjects for this study. We recruited only 11 normal female subjects aged 71–80 in this study, making the results less reliable. The lumbar mobility in males was significantly more than the lumbar mobility among females, being 0.95 cm more on average.

Schober's test results were partitioned into OST, MST, and MMST [7]. Each test is easy to perform in the clinic. Our results indicated that there was a good correlation between the OST and MST results in all the three age groups, $P < 0.001$ (Figures 2 and 3). Thus, one can select either test to perform in daily clinic practice.

According to the report of Cidem et al. [8], the factor of body height was not statistically significant in correlation with OST results; hence we did not measure body heights in our study subjects. In their study in a normal male Turkish population [8], the OST was 5.6 cm in a young male group (ages 20–30), while it was 5.0 cm in the same age group of the normal Taiwanese subjects. It seemed to be not due to the difference of body height in the two races but rather maybe due to hereditary or life style differences, such as exercise.

The lumbar mobility is much less in patients with ankylosing spondylitis (AS). Tan et al. [12] reported that the OST of AS patients with disease duration of 20.0 ± 11.8 years was 3.3 ± 1.2 cm, which is equivalent to our study at ages between 60 and 80 years. In Inanir's report [17], in 48 AS patients with normal heart, having no cardiac disorder of fragmented QRS, the OST was 4.10 ± 1.40 cm. Rezvani et al. [7] reported that the mean OST of AS patients was 4.07 ± 1.88 cm, while the control was 4.57 ± 0.87 cm, being 12.3% more than that of AS patients. The OST of their control is equivalent to that of our report at ages between 21 and 40 years. In patients with acute low back pain, the OST was even less, being only 2 cm, as reported by Konstantinovic et al. [18].

In our study, the subjects with pathological lumbar conditions were strictly excluded, such as functional abnormality, structural abnormality, and lumbar skin problems. The data presented here measuring the normal subjects is purely aimed as reference data to be used for comparison with various diseases in oriental subjects.

5. Conclusion

The anthropometric measurements of Schober's test (OST) in a Taiwanese population showed significant declination with age. The male OST values were significantly more than the OST values in females. There was a good correlation between OST and MST in each of three age groups and between sexes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Inhibitory Effect of a Novel Antirheumatic Drug T-614 on the IL-6-Induced RANKL/OPG, IL-17, and MMP-3 Expression in Synovial Fibroblasts from Rheumatoid Arthritis Patients

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T-614 (also named as iguratimod), a novel antirheumatic drug, could attenuate joint inflammation and articular damage in rheumatoid arthritis (RA) patients, providing a new therapy for RA. Here, we tested the role T-614 on the IL-6-induced receptor activator of nuclear factor κB ligand (RANKL)/osteoprotegerin (OPG), IL-17, and MMP-3 expression in synovial fibroblasts from rheumatoid arthritis (RASFs) patients. T-614 decreased RANKL expression and RANKL/OPG ratio in IL-6-induced RASFs. We confirmed this effect by a decrease of the mRNA and protein RANKL and mRNA RANKL/OPG in RASFs exposed in vitro to T-614 or MTX. Markedly decreased levels of IL-17, retinoid-related orphan receptor C (RORc), and MMP-3 mRNA expression were also observed in IL-6-induced RASFs in the presence of T-614 or MTX compared with those in its absence. Furthermore, T-614 blocked expression of p-ERK1/2 protein without affecting ERK1/2 expression, indicating that the way that T-614 regulated RANKL expression might be ERK1/2 pathway. Our results suggest that T-614 yields a strong improvement in arthritis via exact suppression of RANKL/OPG, IL-17, and MMP-3 expression in RASFs.

1. Introduction

Rheumatoid arthritis (RA) is a complicated autoimmune disease characterized by persistent synovitis and, thereby, bone erosion. Although the etiology is not fully understood, a combination of genetic and environmental risk factors contributes to breaching of the immune tolerance, playing an important role in RA pathogenesis. The goal of RA treatment is remission or low disease activity, ultimately slowing or preventing the progression of joint destruction.

T-614 (also named iguratimod), a novel disease-modifying antirheumatic drug, has been widely used in clinics in China and Japan. T-614 could inhibit the production of various inflammatory cytokines, including interleukin-1, interleukin-6, interleukin-8, and TNF and dramatically improve symptoms in RA patients [1] and collagen-induced arthritis (CIA) mice [2]. Moreover, T-614 could markedly suppress disease progression and bone erosion in CIA mice [3]; however, the exact mechanism remains unclear.

Receptor activator of nuclear factor κB ligand (RANKL) is the most essential factor for osteoclastogenesis by stimulation of osteoclast precursor cells differentiating into osteoclasts and by prompting osteoclasts migration, fusion, activation, and survival [4]. Accumulated evidences have suggested that RANKL level was associated with bone erosion in RA [5]. OPG acts as the natural inhibitor of RANKL, preventing RANKL from binding to its osteoclast receptor. OPG knockout mice exhibit severe osteoporosis and bone erosion [6], implicating the importance of RANKL/OPG balance for maintaining osteoclast homeostasis.

Proinflammatory cytokines including TNF-α, IL-17, IL-1β, and IL-6, derived from synovial fibroblasts of inflamed joints, are the primary trigger for the local or systemic high expression of RANKL in mice and RA patients [7, 8], which is one of the main mechanisms of inflammation-induced bone loss in RA. In addition to RANKL, IL-17 could directly increase cartilage proteoglycan loss and prompt the expansion of osteoclast precursors, participating in joint

degradation in RA [9]. MMP-3 is also involved in bone destruction in RA patients by providing space for RASFs to invade through removing the extracellular matrix [10]. Here, we study the effect of T-614 on the RANKL, OPG, IL-17, and MMP-3 expression in a simulative inflammatory context by RASFs stimulated with IL-6 and conducted a comparative analysis of the antiarthritic of T-614 and MTX, the classic disease-modifying antirheumatic drugs (DMARDs) used for RA therapy.

2. Materials and Methods

2.1. Cell Culture and Reagents. Synovial tissues were obtained from active RA patients who were undergoing surgery for knee replacement surgery. All RA patients fulfilled the American College of Rheumatology revised criteria for the diagnosis of RA [11]. Synovial tissues were harvested and incubated with collagenase type I (1 mg/mL) for 2 hours at 37°C. After digestion, RASFs were washed extensively and cultured in DMEM supplemented with 10% FCS in a humidified 5% CO_2 atmosphere. After overnight culture, nonadherent cells were removed, and adherent cells were cultured in DMEM supplemented with 10% FCS. RASFs were used between passages 3 and 7.

2.2. T-614 and MTX (Methotrexate) Treatment. RASFs ($1 * 10^5$/mL) were cultured in six-well plates and stimulated with IL-6 (Peprotech, Rocky Hill, USA)/sIL-6R (GenWay, San Diego, CA, USA) for 72 h in the presence or absence of T-614 or MTX (4.5 μg/mL), using RASFs without IL-6/sIL-6R treatment as control. Recombinant human IL-6 and sIL-6R were used at 20 ng/mL and 100 ng/mL, respectively. T-614 was synthesized at Simcere Pharmaceutical (Nanjing, China), and it was used as solution in dimethyl sulfoxide (DMSO). The final concentrations of DMSO were less than 0.1% where the cell viability was not affected.

2.3. Cell Viability Assay. RASFs were seeded at a density of $3–5 * 10^3$/0.2 mL/well in 96-well flat bottom. Then, RASFs were stimulated in different concentrations of T-614 (0, 20, 50, 100, and 150 μg/mL) for 72 h. Viability assay was performed using the Cell Counting Kit-8 (Dojindo, Tokyo, Japan) according to manufacturer's instruction. Substrate was added to elicit a colorimetric reaction, and absorbance was measured at 450 nm using a microplate reader.

2.4. Real-Time PCR. RASFs, cultured with cytokines, T-614, and MTX or not, were harvested and total cellular RNA was isolated from cells extracted with the TRIzol reagent (Invitrogen, USA). Precipitated RNA was reverse transcribed by the PrimeScript RT regent Kit (TaKaRa, Japan). mRNA expression of β-actin and the selected molecules were determined by real-time PCR using SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed using specific primers for (β-actin, sense 5′-CCACACTGTGCCCATCTACG-3′ and antisense 5′-AGGATCTTCATGAGGTAGTCAGTCAG-3′; IL-17, sense 5′-GGGCCTGGCTTCTGTCTGA-3′ and antisense 5′-AAGTTCGTTCTGCCCCATCA-3′; RORc, sense 5′-GAAGTGGTGCTGGTTAGGATGTG-3′ and antisense 5′-GCCACCGTATTTGCCTTCAA-3′; RANKL, sense 5′-CAACATATCGTTGGATCACAGCA-3′ and antisense 5′-GACAGACTCACTTTATGGGAACC-3′; OPG, sense 5′-GCGCTCGTGTTTCTGGACA-3′ and antisense 5′-AGTATAGACACTCGTCACTGGTG-3′; MMP-3, sense 5′-CGGTTCCGCCTGTCTCAAG-3′ and antisense 5′-CGCCAAAAGTGCCTGTCTT-3′). Thermocycler conditions included an initial holding at 50°C for 2 min and then 95°C for 10 min; this was followed by a 2-step PCR program: 95°C for 15 s and 60°C for 60 s for 40 cycles. Data were collected and quantitatively analyzed on an ABI PRISM 7900 sequence detection system (Applied Biosystems, CA, USA). β-Actin gene was used as an endogenous control. The amount of gene expression was then calculated as the difference cycle threshold (ΔCT) between the CT value of the target gene and the β-actin. $\Delta\Delta$CT were differences between ΔCT values of test sample and the control. Relative expression of target genes was calculated as $2^{-\Delta\Delta CT}$.

2.5. Western Blot Analyses. Cells were treated with IL-6/sIL-6R for 0, 1, 6, 12, 24, and 48 hours. For another experiment, RASFs were cultured with IL-6/sIL-6R plus T-614 or MTX for 72 hours, using 25 μM PD98059 (CST, USA) before IL-6/sIL-6R was added as control. Proteins were extracted in lysis buffer (30 mM Tris [pH 7.5], 150 mM sodium chloride, 1 mM PMSF, 1 mM sodium orthovanadate, 1% nonidet P-40, 10% glycerol, and phosphatase and protease inhibitors). The proteins were then separated by SDS-PAGE and electrophoretically transferred onto polyvinylidene fluoride membranes. The membranes were probed with RANKL (EPR4999, Abcam, Cambridge, MA, USA), p-ERK1/2 (D13.14.4E, Cell Signaling, MA, USA), ERK1/2 (137F5, Cell Signaling, MA, USA) or GAPDH (D16H11, Cell Signaling, MA, USA), or β-actin (13E5, Cell Signaling, MA, USA) overnight at 4°C and then incubated with an HRP-coupled secondary Ab (Cell Signaling, MA, USA). Proteins were detected with the SuperSignal West Pico Chemiluminescent Kit (Thermo Scientific, Rockford, IL, USA). Densitometry values were analyzed and quantified with ImageLab (Bio-Rad).

2.6. Statistical Analysis. Data are presented as mean ± SD. Statistical analysis was performed using ANOVA test. A probability < 0.05 was defined as significant.

3. Results

3.1. RASFs Cultured In Vitro. A homogeneous population (<1% CD11b, <2.5% CD14+, <1%CD3+, and <1% CD19+) was identified as RASFs [10]. A majority of RASFs at the first or second generation were polygonal or fusiform, with prominent nucleoli and more mitotic figures. The morphology of RASFs at the fourth generation was displayed in Figure 1, which was used in the next experiments.

3.2. Cell Viability Assay. To test the effect of T-614 on cell viability, RASFs were treated with different concentrations of

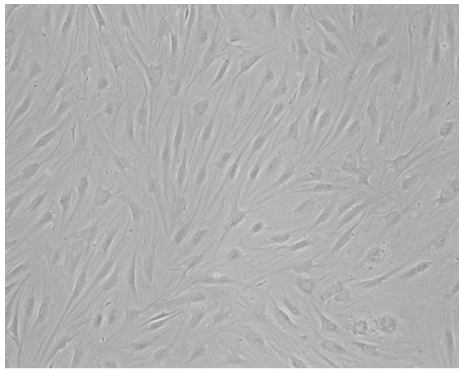

Figure 1: Appearance of human rheumatoid arthritis synovial fibroblasts at generation 4.

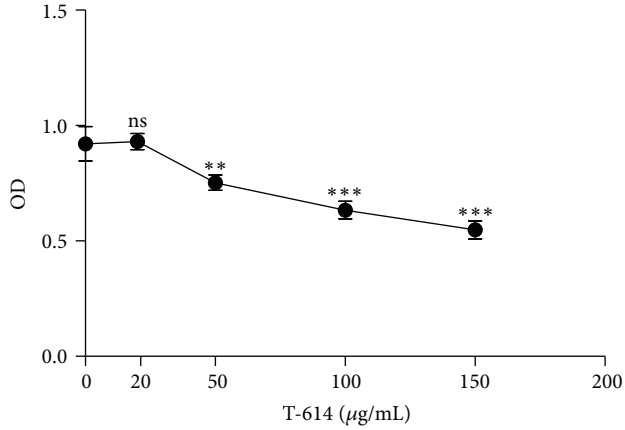

Figure 2: Viability of T-614 on RASFs in vitro. Cells were treated with the indicated concentrations of T-614 for 72 h. Cell viability was determined by the CCK-8 assay. Values are the mean ± SD for three independent experiments, each in triplicate. ns: not significant, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus T-614 (0 μg/mL) alone.

T-614 (0, 20, 50, 100, and 150 μg/mL). After 72 h, CCK-8 was added to cells to assay cell viability. As shown in Figure 2, T-614 could inhibit the cell ability in a dose dependent manner; only the concentration of T-614 less than 20 μg/mL did not markedly affect the growth of RASFs and was then used in current study.

3.3. T-614 Inhibited RANKL/OPG Expression in IL-6-Stimulated RASFs. In the pathological condition of RA, RASFs in the inflamed joints could cause the exaggerated expression of multiple proinflammatory cytokines including TNF-α, IL-6, IL-17, and IL-1β, all of which resulted in an increased RANKL expression in local joint. Given that previous studies reported that only IL-1β or IL-6 plus sIL-6R (but not IL-6 alone) is capable of inducing RANKL expression in vitro after RASFs treatment with different cytokines [12], thus, we stimulated RASFs with IL-6/sIL-6R to induce RANKL expression in current study. As expected, a robust increased RANKL and a modest decreased OPG expression were observed in RASFs upon IL-6 stimulation (Figures 3(a), 3(b), 3(c), and 3(d)). RANKL mRNA (Figure 3(a)) and protein (Figures 3(b) and 3(c)) expression was decreased by T-614 in IL-6-induced RASFs, but OPG expression was slightly enhanced by T-614 (Figure 3(d)), which resulted in a decreased ratio of RANKL/OPG in IL-6-stimulated RASFs (Figure 3(e)). Moreover, the inhibitory role of T-614 on RANKL expression was superior to the traditional disease-modifying antirheumatic drugs (DMARDs) MTX (Figure 3(a)). PD98059, the ERK inhibitor, could also suppress RANKL protein expression (Figures 3(b) and 3(c)), indicating that ERK signal was involved in RANKL expression.

3.4. T-614 Inhibited the Expression of IL-17 and Its Transcription Factor RORc, as well as MMP-3 in IL-6-Stimulated RASFs. Given that previous study reported that IL-17 and MMP-3 also participated in osteoclastogenesis and played a crucial role in inflammation and bone erosion in RA patients and in CIA mice [10, 13, 14], we next evaluated potential effect of T-614 on the production of IL-17 and its transcriptional factor RORc, as well as MMP-3 mRNA expression in IL-6-induced RASFs (Figure 4). Markedly elevated expression levels of IL-17 (Figure 4(a)), RORc (Figure 4(b)), and MMP-3 (Figure 4(c)) mRNA were found in RASFs after stimulation with IL-6, which were significantly suppressed by T-614 or MTX in RASFs. Moreover, the inhibition effect of T-614 on IL-17 and RORc expression was greater than MTX (Figures 4(a) and 4(b)).

3.5. T-614 Inhibited RANKL Expression via ERK1/2 Signal. It is reported that IL-6 exerted its action via JAK/STAT (Janus kinase/signal transducer and activator of transcription) and MAPK (mitogen-activated protein kinase) cascade by binding to its receptors [15]. Previous studies also showed that activation of ERK was necessary for RANKL expression in RASFs [5, 16, 17]. To investigate the mechanism of T-614 on suppression of RANKL expression in IL-6-stimulated RASFs, we tested the p-ERK1/2 and ERK1/2 protein expression in RASFs upon IL-6 and T-614 stimulation. The western blot suggested that IL-6 could induce ERK1/2 phosphorylation in RASFs. The peak of ERK1/2 phosphorylation was at 1 h after the addition of IL-6 into cells (Figure 5(a)). T-614 and MTX could markedly decrease the phosphorylation of p-ERK1/2 ($P < 0.05$; Figures 5(b) and 5(c)), indicating that the inhibitory role of T-614 on RANKL protein expression might be through ERK1/2 pathway.

4. Discussions

The novel disease-modifying antirheumatic drug, T-614, has a reported effect on preventing the inflammatory and destructive processes of RA by inhibiting the production of immunoglobulins and various inflammatory cytokines (IL-1, IL-6, IL-8, and TNF-α), exerting a unique mechanism for RA therapy [18]. Here, we confirmed the anti-inflammatory role of T-614; moreover, our data suggested that T-614 could downregulate the ratio of RANKL/OPG via blocking ERK activation, suggesting a novel mechanism of T-614 on inhibition of bone destruction in RA.

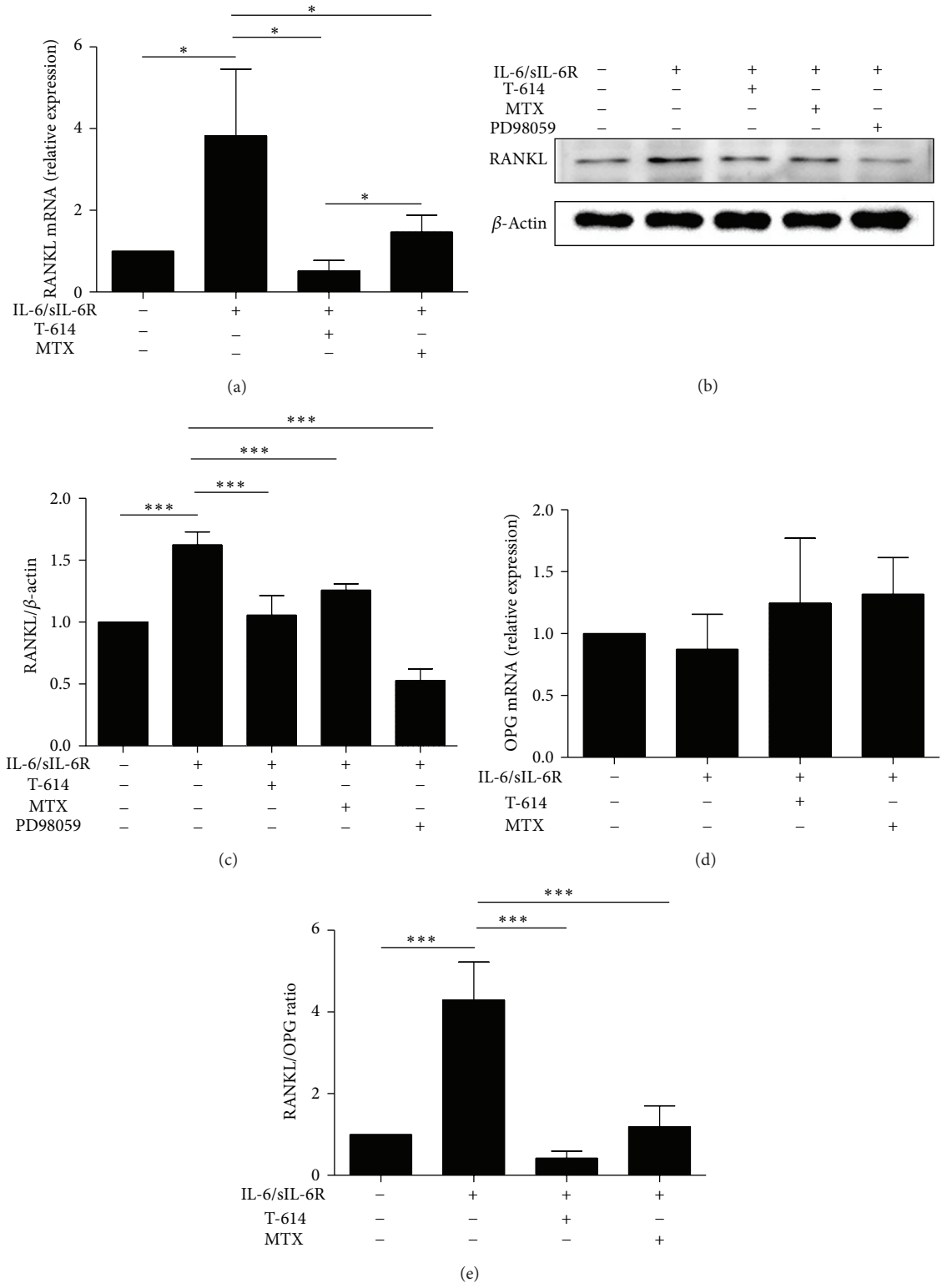

Figure 3: Effect of T-614 and MTX on IL-6-induced RANKL, OPG expression, and the ratio of RANKL/OPG in RASFs. RASFs were stimulated with IL-6/sIL-6R and then treated with T-614 or MTX for 72 h. The effect of T-614 and MTX on the expression of RANKL (a), OPG (d), and RANKL/OPG (e) in IL-6-induced RASFs was tested by real-time quantitative PCR analysis. Total proteins were extracted and expression levels of RANKL protein were determined by western blot (b, c). The data points shown are the mean ± SD for three independent experiments, each in triplicate. $^{***}P < 0.001$ and $^{*}P < 0.05$.

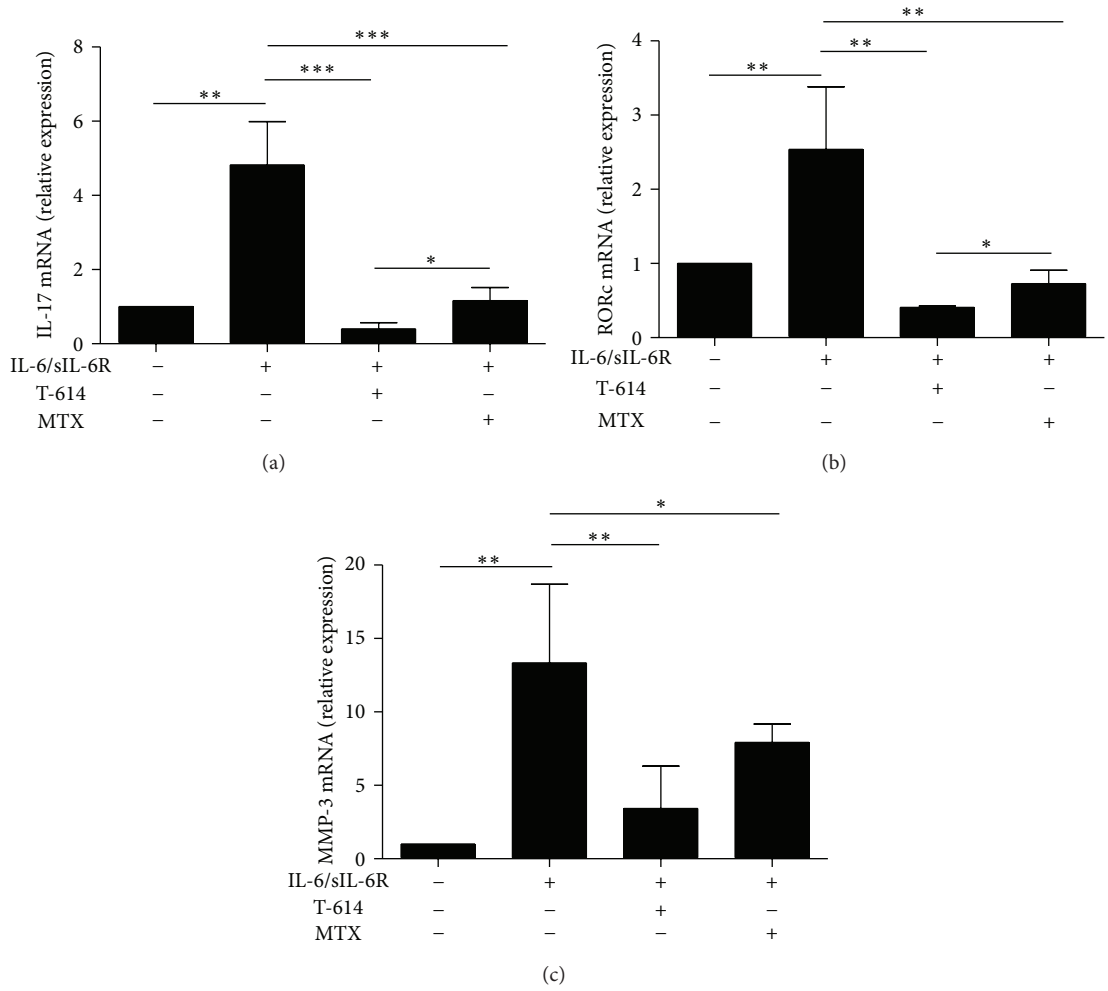

Figure 4: Effect of T-614 and MTX on IL-6-induced MMP-3, IL-17, and RORc mRNA expression in RASFs. RASFs were stimulated with IL-6/sIL-6R, T-614, or MTX for 72 h. The effect of T-614 and MTX on IL-6-induced IL-17 (a), RORc (b), and MMP-3 (c) mRNA expression in RASFs was tested by real-time quantitative PCR analysis. The data shown are the mean ± SD for three independent experiments, each in triplicate. $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$.

Osteoclast is the cell ultimately responsible for destruction in RA. A mass of evidences indicates that RANKL plays an important role in regulating osteoclast development [19]. RANKL is known to be produced by a number of different cell types including T cells, B cells, dendritic cells, macrophages, and synovial fibroblasts in RA [20–22]. OPG acts as its natural decoy receptor by blocking the RANK/RANKL interaction and the elevated ratio of RANKL/OPG may represent a high state of osteoclastogenesis and high activity of bone degradation. Given that RASFs were one of the major cells that express RANKL [23], here, RASFs were stimulated with IL-6 to induce RANKL expression. We found, for the first time, that T-614 has a potent ability to decrease the ratio of RANKL/OPG, which suggested that the therapeutic effect of T-614 on preventing disease progression, at least in part, attributed to its regulation role on RANKL/OPG axis hence contributing to suppress osteoclastogenesis [24].

IL-17, also produced by RASFs [7], plays a crucial role in inflammation and bone erosion in RA patients and in CIA mice [12, 13]. IL-17 could also upregulate the expression of RANKL in osteoblasts and synovial fibroblasts and then amplify the bone destruction in collagen-induced arthritis (CIA) mice. RORc is the transcription factor for IL-17. Consistent with recent report that T-614 could suppress IL-17 signal in RASFs [5], our data proved that T-614 could significantly downregulate the production of IL-17 and RORc in IL-6-induced RASFs. Moreover, T-614 showed a greater inhibitory effect on IL-17 and RORc expression than MTX.

MMP-3 has been suggested to play a pivotal role in the cartilage destruction in RA. Patients with joint injury have been found to have persistently increased proMMP-1 and proMMP-3 levels in synovial fluid, which mainly came from RASFs [25]. Serum MMP3 was correlated with IL-8, IL-6, IFN-γ, CRP and cartilage breakdown in 128 patients [26] and have been suggested to be a good laboratory index to evaluate the joint injury status and therapeutic effect [27]. In our study, IL-6 increased the production of MMP-3 in RASFs, confirming that inflammatory environment of joints was

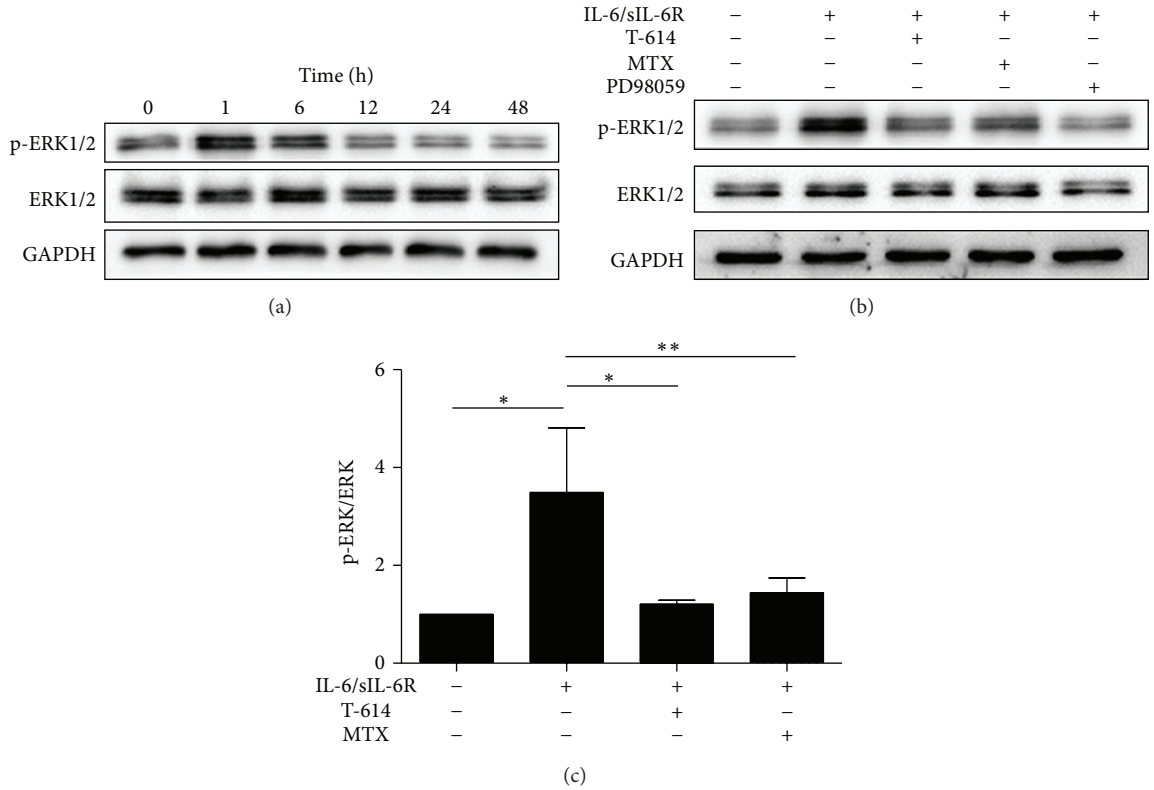

Figure 5: The effect of T-614 on p-ERK1/2, ERK1/2 expression in IL-6-induced RASFs. (a) RASFs were stimulated with IL-6 for 0, 1, 6, 12, 24, and 48 h; total proteins were extracted and expression levels of ERK1/2 and p-ERK1/2 protein were determined by western blot. (b, c) RASFs were cultured with PD98059 (25 μM) for 1 h or not; then IL-6/sIL-6R were added, and incubation was continued for another 1 h. Total proteins were extracted and expression levels of ERK1/2 and p-ERK1/2 protein were determined by western blot. The data shown are the mean ± SD for three independent experiments, each in triplicate. $^{**}P < 0.01$ and $^{*}P < 0.05$.

the major cause of high expression of MMP-3. Here, our data demonstrated that T-614 could decrease MMP-3 production in IL-6-induced RASFs, implying that T-614 could reduce disease activity and bone erosion of RA.

Substantial evidence has suggested that ERK phosphorylation was involved in RANKL [5, 16, 17], IL-17 [28], and MMP-3 [29] expression in RASFs. In order to investigate the potential pathway of T-614 on RAKL/OPG expression in RASFs, we studied the effect of T-614 on ERK1/2 signaling. Our data showed that the phosphorylation of ERK1/2 was triggered after the stimulation of RASFs by IL-6 and reached peak at 1 h after stimulation with IL-6. The level of ERK1/2 phosphorylation could be abolished by T-614 and MTX. The high expression of RANKL protein in IL-6-induced RASFs could be cancelled by ERK inhibitor, PD98059, confirming that T-614 inhibited RANKL expression via ERK1/2 signal. Taken together, our data suggested that T-614 could inhibit RANKL expression in RASFs via ERK1/2 pathway.

5. Conclusions

T-614 has been confirmed as a highly effective drug for RA therapy and has been widely used in clinics in China and Japan. The present study demonstrates, for the first time, that T-614 could decrease the RANKL expression and downregulate the ratio of RANKL/OPG in RASFs via blocking ERK1/2 phosphorylation; we also confirmed that T-614 could suppress IL-17 and MMP-3 expression in IL-6-induced RASFs. This study also identified that the immunosuppressive effects in RANKL and IL-17 expression of T-614 on RASFs were stronger than MTX; our data provide a novel insight into the mechanisms of antiarthritic effect in T-614.

Abbreviations

RANK: Receptor activator of NF-κB
RANKL: Receptor activator of the NF-κB ligand
OPG: Osteoprotegerin
RA: Rheumatoid arthritis
RASFs: Rheumatoid arthritis synovial fibroblasts
IL-17: Interleukin-17
RORc: Retinoid-related orphan receptor C
MMP-3: Matrix metalloproteinase-3
MTX: Methotrexate
TNF: Tumor necrosis factor
CIA: Collagen-induced arthritis
sIL-6R: Soluble IL-6 receptor.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

All authors read and approved the final paper.

Acknowledgments

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Clinical Study

Disorders of MicroRNAs in Peripheral Blood Mononuclear Cells: As Novel Biomarkers of Ankylosing Spondylitis and Provocative Therapeutic Targets

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Background. MicroRNAs can potentially regulate every aspect of cellular activity. In this study, we investigated whether AS pathogenesis involves microRNAs disorders. *Result*. The expression of 2 microRNAs, hsa-miR-126-3p and hsa-miR-29a, was significantly lower in active AS group before etanercept therapy than in control group. Marched fold changes of them were 3.76 and 16.22. Moreover, expressions of hsa-miR-126-3p and hsa-miR-29a were dramatically upregulated after 12-weeks etanercept treatment. Fold changes were 2.20 and 3.18. All regulations of microRNAs expression mentioned before were statistically significant (fold change >2 and $P < 0.05$). The expression disorders of the 2 microRNAs did not statistically significantly correlated with BASDAI, CRP, and ESR. *Conclusion*. AS pathogenesis involved dysregulation of microRNAs. Hsa-miR-126-3p and hsa-miR-29a will probably become the potential biomarkers and provocative therapeutic targets of AS.

1. Background

Ankylosing spondylitis (AS) is a member of a group of rheumatic diseases that affects the axial joints (spine and pelvis), collectively known as spondyloarthropathies. It is a common disease affecting approximately 0.5% of white Europeans and has a global distribution with the exception [1]. The risk of developing the disease is largely genetically determined, and the genetic susceptibility to AS is confirmed by twin study [2]. HLA-B27 is one of the convincing genetic factors [3–5], but it can explain no more than 30% of the overall genetic risks of AS [6]. Much of AS related genetic disorder, outside HLA-B27, still remains to be explored.

MicroRNAs are endogenous ~22 nt RNAs that comprise one of the more abundant classes of gene regulatory molecules in multicellular organisms and likely influence the output of many protein-coding genes [7]. MicroRNAs take part in regulation of gene expression; moreover about one-third of all mRNAs may be regulated by microRNAs [8]. It is now clear that microRNAs can potentially regulate every aspect of cellular activity, including differentiation and development, metabolism, proliferation, apoptotic cell death, viral infection, and tumorigenesis [9].

It was validated that microRNA disorder was related to pathogenesis of rheumatism. Expression of miR-132 and 3 other microRNAs differentiated patients with RA or OA from HC [10], while miR-146a was thought to be biomarker of SLE [11]. The paper also shows that microRNAs may have correlation with pathological changes of AS [12]. Huang et al. found that patients with AS compared to controls had significantly higher levels of miR-21, PDCD4 mRNA, and CTX [13]. To validate whether microRNAs could be biomarkers and therapeutic targets of AS, we investigated the correlation of peripheral blood mononuclear cells (PBMCs)

microRNA disorders and AS activity and measured the regulation of microRNAs expression after etanercept therapy.

2. Methods

2.1. Patients and Control Samples. Forty patients who fulfilled the Modified New York Criteria for Ankylosing Spondylitis (1984) were included in this study. It was demanded that the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [14] and VAS of all patients in this study be at least 4. All of them accepted regular etanercept therapy (50 mg, qw, hypodermic injection) for 12 weeks. Immunosuppressive drugs and other medication were not allowed during etanercept therapy. At least 10 mL peripheral blood was obtained from AS patients at baseline and after 12-week therapy. And their clinical information, including BASDAI, CRP, and ESR, was also collected. Fifty healthy volunteers with age and sex matched were taken as controls (Figure 3). Blood samples were collected with ethylenediaminetetraacetic acid (EDTA) containing tube to separate plasma and were stored in 4°C refrigerator. Ethical approval for this study was granted by the Ethics Committee of Third Affiliated Hospital of Sun Yat-sen University. Written permission was obtained from all subjects who participated in the study.

2.2. Filtrated MicroRNA by Microarray. PBMCs of 35 AS patients and 47 controls were extracted by using lymphocytes separation medium. Total RNA and microRNAs of each sample were isolated according to manufacturer's protocol of TRIzol Reagent (Life Technologies, Inc.) and mirVana microRNA Isolation Kit (Ambion, Inc.). To confirm that the report of microarray is reliable, the OD value (DU520 UV/Vis Spectrophotometer, Beckman Coulter, Ltd) and electrophoresis (Bio-Rad Mini-Sub GT System, Bio-Rad, Ltd) were used to evaluate the quantity and quality of total RNA. Then microRNAs were marked with Monoreactive Cy3 dye (Amersham Pharmacia Biotech, Ltd) and purified according to mirVana microRNA Labeling Kit (Ambion, Inc.) procedure. Prepared microRNAs were hybridized to microarray probes. 428 microRNAs probes were involved, and all of them were 34–44 nt and had the same Tm value. Fluorescent signals were scanned (Generation iii array scanner, Amersham Pharmacia) and translated into digital signals (Imagequant 5.0, Array Vision 6.0). Relative expression levels of target microRNAs were estimated according to digitized intensity of fluorescence. Calibrator was the median of all the valid data. Average microRNA expression level of each group and the ratio of any two of the three groups (AS patients before treatment, AS patients after treatment, and healthy donors) were independently calculated. Because only CY3 was used to mark target microRNA, the ratio >3 or <0.33 was thought to be statistically significant.

2.3. Reverse Transcription and Quantitation of miRNAs by Real-Time PCR. Thirteen microRNAs were chosen for validation, as follows. The relative expression levels of target microRNAs fulfilled being (a) significantly different between AS patients and healthy donors, (b) increasing/decreasing dramatically after regular etanercept therapy, or (c) taking part in the process of inflammation or bone metabolism according to the papers published before. Reverse transcription was performed according to the protocol. Real-time polymerase chain reaction (PCR) was performed on MX-3000P Real-Time PCR Instrument (Stratagen, US) using Beacon Real-Time PCR Universal Reagent (Cat# GMRS-001, GenePharma, Shanghai) and with U6 snRNA as the internal control. Primers were designed as follows. 10 samples of AS patient and 10 control samples were included in pilot real-time PCR experiment. Two microRNAs, hsa-miR-29a and hsa-miR-126-3p, were involved in next step real-time PCR validation. Sample size of each group was enlarged in further study. Relative copy numbers of target microRNAs were obtained. The expression levels of target microRNAs in each sample were calculated according to the copy numbers of target microRNAs. A fold change of >2 was considered significant.

Primers were designed as follows: hsa-miR-let7a (F primer: GGACTGAGGTAGTAGGTT, R primer: CATCAGATGCGTTGCGTA), hsa-miR-let7f (F primer: GGACTGAGGTAGTAGATTG, R primer: CATCAGATGCGTTGCGTA), hsa-miR-let7i (F primer: GGACCTGCGCAAGCTAC, R primer: CATCAGATGCGTTGGCTA), hsa-miR-21 (F primer: GGACTAGCTTATCAGACTG, R primer: CATCAGATGCGTTGCGTA), hsa-miR-26b (F primer: GGACTTCAAGTAATTCAGGA, R primer: CATCAGATGCGTTGCGTA), hsa-miR-27a (F primer: GGACTTCACAGTGGCTAA, R primer: CATCAGATGCGTTGCGTA), hsa-miR-29a (F primer: GGACTAGCACCATCTGAA, R primer: CATCAGATGCGTTGCGTA), hsa-miR-29b (F primer: GGACTAGCACCATTTGAAA, R primer: CATCAGATGCGTTGCGTA), hsa-miR-98 (F primer GGACTGAGGTAGTAAGTTG, R primer: CATCAGATGCGTTGCGTA), hsa-miR-202 (F primer: GGACTTCCTATGCATATAC, R primer: CATCAGATGCGTTGCGTA), hsa-miR-494 (F primer: GGACTGAAACATACACGG, R primer: CATCAGATGCGTTGCGTA), hsa-miR-526a (F primer: GGACCTCTAGAGGGAAG, R primer: CATCAGATGCGTTGCGTA), hsa-miR-126-3p (F primer: GGACTCGTACCGTGAGTA,R primer: CATCAGATGCGTTGCGTA).

2.4. Statistical Analysis. Data were presented as the mean ± standard deviation. Statistical analyses were performed using SPSS 10.0. Differences between two groups were analyzed with Wilcoxon rank sum test. Correlations of clinical presentations and microRNA expression levels were also analyzed. Spearman correlation coefficients were calculated. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Patients and Control Samples. There were 40 AS patients and 50 healthy volunteers included in this study. The male to female ratio and average age in the AS patients group matched with the healthy control group. Means of disease duration, BASDAI, BASFI, CRP, ESR, and medications used were calculated (Table 1).

Table 1: Clinical features of the AS participants.

Characteristics	AS ($n = 40$)
Disease duration, mean ± SD years	7.9 ± 0.8
BASDAI, mean ± SD	5.25 ± 1.62
BASFI, mean ± SD	46.5 ± 23.6
CRP, mean ± SD mg/L	30.5 ± 23.5
ESR, mean ± SD mm	39.9 ± 29.5
Medications before etanercept therapy, taking/not taking	
Steroids, last 3 months	6/34
DMARDs, last 3 months	27/13
NSAIDs, last 1 month	29/11

AS, ankylosing spondylitis; HC, healthy control; SD, standard deviation; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; DMARDs, disease-modifying antirheumatic drugs; NSAIDs, nonsteroidal anti-inflammatory drugs.

3.2. The Result of Microarray. According to the microarray, there were 26 significantly differentially expressed microRNAs. The expression levels of all the microRNAs were significantly higher in AS group than in control group (fluorescence intensity ratio of AS group to control group was >3 : 1) (Figure 1(a)). Furthermore, the first screening with microRNAs discovered 23 microRNAs, expressions of which were significantly different before and after 12-week etanercept therapy. Among them, the expression levels of 6 microRNAs downregulated significantly after regular etanercept therapy, while the other 17 microRNAs upregulated (fluorescence intensity ratio of AS group after treatment to before treatment was <1 : 3 or >3 : 1) (Figure 1(b)).

However, hsa-miR-15a, hsa-miR-515-3p, hsa-miR-198, hsa-miR-494, and hsa-miR-142-3p were probably higher in AS group than in control group (fluorescence intensity ratio of AS group to control group was >2 : 1 but <3 : 1) (Figure 1(a)). Similarly, 13 microRNAs were thought to keep different expression levels in AS group before and after etanercept therapy. The expression levels of 7 among them probably downregulated after regular etanercept therapy (fluorescence intensity ratio of AS group after treatment to before treatment was <0.5 but >0.3). And the levels of the other 6 probably upregulated after therapy (fluorescence intensity ratio of AS group after treatment to before treatment was <1 : 2 but >1 : 3) (Figure 1(b)).

It was necessary to point out that there were 9 differentially expressed microRNAs. All of them had higher expressed levels in AS group than healthy control group. However the expression levels of these microRNAs downregulated after regular etanercept therapy for 12 weeks (Figure 2).

3.3. The Result of Real-Time PCR. Real-time PCR was performed on 13 candidate microRNAs ($n = 10$ for both AS group and healthy control) (Table 2). The expression level of hsa-miR-126-3p, hsa-miR-29a, and hsa-miR-let7i which was validated by real-time PCR was consistent with the result of microarray. The fold change of them was >2, and P value was <0.05.

Table 2: Expression level of microRNAs in both AS and control group validated by real-time PCR.

microRNA	$2^{\Delta\Delta C_T}$	
	AS group/control group (interval)	AS group before/after therapy (interval)
hsa-miR-202	1.28 (0.28~5.88)	0.50 (0.13~2.08)
hsa-miR-21	0.51 (0.11~2.5)	—
hsa-miR-26b**	0.70 (0.26~1.89)	3.43 (1.20~9.85)
hsa-miR-27a*	0.07 (0.01~0.58)	0.8 (0.27~2.43)
hsa-miR-29a**	0.26 (0.12~0.53)	0.35 (0.25~1.24)
hsa-miR-29b**	1.22 (0.67~2.22)	0.43 (0.19~0.97)
hsa-miR-494**	0.58 (0.52–5.6)	0.21 (0.05~0.89)
hsa-miR-526a**	1.20 (0.48~3.03)	0.40 (0.13~1.14)
hsa-miR-98*	0.44 (0.10~1.89)	—
hsa-miR-let7a	0.91 (0.16~4.76)	—
hsa-miR-let7f	0.54 (0.11~2.70)	—
hsa-miR-let7i*,**	0.27 (0.05~1.49)	0.27 (0.05~1.47)
hsa-miR-126-3p*	0.15 (0.03~0.75)	0.51 (0.28~0.93)

*MicroRNAs had statistically significantly different expression in AS group and healthy control group (fold changes >2, $P < 0.05$).
**Expression levels were changed statistically significantly after medicine treatment (fold changes >2, $P < 0.05$).

Further validated study involved the 2 microRNA, hsa-miR-126-3p and hsa-miR-29a. As we anticipated, the result of further study was infusive. The expression levels of these 2 microRNAs were significantly lower in AS group (PCR values: 3.52 ± 3.76 and 7.26 ± 5.18; $n = 40$ and 29 for hsa-miR-126-3p and hsa-miR-29a, resp.) than in control group (PCR values: 5.42 ± 3.71 and 11.28 ± 0.62, $n = 50$ and 30 for hsa-miR-126-3p and hsa-miR-29a, resp.). Marched fold changes of them were 3.76 and 16.22. Moreover, expressions of hsa-miR-126-3p and hsa-miR-29a were dramatically upregulated after 12-week etanercept treatment. Fold changes were 2.20 and 3.18 ($n = 30$ and 29 for hsa-miR-126-3p and hsa-miR-29a, resp.). All the regulations of microRNAs expression mentioned before were statistically significant (fold change >2, $P < 0.05$) (Table 3).

3.4. Correlation Analysis of MicroRNAs and Clinical Presentation. Correlation analysis of 2 microRNAs, hsa-miR-126-3p and hsa-miR-29a, expression and clinical data indicated that hsa-miR-126-3p expression disorders statistically significantly correlated with CRP and ESR (Table 4).

4. Discussion

Since the discovery of microRNA, lin-4, in 1993 at Harvard [15], a great deal of effort had been devoted to annotate their biologic function and the relevance to diseases. Not only had the genomics, biogenesis, mechanism, and function of microRNAs been discovered [16], but also disorders of microRNAs had been associated with certain human disease

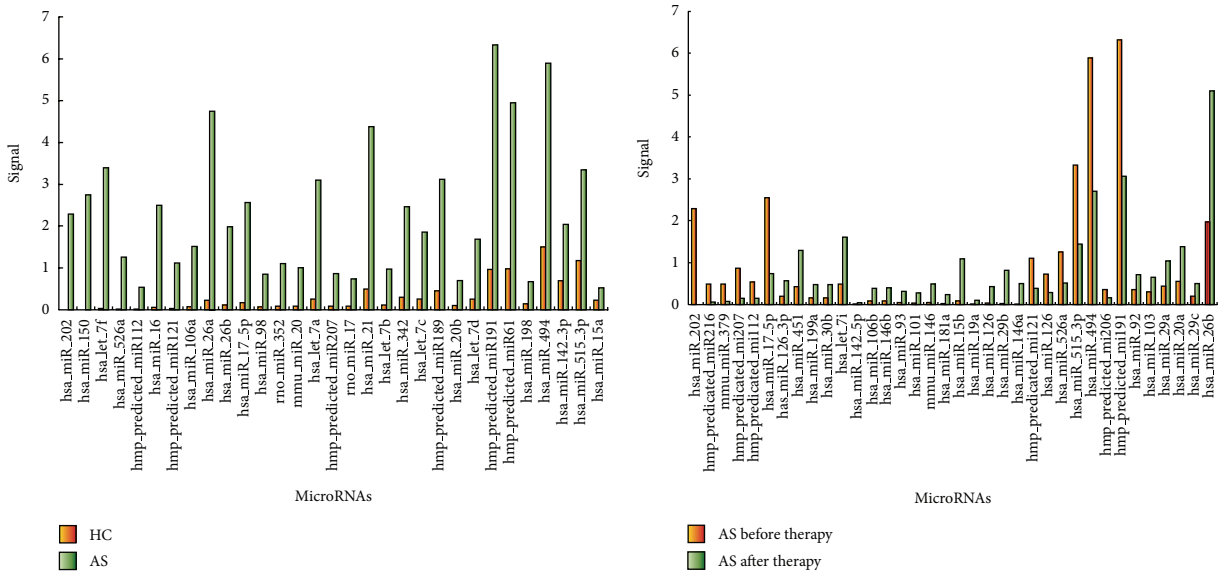

Figure 1: Calibrated fluorescence intensity of microarray in different groups. (a) The microRNAs signal intensity of AS group was compared with that of healthy control. There were 31 microRNAs in this figure. 26 of them, at the left side of the figure, expressed significantly higher in AS group than in HC. The other 5, at the right side of the figure, were probably higher in AS group than in control group. (b) The microRNAs signal intensity of AS group before and after etanercept therapy was compared. There were 36 microRNAs in this figure. 23 of them, at the left side of the figure, had definite expression regulation after therapy. Among them, the expression levels of 6 downregulated significantly after regular etanercept therapy, while 17 upregulated. However, the other 13 microRNAs, at the right side of the figure, were thought to keep different expression levels, not so dramatically changed in AS group before and after etanercept therapy. 7 among them downregulated, while 6 upregulated. AS, ankylosing spondylitis; HC, healthy control.

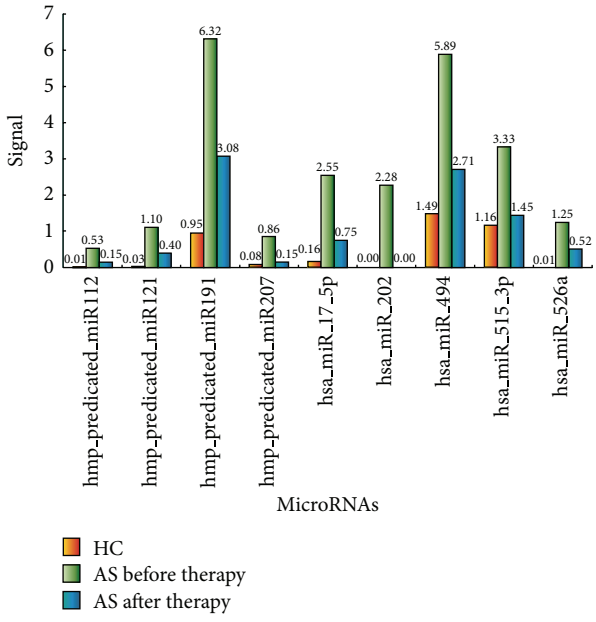

Figure 2: Nine microRNAs had higher expressed levels in AS group than healthy control group, and the expression levels of them downregulated after regular etanercept therapy for 12 weeks. The calibrated signal ratio of AS group before therapy to healthy control group were >3 : 1, and the ratio of AS group after therapy to before therapy were <1 : 3. HC, healthy control; AS, ankylosing spondylitis.

and pathological changes of tissue. MicroRNAs thought to take part in pathologic process of 15 common human disorders [12]. Recent studies suggested that miRNAs in PBMCs could be biomarkers for the diagnosis of heart disease [17] and prostate cancer [18, 19]. MicroRNAs were also suggested to be potential biomarkers for drug-induced liver injury [20] and myocardial injury [21]. Furthermore, microRNAs were proved to play an important role in pathogenesis, diagnosis, and therapy of rheumatism. For examples, synovial fluid and plasma microRNAs had potential as diagnostic biomarkers for RA and OA and as a tool for the analysis of their pathogenesis [10], while miR-146a in lupus patients was relevant to the biologic and clinical behavior of SLE. And microRNA could serve as therapeutic targets for the treatment of SLE via regulation of the type I IFN pathway [11]. An article mentioned that miR-125 and another 6 microRNAs may have correlation with pathological changes of AS [12]. But no further confirmatory experiment had been done. In this report, we showed the PBMCs microRNAs expressions profiles of AS patients were distinct from these of healthy donors. And after regular etanercept therapy, the dysregulation of microRNAs expression could be corrected. Finally, we discussed the possibility of PBMCs miRNAs to be potential biomarkers of AS.

Our hypothesis was that microRNAs took part in pathogenesis of AS. According to this hypothesis, we could infer

Table 3: Expression of two microRNAs by real-time PCR validated.

microRNA	$2^{\Delta\Delta C_T}$ Control group/AS group (fold)	*P* value	$2^{\Delta\Delta C_T}$ AS group before/after therapy (fold)	*P* value
hsa-miR-29a	16.22 (16.22)	0.002	0.31 (3.18)	0.049
hsa-miR-126-3p	3.76 (3.76)	0.046	0.45 (2.20)	0.035

The expression levels of these 2 microRNAs were significantly lower in AS group than in control group. And expressions of them were dramatically upregulated after 12-week etanercept treatment (fold changes >2, $P < 0.05$).

Table 4: Correlation of 2 validated microRNAs and clinical presentation.

	BASDAI		CRP		ESR	
	Correlation coefficients	*P*	Correlation coefficients	*P*	Correlation coefficients	*P*
hsa-miR-126-3p	0.302	0.059	0.317	0.046	0.378	0.016
hsa-miR-29a	−0.038	0.843	0.207	0.282	0.153	0.429

MicroRNAs expression disorders did not statistically significantly correlated with BASDAI, CRP, or ESR. BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; correlation coefficients, Spearman correlation coefficients; *P*, *P* value.

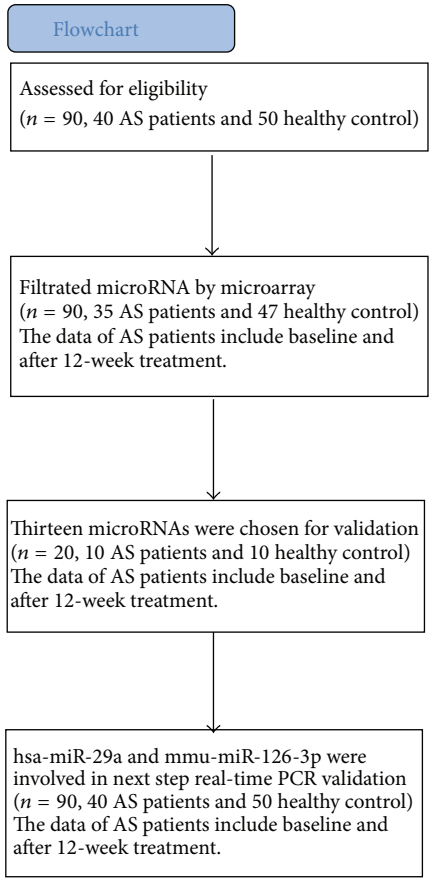

Figure 3

that (a) expression of microRNAs was disorder in active stage of AS; (b) when the clinical presentations were controlled, the expression of microRNAs would tend to be normal. For one thing, the expression levels of hsa-miR-126-3p and hsa-miR-29a were lower in AS group than in healthy control group. The differences between the two groups were statistically significant. And the expressions of hsa-miR-126-3p and hsa-miR-29a were significantly upregulated in AS group after 12-week therapy taking baseline as control. Unfortunately, it was not certified that expression disorders of microRNAs and clinical parameters of AS activity (BASDAI, CRP, and ESR) correlated linearly. At least there were three reasons that shouldered the responsibility. Firstly, microRNAs disorders and alterations of clinical parameters maybe have complex correlation, not linear, which would be affected and regulated by many factors. Secondly, clinical parameters reflected just inflammatory activity of AS, while microRNAs disorders may be related to bone metabolism. Relative clinical parameters could be collected to validate this hypothesis in the future. Thirdly, appearance of microRNAs disorders and clinical presentations may lack temporal concurrence, while data collection was performed at the same time point. Under the circumstances the result would not reflect their real correlation.

On the basis of our hypothesis, microRNAs, hsa-miR-126-3p and hsa-miR-29a, are probably potential diagnostic biomarkers for AS. As reported in the field of malignant tumors [18, 22, 23], disease specific miRNAs for AS are expected. In our study, we proved that expression levels of microRNAs could differentiate AS from healthy control. When the clinical presentations were controlled, the expression of microRNAs would tend to be normal. The variation trend of microRNAs and course of disease were of high degree of consensus. Quantitative analysis of microRNAs mentioned before would be of great value in AS diagnosis, activity evaluating, and curative effect monitoring. Furthermore, regular etanercept therapy could elevate the downregulated microRNAs of AS patient, and this change was also statistically significant. It is suggested that hsa-miR-126-3p and hsa-miR-29a have potential to be new target for AS treatment.

Our research provides clues of further researches. MicroRNAs play a special role in gene regulation [24]. MiR-29 was thought to be involved in the regulation of collagen expression in hepatic stellate cells [25] and fibroblasts [26], though the target site was not revealed. MiR-126 was reported to target TOM1, which was a negative regulator of IL-1b

and TNF-a-induced signaling pathways [27]. There was little known information about the effect of these microRNAs on AS pathogenesis. The functions of these dysregulated microRNAs would be the emphases of sequential studies. We will focus on the target sites they are combining and the pathways they are making effect on. Target genes and their functions are expected to be revealed.

5. Conclusion

PBMCs microRNAs expressions profile of AS patients was distinct from these of healthy donors. Our study first verified AS pathogenesis involved dysregulation of microRNAs. Expression levels of two microRNAs, hsa-miR-126-3p and hsa-miR-29a, were distinct from these of healthy controls. And after regular etanercept therapy, the dysregulation of microRNAs expression could be corrected. They will probably become not only the potential biomarkers for AS diagnosis, activity evaluation, and curative effect monitoring, but also provocative therapeutic targets of AS.

Abbreviation

RNA:	Ribonucleic acid
PBMC:	Peripheral blood mononuclear cell
AS:	Ankylosing spondylitis
PCR:	Polymerase chain reaction
BASDAI:	Bath Ankylosing Spondylitis Disease Activity Index
BASFI:	Bath Ankylosing Spondylitis Functional Index
CRP:	C-reactive protein
ESR:	Erythrocyte sedimentation rate
DMARDs:	Disease-modifying antirheumatic drugs
NSAIDs:	Nonsteroidal anti-inflammatory drugs
HLA:	Human leukocyte antigen
RA:	Rheumatoid arthritis
OA:	Osteoarthritis
SLE:	Systemic lupus erythematosus
VAS:	Visual Analogue Score
EDTA:	Ethylenediaminetetraacetic acid
OD:	Optical density
IFN:	Interferon
IL:	Interleukin
TNF:	Tumor necrosis factor
HC:	Healthy control
SD:	Standard deviation.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Jieruo Gu carried out experimental design, Qing Lv and Qiuxia Li carried out experimental analysis and data statistics, Peizhuo Zhang and Yingjuan Jiang carried out experiment of microarray, Xinwei Wang carried out RNA purification, Qiujing Wei carried out collection of samples, Shuangyan Cao, Zetao Liao, Zhiming Lin, Yunfeng Pan, Jianlin Huang, Tianwang Li, Ou Jin and Yuqiong Wu carried out collection of clinical data. Qing Lv and Qiuxia Li contributed equally to this work.

Foundations

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Research Article

Serum Vitamin D and Pyridinoline Cross-Linked Carboxyterminal Telopeptide of Type I Collagen in Patients with Ankylosing Spondylitis

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Objective. To assess the serum vitamin D and ICTP levels in patients with ankylosing spondylitis (AS) and investigate their relationship with disease activity and bone mineral density (BMD). *Method*. 150 patients and 168 controls were included. Serum 25(OH)D, ICTP, C-reaction protein (CRP), Bath AS Disease Activity Index (BASDAI), Bath AS Functional Index (BASFI), and Hip BMD were assessed in patients. 25(OH)D and ICTP were detected in controls. *Results*. The serum 25(OH)D in AS was 57.92 ± 24.42 nmol/L, significantly lower than controls (91.24 ± 42.02 nmol/L). Serum ICTP in AS was 5.72 ± 3.88 ug/L, significantly higher than controls (3.69 ± 1.26 ug/L). ICTP level was higher in men than in women patients (6.07 ± 4.05 versus 3.84 ± 1.96 ug/L, $P \leq 0.01$); it was also higher in JAS than in AAS (9.52 ± 3.79 versus 5.27 ± 3.65 ug/L, $P \leq 0.01$). Furthermore, 25(OH)D was negatively correlated with ICTP. Low 25(OH)D and high ICTP were one of the reasons of AS patients' low hip BMD. Besides, a significant relationship was found between serum ICTP and CRP. *Conclusion*. There was a high incidence of vitamin D inadequacy in AS. Serum ICTP level was elevated in AS, especially in JAS and male patients. 25(OH)D and ICTP seem to be valuable markers to detect bone loss in AS.

1. Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease that primarily affects the axial skeleton. Although it is characterized by new bone formation, which leads to the formation of syndesmophytes and ankylosis of the spine and sacroiliac joints [1], osteoporosis is also a well-recognized complication of AS. It is also the main reason of the patients' spinal deformity, bone pain, and disability.

It is well-known that, as a secosteroid hormone, vitamin D is crucial in maintaining bone health. Many studies have shown that vitamin D participates in the regulation of the body's immune system, so it is helpful to obtain adequate vitamin D in the patients with rheumatic diseases. The pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is a specific component of type I collagen, only generated from damaged mature bone matrix, and can represent a sensitive indicator of bone resorption in vivo. Till now, lots of researches on ICTP have been done worldwide, but most of them were about its relationship with malignant bone metastases. It was reported that there was a positive correlation between serum ICTP activity and pathological bone resorption [2, 3]. Unexpectedly, few studies were carried out on serum ICTP level in patients with AS and other rheumatic diseases.

The aim of this study was to elucidate the alteration of serum ICTP and vitamin D level in patients with AS and to further investigate the relations between ICTP, vitamin D, disease activity (CRP, BASDAI), and BMD in these patients.

2. Methods

2.1. Patients. From June 2012 to April 2013, 150 AS patients, from both the out-patients and in-patients registered in the Rheumatology Department of the Third Affiliated Hospital, were included in this study. The age ranged from 18 years to 50 years, with a mean disease duration of 8.4 years. All the patients fulfilled the modified New York criteria for AS

[4]. Patients whose onset age was less than 16 years were named juvenile ankylosing spondylitis (JAS), and patients whose onset age was less than 16 years were named adult ankylosing spondylitis (AAS). 168 healthy controls with age and gender matched who visited our hospital in the same period were enrolled in this study.

Patients with the concomitant presence of inflammatory bowel disease, chronic renal or hepatic disease, diabetes mellitus, parathyroid or thyroid disease, recent fractures, malnutrition, or drug intake affecting bone metabolism (bisphosphonates, glucocorticoids, anticonvulsants, coumarin derivatives, or diuretics) were excluded, and postmenopausal, pregnant, and breast-feeding women were also excluded in this research. The study was approved by the local ethical committee, and all patients included in our study were provided with written informed consent to participate in this study. All the patients had received conventional treatment which included nonsteroidal anti-inflammatory drugs (NSAIDs) and/or sulfasalazine, but no one received anti-tumor necrosis factor alpha(anti-TNFα) drugs. Moreover, the patients had to be on stable doses of NSAIDs, sulfasalazine, and calcium and vitamin D supplements for at least 3 months before the assessment.

2.2. Assessment. Laboratory assessment included complete blood count, C-reactive protein (CRP), liver and kidney function tests, HLA-B27, and serum levels of 25(OH)D and ICTP. The blood specimens of all the patients were taken after an overnight fasting. CRP was measured using the nephelometric method, and ELISA was used to detect serum HLA-B27, 25(OH)D, and ITCP. Vitamin D deficiency was defined as 25(OH)D serum level less than 12 ng/mL (50 nmol/L), vitamin D insufficiency as 25(OH)D at a level of 12–32 ng/mL (50–80 nmol/L), and vitamin D sufficiency at a level higher than 32 ng/mL (80 nmol/L) [5, 6].

Clinical assessment included collection of demographic data, disease duration, medication history (NSAIDS, sulfasalazine, and calcium and vitamin D supplements), and visual analogue scale (VAS) of patients assessment of pain and disease activity. Disease activity was evaluated by ESR, CRP, and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [7, 8]. Physical function was assessed by Bath Ankylosing Spondylitis Functional Index (BASFI; on a scale of 0–10) [9]. BMD of lumbar spine (anterior-posterior projection at L1–L4) and hip (total proximal femur) were monitored by dual-energy X-ray absorptiometry (DXA). According to the World Health Organization (WHO) classification, osteopenia was defined as a T-score between −1SD and −2.5SD, and osteoporosis as a T-score < −2.5SD [10].

2.3. Data Analysis. Statistical analysis was performed with SPSS 20.0 software. In independent-sample t-test, the patients were divided into groups according to gender, age, disease duration, 25(OH)D, CRP, HLA, and BASDAI. In the multiple regression analysis, when a parameter is a dependent variable and the others are all the variables, regression analysis was performed with SPSS 20.0 software, and we use the method of "stepwise" to bring in variables (stepping method criteria: Entry 0.05, Removal 0.10).

Table 1: Comparison of indicators between AS and control groups.

Factor	AS	Controls	P value
n	150	168	
Age (years)	29.19 ± 8.94	31.83 ± 9.97	P = NS
Gender (male/female)	127/23	128/40	P = NS
25(OH)D (nmol/L)	57.92 ± 24.42	91.24 ± 42.02	$P < 0.01$
ICTP (ug/L)	5.72 ± 3.88	3.69 ± 1.26	$P < 0.01$

Note: we compared means using t-test and proportions using chi-square test. 25-Hydroxyvitamin D (25(OH)D); pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP); ankylosing spondylitis (AS).

3. Results

3.1. Geographic Data. 150 AS patients involved 127 males and 23 females (Male : female = 5.52 : 1) and the mean age of the patients was 29.83 years (SD ±8.94); 168 controls included 128 males and 40 females (male : female = 3.20 : 1), and mean age of the controls was 31.83 years (SD ± 9.97). The differences of age and gender between AS group and healthy control subjects were not significant ($P > 0.05$). Mean level of 25(OH)D in control group was 91.24 nmol/L (SD ± 42.02), and in AS group it was 57.92 nmol/L (SD ± 24.42), which was significantly lower than the healthy controls ($P < 0.01$). Mean level of ICTP in controls and AS groups was 3.69 ug/L (SD ± 1.26) and 5.72 ug/L (SD ± 3.88), respectively, and the ICTP level was elevated significantly ($P < 0.01$) in patients with AS (Table 1).

3.2. Characteristics of the Study Population. The AS patients were divided into different groups according to gender, age, disease duration, HLA, CRP, 25(OH)D, and BASDAI, and the comparisons were as follows (Table 2). ICTP level was found to be higher in male and JAS patients than in female and AAS patients ($P < 0.01$), when comparing to the other groups. No differences were found in 25(OH)D, ICTP level, and hip BMD T-score in groups which were divided according to disease duration, CRP, and HLA-B27 (P = NS). Patients with lower 25(OH)D level (<50 nmol/L) had higher ICTP and lower hip BMD T-score ($P < 0.01$). Patients with higher BASDAI (>4) had lower lumbar spine and hip BMD T-score ($P < 0.01$).

3.3. Association of Different Clinical Biochemical Indexes and BMD. By partial correlation analysis, we found some correlations in different clinical biochemical indexes and BMD. Serum levels of 25(OH)D were inversely associated with ICTP ($P < 0.01$) and disease duration ($P < 0.05$), and there was a positive association between 25(OH)D level and hip BMD T-scores ($P < 0.05$). Serum levels of ICTP were positively associated with CRP but were inversely associated with hip BMD T-score. Unexpectedly, no association was found between ICTP and disease duration, BASDAI, BASFI, and lumbar spine BMD T-score. Moreover, BASDAI was found to be negatively associated with lumbar spine and hip BMD T-score ($P < 0.05$). CRP and disease duration were found negatively associated with hip BMD T-score ($P < 0.05$) but not with lumbar spine BMD T-score.

Table 2: Comparison of biochemical index and BMD in different groups (AS patients were divided into groups according to different clinical factor).

AS group	n	25(OH)D (nmol/L)	ICTP (ug/L)	Lumbar spine BMD (T-score)	Hip BMD (T-score)
Gender					
Male	127	56.81 ± 22.79	6.07 ± 4.05	−1.64 ± 1.24	−0.90 ± 0.94
Female	23	64.08 ± 31.90	3.84 ± 1.96**	−1.51 ± 1.69	−1.01 ± 0.86
Clinical type					
AAS	134	58.90 ± 25.19	5.27 ± 3.65	−1.58 ± 1.35	−0.88 ± 0.90
JAS	16	49.78 ± 14.81	9.52 ± 3.79**	−1.96 ± 1.03	−1.21 ± 1.08
Disease duration					
>4 y	100	58.44 ± 27.58	5.78 ± 4.05	−1.54 ± 1.36	−1.02 ± 0.91
≦4 y	50	56.88 ± 16.56	5.62 ± 3.55	−1.79 ± 1.23	−0.71 ± 0.93
HLA-B27					
Positive	129	56.98 ± 25.25	5.95 ± 4.03	−1.60 ± 1.34	−0.93 ± 0.93
Negative	21	63.72 ± 17.96	4.32 ± 2.37	−1.78 ± 1.36	−0.82 ± 0.92
CRP (mg/L)					
>6	109	58.00 ± 21.65	5.94 ± 3.96	−1.71 ± 1.40	−0.99 ± 0.94
≦6	41	57.72 ± 30.92	5.16 ± 3.66	−1.37 ± 1.03	−0.72 ± 0.88
25(OH)D (nmol/L)					
>50	91		4.75 ± 2.23	−1.53 ± 1.35	−0.75 ± 0.86
≦50	59		7.23 ± 5.21**	−1.77 ± 1.26	−1.18 ± 0.97**
BASDAI					
>4	29	50.93 ± 17.25	6.70 ± 5.47	−2.21 ± 1.42	−1.31 ± 0.86
≦4	121	59.60 ± 25.62	5.49 ± 3.38	−1.51 ± 1.27*	−0.82 ± 0.92*

Note: independent-sample t-test was used between groups.
Values are in means ± SD.
C-reactive protein (CRP), bone mineral density (BMD), Bath AS Disease Activity Index (BASDAI), 25-hydroxyvitamin D (25(OH)D); pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP).
The comparison between the two groups.
*Statistically significant correlation $P < 0.05$.
**Statistically significant correlation $P < 0.01$.

3.4. Multiple Linear Regression Model Analysis for 150 AS Patients. A multiple regression model was built to assess how biochemical index, disease activity, and clinical assessments could predict the variation of hip BMD in AS patients. ICTP, BASDAI, disease duration, and 25(OH)D contributed independently and significantly to the hip BMD T-score ($P < 0.05$) (Table 3). Similarly, another multiple regression model analysis illuminated that ICTP contributed independently and significantly to the 25(OH)D level in AS ($P < 0.01$) (Table 4). Meanwhile, 25(OH)D and CRP contributed independently and significantly to the serum ICTP level ($P < 0.05$) (Table 5).

4. Discussion

Vitamin D is a secosteroid hormone which is produced in the skin under adequate exposure to sunlight or obtained from the diet. It is metabolized by a hepatic 25-hydroxylase into 25-hydroxyvitamin D (25(OH)D) and by a renal 1 alpha-hydroxylase into the vitamin D hormone calcitriol. Due to its stability and relatively long half-life, the serum 25(OH)D level is the best indicator to assess vitamin D situation in the body [11]. Vitamin D has functions beyond calcium, phosphorus, and bone, and plenty of evidences have shown that vitamin D participates in the regulation of the body's immune system [12, 13]. Deluca et al. reported that vitamin D was extremely effective in blocking the development of autoimmune disorder, and vitamin D deficiency could accelerate the appearance of symptoms and increased the severity of rheumatic disease.

Previous studies have showed the relation between a biochemical marker of type I collagen degradation (urinary CTX-I, reflecting bone resorption) and lower BMD at the hip [1, 14–16]. These studies also demonstrated that higher serum levels of bone resorption markers are associated with bone loss in AS patients with active disease. As the specific ingredients of type I collagen, which is known as the most common protein in the skeleton, comprise about 90% of the organic matrix in bone tissue, ICTP is the degradation product of mature bone matrix but not of newly formed bone. It is a newly discovered indicator of bone resorption and has stable concentration in serum. Moreover, ICTP gives responses slowly and slightly in physiologic bone metabolismbut significantly in pathological bone resorption [2]. So, ICTP can reflect the bone transformation level and is a sensitive marker of bone resorption. In the near future, it will be most probably widely used in clinical field.

So far, there have been few studies on evaluating the vitamin D and ICTP levels and their relationship to BMD in AS patients. This study showed that patients with AS were

Table 3: Multiple linear regression model analysis for hip BMD.

Model	Coefficients[a]				
	Unstandardized coefficients		Standardized coefficients	t	Sig.
	β	Std.Error	Beta		
(Constant)	−0.523	0.265		−1.971	0.051
ICTP	−0.053	0.018	−0.220	−2.852	0.005
BASDAI	−0.106	0.035	−0.224	−3.008	0.003
Disease duration	0.033	0.012	−0.208	−2.795	0.006
25(OH)D	0.007	0.003	0.180	2.332	0.021

[a]Dependent variable: hip BMD T-score.

Table 4: Multiple linear regression model analysis for 25(OH)D.

Model	Coefficients[b]				
	Unstandardized coefficients		Standardized coefficients	t	Sig.
	β	Std.Error	Beta		
(Constant)	71.315	3.552		20.077	0.000
ICTP	−1.526	0.506	−0.242	−3.017	0.003

[b]Dependent variable: 25(OH)D.

more likely to have lower serum 25(OH)D level and higher ICTP level compared to healthy individuals, and there was a common situation of 25(OH)D lacking and bone loss in AS patients. Our study revealed that about 84% AS patients were vitamin D deficient or insufficient in 25(OH)D serum level, which meant less than 32 ng/mL (80 nmol/L), and about 46.7% patients were diagnosed with osteopenia or osteoporosis whose hip BMD T-score was < -1SD. The pathological mechanism may be due to the high levels of plasma TNF-α in AS patients, which downregulates the 24-hydroxylase activity in the vitamin D system in the kidney [17]. The absorption disturbance, caused by the intestinal vitamin D receptor defect, which had been observed in AS, may also cause a decrease in vitamin D level [18]. Unexpectedly, we also found the ICTP level was much higher in men and JAS patients, although the pathological mechanism behind that is still unknown. In this study, we also found that there was no difference of 25(OH)D, ICTP, and hip BMD T-score level in groups of HLA-B27 positive and negative and CRP above 6 mg/L and below 6 mg/L.

Recent studies have found low level of vitamin D in AS patients is extremely common due to high disease activity [19, 20]. But in this study, no significant association was found between 25(OH)D and disease activity in AS and our findings were consistent with other previous reports [1, 5, 21]. We also found that the ICTP level was higher in the group whose 25(OH)D level was below 50 nmol/L, and 25(OH)D was inversely associated with ICTP. Thus, our study has supported the fact about the importance of intaking adequate vitamin D for AS patient in order to avoid or alleviate osteoporosis. The pathological mechanism may be that the 25(OH)D indirectly influences the bone metabolism, or the bone resorption could lead to compensatory increasing consumption of 25(OH)D, which needs more in-depth research to verify in future.

No significant correlations were found between BMD (lumbar spine or proximal femur bone) and turnover markers (such as serum carboxyterminal cross-linked telopeptide of type I collagen (CTX), osteocalcin (OC)) in a cohort study [22] of relatively young males with AS. Since the anterior-posterior lumbar spine BMD measured by DXA can be overestimated by the presence of syndesmophytes, ligament calcifications, and fusion of facet joints in these patients [1, 23–25], we used hip BMD T-score for further analyzing the relevant factors of osteoporosis. While comparing different groups, we found that the hip BMD T-score was lower in the group whose 25(OH)D level was below 50 nmol/L than the group with level above 50 nmol/L and in group whose BASDAI was above 4.

A few studies have found increased bone turnover and disease activity and decreased vitamin D levels were associated with AS-related osteoporosis in AS patients [1, 26]. According to multiple linear regression model analysis, the present study showed 25(OH)D, ICTP, BASDAI, and disease duration were independently related to low hip BMD T-score, which indicated 25(OH)D and ICTP, like BASDAI and disease duration, were valuable markers to detect bone loss in AS. Our findings of ICTP being positively associated with CRP in AS patients underline the importance of ICTP as a monitoring factor for disease activity. Meanwhile, the findings of 25(OH)D being inversely associated with disease duration indicate that more attention should be given to the patients with longer disease course. Besides, CRP was found positively associated with BASFI and BASDAI, and there was also a positive association between BASFI and BASDAI. In the multiple linear regression model analysis, we found no correlation between CRP, BASFI, and hip BMD T-score; however, BASDAI followed by ICTP made the largest contributions to the low hip BMD T-score.

The main limitations of our study are the fact that it is a cross-sectional study. No investigation was made to monitor 25(OH)D level at different periods of disease, and there was no data on the radiographic aspect of the spine and its scores.

Table 5: Multiple linear regression model analysis for ICTP.

Model	Coefficients[c]				
	Unstandardized coefficients		Standardized coefficients	t	Sig.
	β	Std.Error	Beta		
(Constant)	8.990	0.922		9.755	0.000
CRP	0.087	0.017	0.627	5.155	0.000
25(OH)D	−0.042	0.011	−0.262	−3.641	0.000

[c]Dependent variable: ICTP.

Also, other bone turnover markers such as osteocalcin, procollagen type I N-terminal peptide (PINP), pyridinoline, N-telopeptide, and bone specific alkaline phosphatase (BALP) were not taken into consideration. We could not properly assess the influence of drugs intake, dietary calcium supply, and seasonal differences during the study period.

In conclusion, this study indicates that there is a high incidence of vitamin D inadequacy in AS patients. As an indicator of bone resorption, serum ICTP level was elevated in AS, especially in JAS and male patients. 25(OH)D and ICTP seem to be valuable markers to detect bone loss in AS; the serum levels of vitamin D and ICTP may play an important role in the pathophysiology of AS-related osteoporosis, which needs further research on its pathogenesis.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Pingping Zhang and Qiuxia Li contributed equally.

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Research Article

Management of Deep Infection after Instrumentation on Lumbar Spinal Surgery in a Single Institution

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Postoperative surgical site infections (SSIs) are more common complications after spinal surgery. SSIs often require extended hospitalisation and may worsen overall clinical outcomes. A retrospective database review of consecutive patients with traditional open lumbar spinal surgery was performed. SSIs patients were identified and reviewed for clinically relevant details, and postoperative SSIs' incidence was calculated for the entire cohort as well as for subgroups with or without spinal implants. In 15 years, 1,176 patients underwent open lumbar spinal surgery with spinal implants and 699 without. Thirty-eight developed postoperative SSIs. Total SSI rate for the entire group was 2.03%. The incidence of postoperative SSIs in the nonimplant group was relatively low. Patients received antibiotics, hyperbaric oxygen therapy, and wet dressing. We provided the precise rates of postoperative SSIs in traditional open spinal surgery obtained from a single-centre data. Patients with spinal implants had higher SSIs' incidence than those without.

1. Introduction

Postoperative surgical site infection (SSI) is one of the most common complications [1–3] after spinal surgery [4, 5]. The incidence of spinal SSIs reported in the literature is 0.7%–16.0% [6]. These infections often require extended antibiotic therapy, repeated surgery for wound debridement, hardware removal, and prolonged hospitalisation [7]. It dramatically increases utilisation of healthcare resources and worsens overall clinical outcomes [8, 9].

Several complicated procedures result in higher infection rates. Therefore, spinal surgeries carry a higher risk of infection compared with other orthopaedic procedures [10]. Another problem of the increasing complexity of spinal surgeries is increased operation time, which is a well-known intraoperative risk factor for SSIs [11–13]. Besides surgical factors, patient's preoperative characteristics (increased age and body mass index (BMI), smoking, diabetes, steroid use, malnutrition, and previous surgical infection) could also account for an increase in the number of postoperative complications [11–13].

The management of SSIs has become increasingly important. Considering the complexity of spinal procedures, preventive interventions have the potential to improve a patient's overall outcome [14, 15]. Furthermore, these interventions may decrease the duration of hospital stay and postoperative recovery time, thereby lowering medical expenses.

2. Patients and Methods

2.1. Ethics Statement. The approval of local ethics committee for this research was permitted. The protocol conforms to the Declaration of Helsinki and the Institutional Review Board of Chung Shan Medical University (Taichung, Taiwan) approved the study by expedited review (Approval Reference number: CS15026).

2.2. Retrospective Database Review. We performed a retrospective review of prospectively collected databases of

consecutive patients who underwent open lumbar spinal surgery between February 1998 and December 2012 by experienced surgeons at our hospital. During this 15-year period, 1,176 lumbar spinal surgeries were performed, and the procedures included the following: simple decompression procedures such as micro- or endoscopic discectomy or foraminotomy or decompression of stenosis, arthrodeses (e.g., posterolateral interbody, posterior/transforaminal interbody, and lateral interbody), filum detethering, or syrinx shunting. All procedures were performed using a standard surgical scrub and preparation and draping of patients after administering general anaesthesia were carried out. All patients received a single dose of intravenous antibiotics immediately before surgery (1-2 g of cefazolin or 1 g of vancomycin in those reporting an allergy to penicillin or cephalosporin). This regimen was repeated as required during surgeries lasting > 4 h.

The databases included documentation of all perioperative complications. Identification of SSIs as classified according to the criteria set by the Centres for Disease Control and Prevention was studied. An infection was considered to be SSI if it occurred at the site of the surgery within 30 days postoperatively or within 1 year if the procedure included placement of a foreign body (e.g., an implant). Cases with SSI were identified and confirmed through microbiological cultures. The incidence of postoperative SSIs was calculated for the entire cohort as well as for subgroups with or without spinal implants. Positive cases of SSI were reviewed for clinically relevant details.

Demographic and preoperative variables were collected from medical records using a standardised data collection form by an investigator who was not involved in the initial treatment. Information regarding preoperative risk factors was derived from standardised and routinely recorded data as reported in the patient charts. Surgical-level risk factors that could be considered possible risk factors for infection were derived from surgical reports of the surgeons' database. When the data collection was completed, all data were checked by a second investigator.

Preoperative patient-level risk factors that were reviewed included age at the time of surgery, sex, height, weight, and diagnosis. Additionally, smoking habits, comorbidity, and previous lumbar surgeries were recorded, and BMI was calculated. Registered type of comorbidity included diabetes, rheumatoid arthritis, and cardiovascular and pulmonary diseases.

2.3. Statistical Analysis. Two-tailed independent t-tests, Chi-square tests, or appropriate nonparametric alternatives were used to identify differences between the groups of patients with or without implants. Probability values of < 0.05 were considered statistically significant. All data were analysed using SigmaPlot statistical software (SAS Institute, San Jose, CA, USA).

3. Results

3.1. Deep Infection after Instrumentation on Lumbar Spinal Surgery. Over the past 15 years, 1,875 open lumbar spinal surgeries (1,176 patients with spinal implants and 699 patients without spinal implants) were performed at our medical centre. Thirty-eight postoperative SSIs were detected. The total SSI rate for the entire group was 2.03% (31 (2.64%) in the implant group and 7 (1.00%) in the nonimplant group). The incidence of wound infection and operation time in the nonimplant group was relatively low ($P < 0.05$). No significant differences in age, sex, extent of bleeding, amount of irrigation, indwelling of drainage tube, days of the initial onset of infection, and duration of hospital stay were ascertained for both groups (Table 1).

Table 1: Demographic data of patients with postoperative infection.

	Implant group	Nonimplant group	P value
Postoperative infection [n/total (%)]	31/1176 (2.64)	7/699 (1.00)	<0.05
Age (years, mean ± SD)	65.5 ± 12.9	67.6 ± 11.8	0.702
Gender, male/female	16/15	2/5	0.410
Operation time (hours)	3.4 ± 0.8	2.7 ± 0.8	<0.05
Bleeding amount (mL)	826.5 ± 361.7	564.3 ± 319.2	0.086
Irrigation amount (mL)	2080.6 ± 817.5	1464.3 ± 625.4	0.071
Drainage tube indwelling, Y/N	30/1	6/1	0.339
Days for infection start	5.2 ± 0.6	5.0 ± 0.0	0.344
Hospital stay (days)	32.6 ± 3.4	33.7 ± 7.7	0.695

3.2. Underlying Disease, Infection Germ, Treatments, and Outcome. Approximately 50% patients with postoperative infection have comorbidity associated with diabetes and hypertension. There was no significant difference between any type of underlying diseases and BMI between these two groups. The type of bacteria and sample cultures in different groups are listed in Table 2. The most common organism isolated from wound cultures was *Staphylococcus aureus*, followed by methicillin-resistant *S. aureus* (MRSA). Patients received antibiotics, hyperbaric oxygen (HBO) therapy, and wet dressing, and most showed good outcome. However, some patients developed low back pain and neuralgia after postoperative infection.

4. Discussion

Postoperational SSIs after spinal surgery remain a serious condition, leading to major complications and worse outcomes [6, 16]. Although the generalized adoption of preoperative antibiotic prophylaxis has served to decrease SSI rates to as much as 50%, it has not completely eliminated them [17–19]. Recently, a review of 2,316 patients who underwent a wide variety of open spinal surgeries over 5 years found an overall infection rate of approximately 2% [20]. Our data are consistent with those results. The most common pathogen isolated from wound cultures was *Staphylococcus* species (predominantly *S. aureus*) [6, 11, 21], followed by MRSA [22, 23],

Table 2: Underlying diseases, infection germ, treatments, and outcome in patients with postoperative infection.

	Implant group	Nonimplant group
Underlying diseases		
Diabetes	15	4
Hypertension	15	3
Osteoporosis	9	1
Poor nutrition	2	1
Traumatic injury	2	0
Coronary artery disease	2	0
Uremia	2	0
Rheumatoid arthritis	1	1
High BMI	1	0
Low BMI	1	0
Cancer	0	2
Hyperthyroidism	0	1
Nil	4	0
Infection germ		
Staphylococcus	17	2
MRSA	9	3
ORSA	0	2
E. coli	2	0
Pseudomonas	2	0
Proteus	1	0
Treatments		
Antibiotics	31	7
Hyperbaric oxygen	31	6
Wet dressing	31	7
Nutrition supply	1	1
Outcome		
Good	20	6
Low back pain	9	0
Neuralgia	1	0
Nutrition supply	1	0
Expiry (sepsis)	0	1

which was responsible for several difficult-to-treat infections [24]. Our database provided additional documentation of several factors that may be related to the occurrence of wound infection based on the administration of prophylactic antibiotics, duration of operation time, estimated blood loss, length of hospitalisation, or patient comorbidities. In addition, there was documentation of causative organisms on the management of infection or surgical outcomes.

Recently, another retrospective review also provided the rates of postoperative wound infection after a broad range of spinal procedures based on the cases predominantly performed by fellowship-trained spinal surgeons [25]. The large number of cases in that review enabled assessment of infection rates for relatively uncommon procedures, including those performed on paediatric patients. Their database also enabled stratification of cases and assessment of the corresponding infection rates based on surgical factors, including primary *versus* revision status, use of implants, and fusion approach, and whether minimally invasive techniques were used. The 2% total infection rate in this series was comparable with that in previously reported series, which included a diverse representation of spinal procedures, with the infection rate ranging from 0.9% to 4.4% [11, 20, 26, 27]. The rates in the present study, in general, were comparable with those in the previous reports.

The risk factors for infection included diabetes, elevated serum glucose levels, and inappropriate timing or dosing of preoperative antibiotics [6]. A case-control study identified incontinence, posterior surgical approaches, tumour resection procedures, and obesity as independent risk factors for postoperative SSIs [13]. The role of diabetes and high BMI as risk factors has been supported in other studies [28–31]. Information on preoperative risk factors for SSI, such as diabetes, previous surgery, obesity, previous exposure to radiation, and smoking, was not consistently recorded in our databases. Therefore, comparison of the group of patients in our study with those in other published cohorts based on these variables was not possible. More formal case-control or randomized studies on this topic could better answer these important questions.

HBO has been reported to heal postoperative spinal infections in adults with intact osteosynthesis material [32]. The therapeutic effect of HBO treatment with regard to infections is mainly attributable to reduction of hypoxia in tissues with significant improvement in leucocyte phagocytic killing capacity [33]. Larsson et al. have evaluated possible benefits of HBO therapy in the treatment of deep postoperative infections in patients with neuromuscular spine deformity, suggesting that HBO therapy is a safe and potentially useful adjuvant treatment to the standard therapy of early postoperative deep infections [34, 35].

The present study also cannot answer the possible reasons for lower infection rates after open lumbar spinal surgery. However, several potential mechanisms may have important roles [6]. To avoid SSIs after spinal surgery, ultimately, there is no replacement for sterile methods, meticulous hemostasis and closure, and appropriate preoperative antibiotic prophylaxis. Further study is required to validate these findings through more direct comparisons of traditional open versus minimally invasive approaches.

Based on our data and those from previous studies [25], the rates of postoperative wound infections were significantly higher for cases that included fusion or implants than those that did not. It is important to recognize that these data do not necessarily suggest a causative link between infection and performance of fusion or implantation but rather likely reflect a greater complexity and associated risk for cases that require fusion or implantation. The overall infection rate for procedures performed with a minimally invasive approach was significantly lower compared with that for those without. Importantly, several procedures that are commonly performed using a minimally invasive approach (e.g., lumbar discectomy), in general, have a relatively low infection rates, whereas more complex procedures that are typically conducted using a traditional open approach (e.g., degenerative scoliosis or neuromuscular kyphosis) have substantially higher infection rates [28].

5. Conclusions

We detected a low rate of SSIs in this large series of patients who underwent open lumbar spinal surgery. In addition, we provided the precise rates (2.03%) of postoperative wound infections in traditional open spinal surgery from a single-center data. The incidence of SSIs in patients with spinal implants was higher than that in those patients without spinal implants. Further large-scale prospective studies using a clear definition of complication are necessary to ascertain the true incidence of postoperative complications in spinal surgery. Our data provide a general benchmark of infection rates as a basis for ongoing efforts to improve safety of care.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Clinical Features in Juvenile-Onset Ankylosing Spondylitis Patients Carrying Different B27 Subtypes

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Background. Ankylosing spondylitis (AS) is a common rheumatic disease and is characterized by inflammation of the axial skeleton. HLA-B27 is strongly associated with AS. Juvenile-onset AS (JAS) with disease onset before 16 years of age differs from adult-onset AS (AAS) in many respects. *Objective*. To compare the clinical features in JAS with different B27 subtypes and analyze the differences between JAS and AAS. *Methods*. 145 JAS and 360 AAS patients were included. The demographic data, clinical manifestations, laboratory markers, Bath AS indices, and B27 subtypes were recorded. *Results*. Peripheral arthritis, enthesitis, BASDAI, ESR, and CRP were significantly higher in JAS patients with HLA-B*2704 than those with B27-negative. Enthesitis and ESR were significantly higher in patients with HLA-B*2705 than those with B27-negative. The onset age of HLA-B*2715 group was much earlier than the other groups. The peripheral arthritis, enthesitis, and hip joint involvement in JAS with HLA-B*2704 were significantly higher than those in AAS with HLA-B*2704. *Conclusion*. JAS with different B27 subtypes had similar features in most of manifestations; JAS and AAS patients with the same subtype could have distinctive courses. Early diagnosis, hip detection, and control of systemic active inflammation in JAS patients will be helpful for improving the prognosis.

1. Introduction

Ankylosing spondylitis (AS) is an inflammatory disorder mainly affecting the axial joints and distinguished by a significant association with HLA-B27 [1, 2]. Juvenile-onset AS (JAS), that is, having onset of symptoms before 16 years of age, is the major part of juvenile spondyloarthropathies (JSpA). In China, JAS accounts for 27.8%~29.8% [3, 4]. Because the individual differences in the clinical manifestations are large and sacroiliac joints of children are in the developmental stage, so imaging diagnoses are limited, and diagnosis may be delayed. JAS has different phenotype and prognosis than adult-onset AS (AAS) [5]. Many studies report about clinical features of JAS [3, 6], and little about those in JAS patients carries different B27 subtypes.

We suspected whether there was different pathological mechanism in JAS. In our previous studies, a group of JAS patients have been typed into B27 subtypes; the positive rate of B27 subtypes in JAS group had no statistical difference compaired with AAS group [7]. We further analysed the clinical manifestations of JAS with different B27 subtypes in this study, so as to investigate the clinical and pathological mechanism of JAS.

2. Patients and Methods

2.1. Patient Population. From June 2005 to April 2013, 145 JAS and 360 AAS outpatients and inpatients registered in rheumatology department of our hospital were included in this study. All the patients fulfilled the modified New York criteria for AS. Patients with the concomitant presence of chronic renal or hepatic disease, blood disorders, endocrine system diseases, and various acute infections or other infectious diseases were excluded.

Informed consent was obtained from each patient involved in the study.

Table 1: Distribution of patients with different B27 subtype and B27-negative in JAS and AAS groups.

	JAS	AAS	P
HLA-B27-negative	11/145 (7.59%)	41/360 (11.39%)	1.000
HLA-B*2704	120/145 (82.76%)	275/360 (76.39%)	1.000
HLA-B*2705	11/145 (7.59%)	38/360 (10.56%)	1.000
HLA-B*2715	3/145 (2.07%)	3/360 (0.83%)	1.000
HLA-B*2702	0/145 (0)	3/360 (0.83%)	0.561

2.2. Biochemical Parameters. C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured by standard laboratory methods.

2.3. Baseline Disease Activity Measures. The following measures were obtained at baseline visit: Bath ankylosing spondylitis disease activity index (BASDAI) score (0 = none, 10 = worst) and Bath ankylosing spondylitis functional index (BASFI) score (0 = none, 10 = worst).

2.4. Joint Counts. In the 44 joints, which include bilateral proximal interphalangeal joints, metacarpophalangeal joints, wrist, elbow, shoulder, acromioclavicular, sternoclavicular joint, knee, ankle joints, and metatarsophalangeal joint, the number of joints with swelling and tenderness was recorded.

2.5. Statistical Analysis. Statistical software SPSS 19.0 for Windows was used to analyze data. Normally distributed measurement data was shown as "mean ± standard deviation," non-normally distributed data as "median (lower quartile-upper quartile)." The original data of BASFI, ESR, and CRP were skewed distribution, respectively, but after logarithmic transformation, the data became normal distribution, and then they were analyzed. The mean values of two independent samples were compared using t-test; comparison between multiple groups was analyzed using one-way ANOVA, while pairwise comparisons between groups were analyzed using LSD-t test. Chi-square test was used to analyze constituent ratio. Kruskal-Wallis test was used for comparison in multigroups; Mann-Whitney U test was used for comparison between two groups. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Characteristics of the JAS and AAS Patients. The mean age of 145 JAS patients, including 136 males and 9 females (male : female = 15.1 : 1), was 21.3 ± 7.6 years (from 10 to 43 years). Among them, 35 patients were ≤ 16 y and 110 > 16 y. The onset age was 5 to 16 years, and the average onset age was 13.49 ± 2.36 years. The median duration of disease was 6 years (0.04 to 27 years). 104 patients were sporadic and 41 individuals had family histories (sporadic cases: cases with family histories = 2.54). Mean age of the 360 AAS patients (306 male, 54 female) was 30.5 ± 7.6 years, with mean duration of disease 6.9 ± 5.7 years (0.3 to 35 years).

3.2. Comparison of the B27 Subtype Distribution and Clinical Phenotype between JAS and AAS Groups. There was no significant difference in distribution of B27-negative patients and different B27 subtypes between JAS and AAS groups, shown in Table 1. The preliminary study of our research team has shown no significant difference in the constituent ratio of B27 subtypes between the two groups [7], and in the present study B27-negative patients were brought into this table, and there was also no significant difference in the distribution between JAS and AAS groups.

3.3. Comparison of Clinical Features between Different B27 Subtypes in the JAS Group. Because the data of BASFI, ESR, and CRP was not normally distributed, comparison was done after logarithmic transformation. As shown in Tables 2 and 3, there was no significant difference in the diagnostic age, male preponderance, family histories, hip arthritis, iridocyclitis, and BASFI among the four different groups in JAS patients. Peripheral arthritis, enthesitis, BASDAI, ESR, and CRP were significantly higher in patients with HLA-B*2704 than in those with B27-negative. Enthesitis and ESR were significantly higher in patients with HLA-B*2705 than in those with B27-negative. The Incidence of waxy digitus was more common in HLA-B*2705 group than that in HLA-B*2704 group. The onset age of HLA-B*2715 group (5, 9, and 13 years) was much earlier than that in the other three groups.

3.4. Comparison of Clinical Features between JAS and AAS Groups. The group of JAS had higher male preponderance than AAS. And peripheral arthritis, enthesitis, and hip arthritis were more common in patients with JAS than AAS, shown in Table 4.

*3.5. Comparison of Clinical Features in JAS and AAS Patients with HLA-B*2704 and HLA-B*2705.* The comparison of clinical features in JAS and AAS patients with HLA-B*2704 and HLA-B*2705 was shown in Table 5. In patients with HLA-B*2704, male preponderance was more obvious in JAS, and peripheral arthritis, enthesitis, and hip arthritis were also more common in JAS patients. There was no significant difference in these indicators between JAS and AAS patients with HLA-B*2705.

4. Discussion

Among JAS patients with HLA-B*2704, HLA-B*2705, and HLA-B*2715, there were no significant difference in male ratio, positive family history, peripheral arthritis, enthesitis, hip arthritis, iridocyclitis, and indicators, for example, BASDAI, BASFI, ESR, and CRP, which showed that the pathogenesis of different subtypes might be similar.

In the comparison between JAS patients with HLA-B27-positive and HLA-B27-negative, patients with peripheral arthritis and enthesitis in the HLA-B27-negative group were less than those in HLA-B*2704 group; the inflammatory indicators, such as BASDAI, ESR, and CRP, in patients with HLA-B27-negative were lower than those in patients with HLA-B*2704, which indicated the milder level of inflammation in HLA-B27-negative patients. Our previous research on

Table 2: Comparison of clinical features, Bath AS indices, and inflammatory markers in JAS patients with B27-negative and different B27 subtypes.

HLA-B27 subtype	Age (y)	Onset age (y)	Disease duration (y)	BASDAI (score)	BASFI (score)	ESR (mm/h)	CRP (mg/L)
HLA-B27-negative	18.09 ± 3.65 (n = 11)	14.09 ± 2.11 (n = 11)	4.01 ± 4.39 (n = 11)	2.05 ± 1.42 (n = 11)	1.55 (1.21, 3.43) (n = 8)	7.00 (4.00, 9.40) (n = 11)	1.10 (0.60, 5.00) (n = 7)
HLA-B*2704	21.79 ± 8.02 (n = 120)	13.54 ± 2.31 (n = 120)	8.25 ± 8.06 (n = 120)	**4.22 ± 1.95*** (n = 112)	3.00 (1.90, 5.20) (n = 112)	**32.00 (14.00, 48.75)*** (n = 120)	**18.55 (5.9, 49.78)*** (n = 120)
HLA-B*2705	18.36 ± 3.96 (n = 11)	13.55 ± 1.77 (n = 11)	4.82 ± 4.46 (n = 11)	3.61 ± 1.26 (n = 9)	1.6 (1.00, 4.20) (n = 9)	**14.0 (7.00, 48.00)*** (n = 11)	5.80 (1.45, 34.30) (n = 11)
HLA-B*2715	24.33 ± 6.43 (n = 3)	**9.00 ± 4.00**$^{*\#a}$ (n = 3)	**15.33 ± 4.16**^{*a} (n = 3)	3.28 ± 2.79 (n = 2)	1.125 (1.00, 1.25) (n = 2)	**65.00 (4.00, 135.00)*** (n = 3)	15.53 (1.50, 113.45) (n = 3)

Note: (1) data in the table was mean ± standard deviation or median (upper quartile, lower quartile); (2)* comparison between groups of different B27 subtypes and group of B27-negative, $P < 0.05$; (3)$^{\#}$ comparison between HLA-B*2715 and HLA-B*2704 group, $P < 0.05$; (4)^{a} comparison between HLA-B*2715 and HLA-B*2705 group, $P < 0.05$.

Table 3: Comparison of clinical features in JAS patients with B27-negative and different B27 subtypes.

HLA-B27 subtype	Gender (male)	Peripheral arthritis	Enthesitis	Hip arthritis	Family histories	Waxy digitus	Iridocyclitis
HLA-B27-negative	11/11	1/11	2/11	2/11	2/11	0/11	0/11
HLA-B*2704	112/120	**73/120***	**82/120***	49/120	34/120	1/118	6/120
HLA-B*2705	10/11	6/11	**6/11***	4/11	3/11	**1/11**$^{\#}$	2/11
HLA-B*2715	3/3	2/3	2/3	0/3	2/3	0/3	0/3

Note: (1) data in the table for each index number of positive cases and total cases; (2)* comparison between groups of different B27 subtypes and group of B27-negative, $P < 0.01$; (3)$^{\#}$ comparison between HLA-B*2705 and HLA-B*2704 group, $P < 0.05$.

Table 4: Comparison of clinical features between JAS and AAS groups.

	JAS	AAS	P
Gender (male)	136/145 (93.79%)	306/360 (85.00%)	**0.007**
Peripheral arthritis	82/145 (56.55%)	87/310 (28.06%)	**<0.001**
Enthesitis	92/145 (63.45%)	155/310 (50.00%)	**0.003**
Hip arthritis	55/145 (37.93%)	61/305 (20.00%)	**<0.001**
Family histories	41/145 (28.28%)	63/310 (20.32%)	0.086
Waxy digitus	2/143 (1.40%)	8/231 (3.46%)	0.230
Iridocyclitis	8/145 (5.52%)	26/310 (8.39%)	0.417

a group of AAS patients showed that the onset age of patients with HLA-B27-positive was less than that in those with HLA-B27-negative [8]. Both of the two studies above showed the important role of HLA-B27 in the pathogenesis of AS. A lot of evidence illuminated that HLA-27 directly participated in the pathogenesis of AS, and the direct evidence was from the study of transgenic rats, in which human B27 gene was inserted into rats; then symptoms similar to AS appeared in the transgenic rats; and the more gene replication fragment of B27 in rats, the more obvious symptoms [9]. As the ancestor subtype, HLA-B*2705 was most common type in Europe and America, so most basic researches including gene cloning and transgenic animals were about HLA-B*2705 subtype. As for HLA-B*2704 and HLA-B*2715 subtypes, our research focused mainly on clinical aspects; further basic researches are still needed, so as to explore the pathogenesis and to find new methods of diagnosis and treatment.

The onset ages of the three patients with HLA-B*2715 were 5, 9, and 13 years and were significantly earlier than HLA-B*2704, HLA-B*2705, and B27-negative group; meanwhile the duration of HLA-B*2715 group was longer than that of HLA-B*2705 and B27-negative groups. As a rare subtype, HLA-B*2715 was first reported in 2001 and only found in Asia [10, 11], and there were no more than 20 cases found so far in our previous study [7, 8]; most of them were AS patients. Six sporadic cases were found in present research, and three of them were JAS patients with early onset age, indicating that this subtype was related to the disease.

There were significant difference in male ratio, peripheral arthritis, enthesitis, and hip arthritis in JAS than AAS patients with HLA-B*2704 (shown in Table 5). The different phenotypes in juvenile and adult patients with the same subtype showed the complexity of AS pathogenesis. The study about pedigree and twins showed the multigenic mode of AS hereditary susceptibility. Hence, the difference between JAS and AAS with the same B27 subtype may be related to other genes and the different genetic expression of B27 under the influence of environment. As HLA-B*2704 is the predominant subtype in Han population [7], the numbers of patients in HLA-B*2705 and HLA-B*2715 groups were small. There were no significant difference in male ratio, peripheral arthritis, enthesitis, and hip arthritis between JAS and AAS patients with HLA-B*2705, and this was not consistent with

Table 5: Comparison of clinical features in JAS and AAS patients with HLA-B*2704 and B*2705.

	HLA-B*2704			HLA-B*2705		
	JAS	AAS	*P*1	JAS	AAS	*P*2
Gender (male)	112/120 (93.33%)	237/275 (86.18%)	**0.042**	10/11 (90.91%)	33/38 (86.84%)	0.720
Peripheral arthritis	73/120 (60.83%)	65/238 (27.31%)	**<0.001**	6/11 (54.54%)	14/37 (37.84%)	0.329
Enthesitis	82/120 (68.33%)	114/238 (47.90%)	**<0.001**	6/11 (54.54%)	24/37 (64.86%)	0.539
Hip arthritis	49/120 (40.83%)	48/241 (19.92%)	**<0.001**	4/11 (36.36%)	5/32 (15.62%)	0.303
Family histories	34/120 (28.33%)	54/238 (22.69%)	0.207	3/11 (27.27%)	7/37 (18.92%)	0.553
Waxy digitus	1/118 (0.85%)	7/177 (3.95%)	0.161	1/11 (9.09%)	0/27	0.117
Iridocyclitis	6/120 (0.05%)	21/238 (8.82%)	0.385	2/11 (18.18%)	3/37 (8.11%)	0.342

many studies in Caucasians [12, 13]; our results should be further estimated in larger samples.

Our study showed that hip involvement was more common in JAS than AAS (shown in Tables 4 and 5); this was similar to the studies in China, Taiwan, and India [3, 6], and there was no significant difference in the B27-negative and B27-positive groups. Forty percent of patients with juvenile spondyloarthropathy progressed to functional disability in 10 to 15 years [14], and the hip involvement closely associated with poor prognosis [15]. Recent researches show that abnormality of bone loss can appear in early spondyloarthropathies and JAS; hip BMD significantly negatively correlated with BASDAI [16, 17]. These findings emphasize the need for more alertness for hip involvement at an early stage of JAS.

5. Conclusions

Our study showed that JAS patients with different B27 subtypes had similar features in most of manifestations, and JAS and AAS with the same subtype could have distinctive courses. Therefore, early diagnosis, hip detection, and control of systemic active inflammation will be helpful for improving the prognosis of JAS patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Yikun Mou and Pingping Zhang contributed equally.

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Research Article

Association between Hyperuricemia and Metabolic Syndrome: An Epidemiological Study of a Labor Force Population in Taiwan

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The increasing prevalence of metabolic syndrome (MetS) has become an important issue worldwide. Metabolic comorbidities of hypertension, obesity, and hyperlipidemia are shown as important risk factors for incident gout. The purpose of this study was to investigate the relationship between hyperuricemia and MetS. This is a cross-sectional study. The effective sample included 21,544 individuals who received worker health examinations at a local teaching hospital in Changhua County from 2008~2012. We used multiple logistic regression analysis to investigate the influences of hyperuricemia on MetS. The results showed that individuals with MetS had significantly higher blood pressure, fasting plasma glucose, triglycerides, waist circumference, and high-density lipoprotein cholesterol than those without MetS ($P < 0.001$). Multiple logistic regression analysis revealed hyperuricemia to be an important factor of MetS. The risk of developing MetS is higher with high levels of serum uric acid (SUA) and the odds ratio (OR) of having MetS is 4.98 times higher for Tertile 3 than for Tertile 1 (95% CI = 4.16–5.97) and 4 times higher for Quartile 4 than for Quartile 1 (95% CI = 3.59–4.46). In conclusion, males are more likely to develop MetS than females, and the risk of having MetS increases with age and SUA concentration.

1. Introduction

Gouty arthritis is a common type of chronic arthritis. Hyperuricemia and crystallization of monosodium urate in joints are considered as the important risk factors of gouty arthritis [1]. The relations between hyperuricemia and comorbidities, for instance, hypertension, obesity, and MetS, have been demonstrated in many epidemiologic studies [2, 3].

Previous report suggested the high prevalence of MetS in gouty patients, and also it defined the association between insulin resistance and gouty arthritis [4]. Recent studies showed that the prevalence of MetS was high among patients with gout, 44% in Korean men and 82% in Mexican men compared with 5% in the Korean general population [5–7]. Therefore, it is highly suggested to diagnose and treat gout as one of the metabolic diseases [5, 8]. Gout is not considered

as a simple joint disease and is associated with obesity, cardiovascular disease, hypertension, and MetS with increased mortality [9–11].

There is a tendency for younger individuals to have MetS. Also, MetS is closely related to the occurrence and mortality of chronic conditions such as cardiovascular diseases (CVD), cardiac diseases, diabetes mellitus (DM), and hypertension [12–14].

Epidemiological studies on MetS in different countries and regions show drastically different results. The primary reasons for such drastic differences can be the different characteristics of the participants (e.g., age, ethnicity, socioeconomic status, and abnormal risk factors), the study periods, or different diagnostic definitions. In the United States, the prevalence of MetS is estimated to be 27% (25.2% in men and 29% in women) [15]; and in Taiwan, it is 15.7% (18.3% in men and 13.6% in women) [16].

Previous epidemiological surveys showed that the hyperuricemia is related to the prevalence of MetS [17, 18]. However, previous studies, for the most part, recruited the general public as research participants and few studies investigated worker populations. Therefore, the purpose of this study was to investigate the relationship between hyperuricemia and MetS in a labor force population. Thus, identifying asymptomatic individuals with a high risk of developing MetS may lead to improvements in prevention and treatment of the condition and of subsequent cardiovascular events and gouty arthritis.

2. Methods

We conducted a cross-sectional study on individuals that had undergone worker health screening at a local teaching hospital in Changhua County from 2008~2012. The physical examination document includes physical examination and biochemical blood tests. All individual's examination collecting progress and analysis progress were in accordance with the hospital standard operating procedures, and the laboratory analysis was in accordance with the quality assurance (QA) and quality control (QC). The total sample size was 33,776 with a valid sample size of 21,544 after excluding those with incomplete data on physical examination and biochemical blood tests.

Definition of terms is as follows. (I) In order to understand the current international classification of SUA as criteria of elevated SUA, the present study classified serum SUA levels into (A) SUA > 7.0 mg/dL for males and SUA > 6.0 mg/dL for females [19, 20]; (B) three subgroups in light of the concentration of SUA: (a) SUA < 7 mg/dL, (b) 7 mg/dL ≦ SUA < 9 mg/dL, and (c) SUA ≧ 9 mg/dL [21]; (C) four subgroups among males: (a) SUA < 5 mg/dL, (b) 5 mg/dL ≦ SUA < 6 mg/dL, (c) 6 mg/dL ≦ SUA < 7 mg/dL, and (d) SUA ≧ 7 mg/dL; and four subgroups among females: (a) SUA < 4 mg/dL, (b) 4 mg/dL ≦ SUA < 5 mg/dL, (c) 5 mg/dL ≦ SUA < 6 mg/dL, and (d) SUA ≧ 6 mg/dL [22]. (II) MetS status was defined according to the criteria set in the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III, ATP-III) [23]. We utilized previously established modifications for Asian populations, using waist circumference (WC) cut-off points [24]. Any three of the following five criteria were grounds for identifying MetS: (a) abdominal obesity: WC: ≧90 cm in men and ≧80 cm in women; (b) raised triglyceride (TG): ≧150 mg/dL; (c) reduced high-density lipoprotein cholesterol (HDL-C): <40 mg/dL in men and <50 mg/dL in women; (d) raised blood pressure (BP): BP of at least 130/85 mmHg or taking antihypertensive medication; and (e) raised fasting plasma glucose (FPG): ≧110 mg/dL and/or taking antiglycemic medication.

The physical examination comprised BP and anthropometric measurements, including height, weight, and body mass index (BMI). Height was measured to the nearest 0.1 cm, without shoes, using a stadiometer. Weight was measured in light clothing, without shoes, using a beam balance scale, and was recorded to the nearest 0.1 kg. BMI was calculated as weight (kg) divided by height^2 (m^2). Well-trained nurses measured systolic BP (SBP) and diastolic BP (DBP) two times in the left arm of seated participants, according to a standardized protocol. A third BP measurement was made if the first two BP readings differed by more than 10 mm Hg. The average of the two closest readings was calculated to determine the reported BP for each participant. The items in the blood examination included TG, SUA, FPG, and HDL-C. The sample was venous blood drawn after 8 hours of fasting and was sent to the lab within an hour and analyzed by a Hitachi-7070 biochemical analyzer.

Data in the present study were analyzed using SPSS 17.0 (SPSS for Windows release 17.0), with a significance level of $\alpha = 0.05$. Descriptive statistics used the average value, the standard deviation, the frequency distribution, and the percentage criterion. Inferential statistics used the Chi-square and multiple logistic regression models for analysis.

3. Results

Study participants included 21,544 individuals aged over 21 who were receiving a worker health examination in Changhua County. The mean age was 38.4 ± 9.5, with 4,881 individuals (27.0%) in the range of 21–30 years old, 8,940 (41.5%) individuals in the range of 31–40 years old, 4,780 (22.2%) individuals in the range of 41–50 years old, and 2,943 (13.7%) individuals over 51 years old. The majority of the participants were male, with 13,009 males (60.4%) and 8,535 females (39.6%).

Demographic characteristics such as age and gender differed significantly between individuals with and without MetS ($P < 0.001$). Individuals at an older age had high prevalence of MetS, and males had higher prevalence of MetS than females (15.8% versus 6.7%). There were significantly higher ratios of abnormal BP, FPG, TG, WC, and HDL-C in individuals with MetS than in those without MetS; the ratios were 28.0%, 60.5%, 44.7%, 37.8%, and 49.8%, respectively (Table 1).

The ratio of hyperuricemia in individuals with MetS was 24.7%, which is significantly higher than the 8.3% in

Table 1: Correlation analysis of demographic characteristics, biochemistry exam results, and metabolic syndrome (n = 21,544).

Variables	No MetS (n = 18,927, 87.9%)		MetS (n = 2617, 12.1%)		P value
	Number	Percentage	Number	Percentage	
Gender					<0.001
Male	10960	84.2	2049	15.8	
Female	7967	93.3	568	6.7	
Age (years)					<0.001
21–30	4631	94.9	250	5.1	
31–40	7935	88.8	1005	11.2	
41–50	4034	84.4	746	15.6	
≧51	2327	79.1	753	20.9	
WC (cm)					<0.001
Normal	15327	97.3	431	2.7	
Abnormal (male: ≧90; female: ≧80)	3600	62.2	2186	37.8	
Raised blood pressure (mmHg)[a]					<0.001
Normal	13016	97.6	316	2.4	
Abnormal (≧130/85)	5911	72.0	2301	28.0	
TG (mg/dL)					<0.001
Normal	16227	97.4	436	2.6	
Abnormal (≧150)	2700	55.3	2181	44.7	
Raised FPG (mg/dL)[b]					<0.001
Normal	18524	90.3	1999	9.7	
Abnormal (≧110)	403	39.5	618	60.5	
HDL-C (mg/dL)					<0.001
Normal	17547	93.4	1247	6.6	
Abnormal (<40 in men and <50 in women)	1380	50.2	1370	49.8	

Note. Analyzed by Chi-square test, 2-tailed test, and significance level α = .05.
[a]Raised blood pressure ≧130/85 mmHg or currently taking antihypertensive drugs.
[b]Raised fasting plasma glucose ≧110 mg/dL or currently taking oral hypoglycemic agent.

individuals without MetS ($P < 0.001$). After dividing the SUA concentration into 3 groups, we found that Tertile 3 had an abnormally high ratio of SUA compared to Tertiles 2 and 1 (35.3% versus 22.9% and 8.4%). In addition, we divided SUA concentration into 4 groups, and we found that Quartile 4 had a significantly and abnormally high ratio of SUA compared to Quartile 1 (24.0% versus 5.6%) ($P < 0.001$) (Table 2).

In this study, we added variables that were statistically significant by univariate analysis (i.e., age, gender, and SUA) into multiple logistic regression analysis. The results (Table 3) show that SUA was a significant contributing factor to MetS in multiple logistic regression models I, II, and III. For model I, the OR of individuals with hyperuricemia having MetS was 3.08 times that of individuals with normal SUA (95% CI = 2.82–3.37). With increased age, the ORs of having MetS increased accordingly. For example, the OR of individuals over 51 years old having MetS was 4.46 times that of individuals 21–30 years old (95% CI = 3.81–5.23), and the OR of males having MetS was 1.80 times that of females (95% CI = 1.63–2.00). These ORs are all statistically significant ($P < 0.001$). For model II, we divided the SUA concentration into 3 groups and found that a higher SUA concentration was associated with a higher risk of MetS. For those individuals with SUA ≧ 9 mg/dL, the risk of having MetS was 4.98 times higher (95% CI = 4.16–5.97). With increased age, the odds of MetS increased 2.24~4.59 times (ORs = 2.24~4.59). Males had 1.59 times higher OR of MetS than females (95% CI = 1.42–1.77). In addition, in model III, we divided the SUA concentration into 4 groups and found that a higher SUA concentration was associated with a higher risk of MetS. Individuals with a SUA concentration in the Quartile 3 and Quartile 4 groups had 1.70~4.00 times ORs of MetS, and the ORs associated with increased age were 2.25~4.48 times. As for gender as a risk factor, males had a risk of developing MetS that was 1.65 times (95% CI = 1.49–1.83) higher, and significantly so, than that of females ($P < 0.001$).

4. Discussion

Studies show that hyperuricemia not only can cause gout, but also is a high risk factor for atherosclerotic diseases such as CVD and carotid atherosclerosis [25], hypertension [26], DM [27], MetS [28], and cardiac diseases and chronic diseases such as cerebral vascular diseases. Further analysis showed that elevated SUA is associated with a higher risk of developing CVD and mortality as a result of CVD [29]. Our study shows that MetS is associated with age, and increased age is associated with a higher rate of MetS. We observed that individuals over 51 years old had the prevalence rate of 20.9% of developing MetS. Our result is consistent with many previous

Table 2: Correlation analysis of hyperuricemia and metabolic syndrome (n = 21,544).

Variable	No MetS (n = 18,927, 87.9%)		MetS (n = 2617, 12.1%)		P value
	Number	Percentage	Number	Percentage	
Hyperuricemia					<0.001
Normal	15074	91.7	1356	8.3	
Abnormal (male > 7; female > 6 mg/dL)	3853	75.3	1261	24.7	
Subgroups of SUA[a]					<0.001
Tertile 1	15190	91.6	1402	8.4	
Tertile 2	3325	77.1	990	22.9	
Tertile 3	412	64.7	225	35.3	
Subgroups of SUA[b]					<0.001
Quartile 1	3274	94.4	194	5.6	
Quartile 2	6007	93.6	411	6.4	
Quartile 3	5406	88.9	673	11.1	
Quartile 4	4240	76.0	1339	24.0	

Note. Analyzed by Chi-square test, 2-tailed test, and significance level $\alpha = .05$.
[a]Subgroups of SUA Tertile 1: SUA < 7 mg/dL, Tertile 2: 7 mg/dL ≦ SUA < 9 mg/dL and Tertile 3: SUA ≧ 9 mg/dL.
[b]Subgroups of SUA Quartile 1: male SUA < 5 mg/dL, female SUA < 4 mg/dL; Quartile 2: male 5 mg/dL ≦ SUA < 6 mg/dL, female 4 mg/dL ≦ SUA < 5 mg/dL; Quartile 3: male 6 mg/dL ≦ SUA < 7 mg/dL, female 5 mg/dL ≦ SUA < 6 mg/dL; and Quartile 4: male SUA ≧ 7 mg/dL, female SUA ≧ 6 mg/dL.

Table 3: Regression analysis of risk factors of metabolic syndrome.

Item	β	Wald	OR (95% CI)	P value
Model I				
Gender[a]	0.59	127.37	1.80 (1.63–2.00)	<0.001
Age 2[b]	0.80	117.39	2.23 (1.93–2.58)	<0.001
Age 3[b]	1.21	246.29	3.38 (2.90–3.39)	<0.001
Age 4[b]	1.49	342.42	4.46 (3.81–5.23)	<0.001
Hyperuricemia[c]	1.12	616.32	3.08 (2.82–3.37)	<0.001
Model II				
Gender	0.46	70.50	1.59 (1.42–1.77)	<0.001
Age 2	0.80	118.71	2.24 (1.94–2.59)	<0.001
Age 3	1.22	249.78	3.41 (2.92–3.97)	<0.001
Age 4	1.52	354.88	4.59 (3.92–5.38)	<0.001
Tertile 2[d]	0.98	383.48	2.67 (2.42–2.95)	<0.001
Tertile 3[d]	1.60	305.04	4.98 (4.16–5.97)	<0.001
Model III				
Gender	0.50	90.08	1.65 (1.49–1.83)	<0.001
Age 2	0.81	119.36	2.25 (1.94–2.60)	<0.001
Age 3	1.22	247.95	3.40 (2.92–3.96)	<0.001
Age 4	1.50	344.13	4.48 (3.82–5.25)	<0.001
Quartile 3[e]	0.53	78.90	1.70 (1.51–1.91)	<0.001
Quartile 4[e]	1.38	625.44	4.00 (3.59–4.46)	<0.001

*Note*1. Analyzed by stepwise regression analysis. Considered variables include age, gender, and SUA.
*Note*2. Dependent variable: MetS, 1, with **MetS**; 0, without MetS.
[a]Gender: female = 0, [b]age: 21–30 = 0.
[c]Abnormal (male > 7; female > 6 mg/dL) = 1; normal = 0.
[d]Subgroups of SUA Tertile 1: SUA < 7 mg/dL, Tertile 2: 7 mg/dL ≦ SUA < 9 mg/dL and Tertile 3: SUA ≧ 9 mg/dL.
[e]subgroups of SUA Quartile 1: male SUA < 5 mg/dL and female SUA < 4 mg/dL.
Quartile 2: male 5 mg/dL ≦ SUA < 6 mg/dL and female 4 mg/dL ≦ SUA < 5 mg/dL.
Quartile 3: male 6 mg/dL ≦ SUA < 7 mg/dL and female 5 mg/dL ≦ SUA < 6 mg/dL.
Quartile 4: male SUA ≧ 7 mg/dL and female SUA ≧ 6 mg/dL.

studies that show that the prevalence of MetS increases with increased age [30]. We also found that males have a higher rate of MetS than females (15.8% versus 6.7%), a result similar to other studies. For example, Balkau et al. (2003) found that prevalence rates of MetS in males and females among 4,293 individuals in the 2001 French National Cholesterol Education Program were 10% and 7%, respectively [31]. Hu et al. (2004) found that among 11,512 Europeans aged from 30 to 89 who did not have DM males had higher prevalence of MetS than females (15.7% versus 14.2%) [32]. Chuang et al. (2004) found that among those receiving health examinations in Taipei City males had higher prevalence of MetS than females (15.5% versus 10.5%). The prevalence rates were 15.7% versus 12.0% after correcting for variables [33].

MetS is commonly accompanied with hypertension, type II DM, hyperlipidemia, stroke, or CVD. MetS is a cluster of three diseases—hypertension, hyperglycemia, and gout. At present, its etiological mechanisms are yet to be understood. But it is clear that MetS is associated with obesity, insulin resistance, and abnormal blood lipids that lead to the above-described three diseases [34]. From our study, it is obvious that individuals with MetS have a significantly higher ratio of abnormal BP, FPG, TG, WC, and HDL-C than those without MetS.

To understand the effects of SUA concentration on MetS, we used multiple logistic regression and conducted analyses using three models, I, II, and III, based on SUA concentration. Variables that showed statistically significant differences in univariate analysis (i.e., age, gender, and SUA) were used in regression models. For different regression models, after controlling other variables, the results showed that the OR of individuals with abnormal SUA having MetS was 3.08 times (95% CI = 2.82–3.37) that of individuals with normal SUA in model I. In models II and III, the ORs of having MetS were higher in those with an elevated SUA concentration, with ORs of 2.65–4.98 and 1.70–4.00, respectively.

A large-scale epidemiological study reported that elevated SUA concentrations are associated with an increased mortality rate; further analyses showed that hyperuricemia seems to be related to hypertension, obesity, MetS, renal diseases, and CVD [35]. A recent study supported the role of SUA itself as an independent risk factor for CVD [26]. Previous studies reached similar conclusions that SUA is significantly associated with cardiovascular risk factors such as hypertension [36], MetS [37], and insulin resistance [38]. Other studies also found that high SUA concentrations are associated with an increased risk of MetS [39]. Follow-up analysis of American residents with hyperuricemia found that males with a SUA value $\geqq$6.5 mg/dL had a 1.6 times higher risk of developing MetS than males with a value <5.5 mg/dL, and females with a SUA value $\geqq$4.6 mg/dL had a 2 times greater risk of developing MetS.

Although the relationship between hyperuricemia and CVD was established in the 19th century, there is still controversy as to whether hyperuricemia plays a role as an indicator or a risk factor [40]. The strong relationship between hyperuricemia and other cardiovascular risk factors such as hypertension, insulin resistance, obesity, and renal insufficiency makes it difficult to establish a causal relationship in epidemiological studies. Recent experiments have made significant contributions in providing important knowledge pertaining to the pathological meaning of hyperuricemia. Symptoms resulting from hyperuricemia can be alleviated by xanthine-oxidase inhibitors or uricosuric agents that reduce SUA concentrations [41]. These results show that lowering SUA can play an important role in preventing CVD. The consensus is that MetS can be considered as a predictive factor for CVD [14]. The traditional view is that impaired renal clearance induced by compensatory hyperinsulinemia can cause hyperuricemia [42]. However, recent studies provide a different perspective, implying that hyperuricemia can be an independent factor predicting the occurrence of MetS and DM, even after controlling for different genders and basic BMI [28].

The findings in previously described studies are similar to those in our study. That is, SUA concentration can be used as an important predictive factor for MetS. The risk of MetS increases with elevated SUA concentrations.

Admittedly, with the cross-sectional nature of this study, it is not possible to establish a causal relationship between hyperuricemia and MetS. This study could not eliminate the potential effects of underlying diseases, medications used, and dietary habits for these diseases among participants on the present findings. It is possible that residual confounding by these factors may also affect the MetS and SUA link. Further population-based prospective studies are needed to elucidate a cause-effect relationship between hyperuricemia and MetS. By using these epidemiologic study findings, it will bring more benefits for health management and prevention of clinical disease.

In conclusion, although hyperuricemia is still not one of the determining factors for MetS, more and more studies are showing that high SUA concentrations are associated with and have negative effects on CVD, insulin resistance, hypertension, and abdominal obesity. Our study also showed that individuals with MetS have higher ratio of having abnormal BP, FPG, TG, WC, and HDL-C than those without MetS. Age, gender, and abnormal SUA were risk factors for MetS as analyzed by the multiple logistic regression models. In addition, a higher SUA concentration is associated with a higher risk of MetS, no matter if the SUA concentrations were divided into three or four groups.

We recommend that dietitians should pay attention to the SUA value in individuals receiving health education for their MetS-related diseases. Related health education should be provided when necessary. As for patients who only have hyperuricemia, dietary health education that is related to MetS should be provided to help the patients control their uric acid value better.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

Predictive Factors of Clinical Response of Infliximab Therapy in Active Nonradiographic Axial Spondyloarthritis Patients

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Objectives. To evaluate the efficiency and the predictive factors of clinical response of infliximab in active nonradiographic axial spondyloarthritis patients. *Methods.* Active nonradiographic patients fulfilling ESSG criteria for SpA but not fulfilling modified New York criteria were included. All patients received infliximab treatment for 24 weeks. The primary endpoint was ASAS20 response at weeks 12 and 24. The abilities of baseline parameters and response at week 2 to predict ASAS20 response at weeks 12 and 24 were assessed using ROC curve and logistic regression analysis, respectively. *Results.* Of 70 axial SpA patients included, the proportions of patients achieving an ASAS20 response at weeks 2, 6, 12, and 24 were 85.7%, 88.6%, 87.1%, and 84.3%, respectively. Baseline MRI sacroiliitis score (AUC = 0.791; $P = 0.005$), CRP (AUC = 0.75; $P = 0.017$), and ASDAS (AUC = 0.778, $P = 0.007$) significantly predicted ASAS20 response at week 12. However, only ASDAS (AUC = 0.696, $P = 0.040$) significantly predicted ASAS20 response at week 24. Achievement of ASAS20 response after the first infliximab infusion was a significant predictor of subsequent ASAS20 response at weeks 12 and 24 (wald $\chi^2 = 6.87$, $P = 0.009$, and wald $\chi^2 = 5.171$, $P = 0.023$). *Conclusions.* Infliximab shows efficiency in active nonradiographic axial spondyloarthritis patients. ASDAS score and first-dose response could help predicting clinical efficacy of infliximab therapy in these patients.

1. Introduction

The spondyloarthritis (SpA) is a group of related inflammatory diseases including ankylosing spondylitis (AS), reactive arthritis, psoriatic arthritis, inflammatory bowel disease-associated arthritis, juvenile spondylitis, and undifferentiated spondylitis [1]. The occurrence of SpA is common in many countries; in China the pooled prevalence of SpA from civilian surveys is 0.93%, and for AS is 0.24% [2].

Axial SpAs comprise AS and nonradiographic axial SpA. A previous study showed that the frequency of HLA-B27 positivity, inflammatory back pain, arthritis, enthesitis, uveitis, and levels of disease activity are highly comparable between patients with these two types of diseases, thus suggesting that these two entities are part of the same disease [3]. Thus, the axial SpA patients without radiographic change would partly include the early stage of AS patients.

Following preclinical studies identified the key role of TNFα in the immune-mediated inflammatory response observed in AS [4], and anti-TNFα agents have been evaluated and approved as for treatment of AS [5]. While numerous studies have assessed anti-TNFα agents in patients with established disease per the modified New York criteria, that is, structural changes in the sacroiliac joint are visible on X-ray, few studies have been conducted to ascertain the benefits to treat patients in the early stages of AS or nonradiographic axial SpA [6, 7].

In addition, anti-TNF-α agents can be effective in approximately 60%–80% of AS patients [5]; however, the cost of such therapy must be considered in assessing available treatment options, especially in China. Identifying baseline disease characteristics with strong ability to predict efficacy would be quite important in lessening the economic burden of effective treatment for both the patients and the healthcare system in general.

As such, we conducted the current study to evaluate the efficacy of infliximab (REMICADE, Centocor Ortho Biotech Inc, Horsham, PA), an anti-TNF-α agent approved for the treatment of active nonradiographic axial spondyloarthritis patients, in patients to assess (1) the ability of baseline disease characteristics and initial clinical response at week 2 to predict the clinical efficacy of infliximab at week 12 and (2) the clinical efficacy of infliximab in active nonradiographic axial spondyloarthritis patients through week 24.

2. Patients and Methods

2.1. Patients. All patients were recruited by the Department of Rheumatology of the Third Affiliated Hospital of Sun Yat-Sen University from June 2007 to December 2008. In this study, all patients were required to meet the European Spondyloarthropathy Study Group (ESSG) criteria for SpA [1] but could not meet the modified New York criteria for AS [8]. Specifically, patients could not have displayed X-ray evidence of structural changes in the sacroiliac joint (bilateral grade 2 or unilateral grade 3). All axial SpA patients were also required to have less than two-year disease duration and inflammatory back pain (Calin's criteria). In addition, active inflammatory lesions in the sacroiliac joints by MRI were required to be detected in all patients. All patients were required to have a Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score ≥30 mm (based on a visual analog scale (VAS) ranging from 0 to 100 mm) [9] and to have been receiving stable doses (for at least 4 weeks before baseline) of a single nonsteroidal anti-inflammatory drug (NSAID), if an NSAID was being used; no additional AS therapy was permitted during the 24 weeks preceding baseline.

In addition, if patients with or without peripheral symptoms met the above inclusion criteria, they would be included in our study. Our clinicaltrials.gov identifier number is NCT00936143.

This study was conducted at a single center in China. The independent ethics committee at the study site reviewed and approved the study protocol. Patients provided written informed consent before any study-related procedures were performed.

2.2. Patient Treatment and Evaluations. Patients received infliximab 5 mg/kg by intravenous infusion at weeks 0, 2, 6, 12, 18, and 24. Infliximab is a recombinant IgG1-κ human-murine chimeric monoclonal antibody that specifically binds to both soluble and membrane-bound forms of TNFα. Infliximab is supplied as a sterile, white, lyophilized powder in single-use 20 mL vials.

The following clinical and laboratory determinations were made at weeks 0, 2, 6, 12, 18, and 24: ASDAS, BASDAI, Bath Ankylosing Spondylitis Functional Index (BASFI) [10], erythrocyte sedimentation rate (ESR), and serum C-reaction protein (CRP) concentration. The BASDAI score, which ranges from 0 to 100 mm, is a combined assessment of fatigue, spinal pain, joint pain, enthesitis, and morning stiffness [9]. The BASFI score, which also ranges from 0 to 100 mm, includes 8 questions relating to the patient's function and 2 questions relating to a patient's ability to cope with everyday life [10].

The SPARCC MRI scoring method for spine bone edema was assessed on sagittal slices in two segments (C1-T10 and T10-S2), and active nonradiographic axial spondyloarthritis patients also had X-ray and MRI evaluations of the sacroiliac joints (SIJ) and lower spine (T10-S2 vertebrae) at baseline and week 24 in our study. Active inflammatory lesions of the SIJ and lower spine (T10-S2 vertebrae) were scored according to the SPARCC score system [11–13]. The SPARCC score system for active inflammatory lesions relies on the use of a T2-weighted sequence that incorporates suppression of normal marrow fat signal. All scores are based on abnormal increased signals on the STIR sequence representing increased concentration of "free water" otherwise referred to as "bone marrow edema." The scoring method described below assumes that images have been acquired according to our MRI acquisition protocol described in this website (available at http://www.arthritisdoctor.ca/) [11–13].

We also determined the proportions of patients achieving at least 20% improvement in the Assessment of Ankylosing Spondylitis (ASAS) International Working Group response criteria [14]. An ASAS20 response was defined as at least 20% improvement (and an absolute improvement from baseline of at least 10 units, on a scale of 0–100 mm) in at least 3 of the following 4 assessment domains: patient's global assessment, spinal pain, physical function according to the BASFI, and morning stiffness (the average of the last 2 questions of the BASDAI). These ASAS20 responders also must not have had deterioration from baseline (defined as a worsening of at least 20% and an absolute worsening of at least 10 units, on a scale of 0–100 mm) in the potential remaining assessment domain. ASAS40 criteria (at least 40% improvement and 20 units of absolute change in 3 of 4 domains, using the same domains as the ASAS response criteria, without any worsening in the fourth domain) and ASAS partial remission (defined as a value of 20 on a 0–100 mm scale in each of the 4 ASAS20 domains (patient's global assessment, pain, function, and inflammation) were also used for assessing the efficacy [15]. Clinical response was also assessed using the recently developed Ankylosing Spondylitis Disease Activity Score (ASDAS) which was developed specifically for the patients with AS [16, 17].

2.3. Statistical Methods. For analyzing the efficacy of treatment during the 24 weeks, we considered those failing to complete 24 weeks' treatment as treatment failure and conducted analysis by nonresponder imputation.

We assessed the ability of baseline indicators of inflammation and disease severity, that is, ESR, CRP concentration, and ASDAS, BASDAI, BASFI, and MRI sacroiliitis score, to predict attainment of an ASAS20 response at weeks 12 and 24 using Receiver Operating Characteristic (ROC) curve methodology. P value less than 0.05 was considered statistically significant. We also assessed the ability of ASAS20 response at week 2 to predict the attainment of such a response at week 12 and week 24 using logistic regression analysis.

3. Results

3.1. Patient Disposition and Baseline Characteristics. Seventy active nonradiographic axial spondyloarthritis patients were enrolled at a single study center. All patients enrolled completed the week-12 visit, and 61 patients completed the week-24 visit. Nine patients discontinued infliximab treatment at week 12: 3 patients due to adverse events (2 with allergies, 1 with tuberculosis) and 6 patients due to an unsatisfactory therapeutic response.

In this study, 82.9% of patients were men, 90.0% were HLA-B27-positive, and 18.6% had a family history of AS. The disease duration of active nonradiographic axial spondyloarthritis patients was 1.41 ± 0.57 years. MRI scores for the sacroiliac joint and lower spine (T10-S2 vertebrae) were 20.40 ± 10.44 and 1.86 ± 3.85, respectively (Table 1). 100% and 90% of patients fulfilled the new ASAS axial spondyloarthritis classification criteria set 1 and set 2, respectively [18]. In other words, all patients that were included were nonradiographic axial spondyloarthritis patients.

Table 1: Baseline patient characteristics.

	Axial SpA with less than two-year disease duration (n = 70)
Age (years)	21.00 ± 7.05
Sex	
Male	58 (82.9%)
Female	12 (17.1%)
Family history of AS	13 (18.6%)
HLA-B27(+)	63 (90.0%)
Peripheral symptoms (arthritis or enthesitis)	17 (24.3%)
Disease duration (years)	1.41 ± 0.57
BASDAI (0–100 mm VAS)	46.86 ± 10.48
BASFI (0–100 mm VAS)	23.41 ± 19.46
BAS-G (0–100 mm VAS)	49.95 ± 19.16
ASDAS	3.02 ± 1.25
MRI score	
Sacroiliac joint	20.40 ± 10.44
Lower spine (T10-S2)	1.86 ± 3.85
Modified Schober test (cm)	4.63 ± 1.69
CRP (mg/L)	31.43 ± 38.33
ESR (mm/hr)	34.75 ± 30.48

Data reported are mean (standard deviation) or number (%) of patients. BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, BASFI: Bath Ankylosing Spondylitis Functional Index, BAS-G: Bath Ankylosing Spondylitis Global assessment, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, HLA: human leukocyte antigen, MRI: magnetic resonance imaging, and VAS: visual analog scale.

3.2. Infliximab Clinical Efficacy in Active Nonradiographic Axial Spondyloarthritis Patients. Efficacy endpoints were assessed at week 12 and week 24.

3.2.1. Week 12. Among the 70 enrolled active nonradiographic axial spondyloarthritis patients, similar proportions of patients achieved an ASAS20 response at weeks 2, 6, and 12 (85.7%, 88.6%, and 87.1%, resp., P = NS). Proportions of patients who achieved an ASAS40 response at weeks 2, 6, and 12 were 61.43%, 62.86%, and 67.14%, respectively (P = NS). Proportions of patients who achieved an ASAS partial remission at weeks 2, 6, and 12 were 57.14%, 58.58%, and 60.0%, respectively (P = NS). Proportions of patients who achieved BASDAI50 response at weeks 2, 6, and 12 were 67.14%, 71.43%, and 74.29%, respectively (P = NS). ASDAS, which decreased from 3.02 ± 1.25 at baseline (high disease activity) to 0.83 ± 0.63 at week 2, 0.75 ± 0.54 at week 6, and 0.76 ± 0.68 at week 12, suggested clinically significant improvement in these patients (P = 0.000) and continuing through week 12. Significant improvements were also observed in the BASDAI score, BASFI score, CRP concentration, and ESR at the beginning of week 2, compared to baseline (P = 0.000) and continued to week 12.

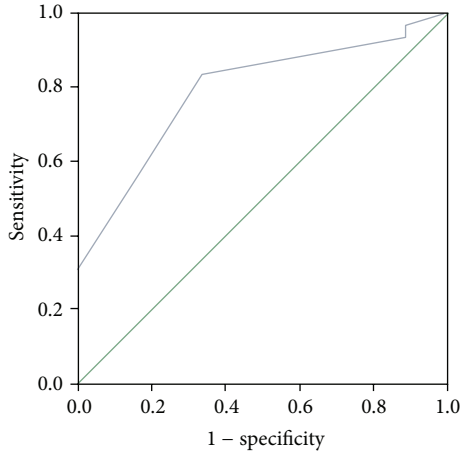
3.2.2. Week 24. For analyzing the efficacy of treatment during the 24 weeks, we considered nine patients who failed to complete 24 weeks' treatment as treatment failure and conducted analysis by nonresponder imputation.

At week 24, 84.3% of patients achieved an ASAS20 response. Besides, the proportions of the patients achieving an ASAS40 response, ASAS partial remission, and BASDAI50 response were 64.17%, 61.43%, and 68.57%, respectively.

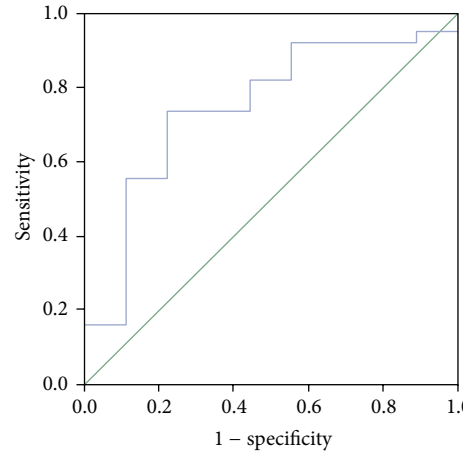
3.3. Ability of Baseline Disease Characteristics and Early Clinical Response to Predict Infliximab Clinical Efficacy in Active Nonradiographic Axial Spondyloarthritis Patients

3.3.1. Week 12. Results of ROC curve analysis indicated that the baseline MRI sacroiliitis score was a significant predictor of achievement of an ASAS20 response at week 12 after 3 infliximab infusions (AUC = 0.791; P = 0.005) (Figure 1(a)). Baseline CRP (AUC = 0.75; P = 0.017) (Figure 1(b)) and baseline ASDAS (AUC = 0.778, P = 0.007) (Figure 1(c)) were also significant predictors of ASAS20 response at week 12. The other baseline parameters assessed, including BASDAI (AUC = 0.556; P = 0.593), BASFI (AUC = 0.644; P = 0.166), and ESR (AUC = 0.618; P = 0.254), were not significant predictors of ASAS20 response at week 12.

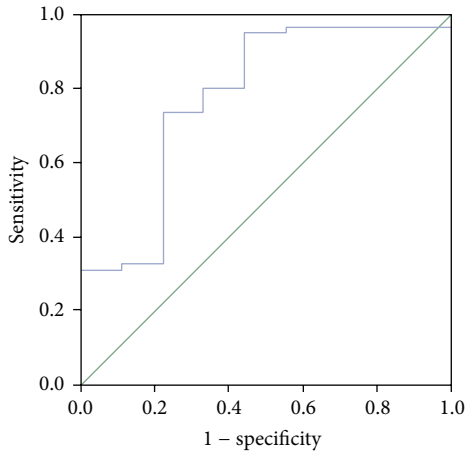
Considering CRP, ASDAS, SIJ MRI score at baseline, and age as corrected factors, we analyzed ASAS20 response at



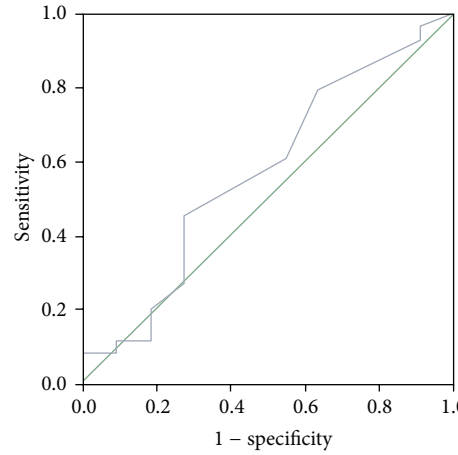
(a) ROC curve result of baseline MRI sacroiliitis score after 12 weeks' infliximab treatment



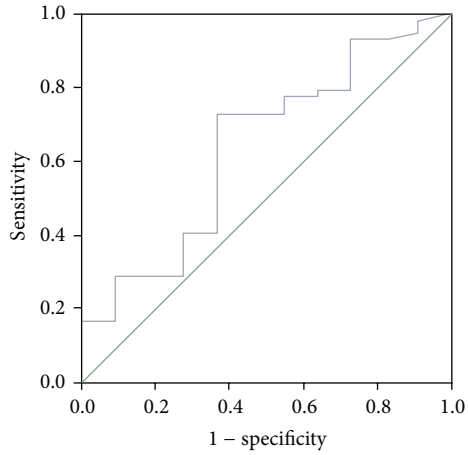
(b) ROC curve result of baseline CRP after 12 weeks' infliximab treatment



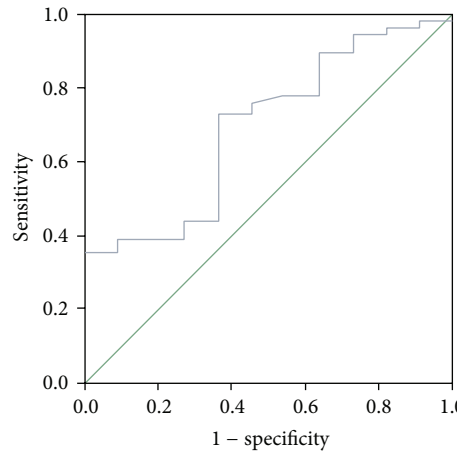
(c) ROC curve result of baseline ASDAS score after 12 weeks' infliximab treatment



(d) ROC curve result of baseline MRI sacroiliitis score after 24 weeks' infliximab treatment



(e) ROC curve result of baseline CRP after 24 weeks' infliximab treatment



(f) ROC curve result of baseline ASDAS score after 24 weeks' infliximab treatment

Figure 1: Ability of baseline sacroiliac joint magnetic resonance imaging (MRI) score (Panel (a)), serum C-reactive protein (CRP) concentration (Panel (b)), and Ankylosing Spondylitis Disease Activity Score (ASDAS) (Panel (c)) to predict at least 20% improvement in the Assessment of Ankylosing Spondylitis International Working Group response criteria (ASAS20) from baseline to week 12, as determined by area under the Receiver Operating Characteristics (ROC) curve. The same analysis results of sacroiliac joint magnetic resonance imaging (MRI) score, serum C-reactive protein (CRP) concentration (Panel (b)), and Ankylosing Spondylitis Disease Activity Score (ASDAS) at week 24 were shown in Panel (d), Panel (e), and Panel (f).

Table 2: Considering CRP, ASDAS, SIJ MRI score at baseline, and age as corrected factors to analyze ASAS20 response at week 2 by stepwise regression method (logistic regression) after 12 weeks' treatment and 24 weeks' treatment.

	After 12 weeks' treatment	After 24 weeks' treatment
Wald χ^2	6.870	5.171
P value	0.009	0.023
OR	12.077	6.764

week 2 by stepwise regression method (logistic regression) after 12 weeks' treatment and 24 weeks' treatment.

Finally, considering CRP, ASDAS, SIJ MRI score at baseline, and age as corrected factors, we analyzed ASAS20 response at week 2 by stepwise regression method (logistic regression) after 12 weeks' treatment. The result showed that an ASAS20 response at week 2 was a significant predictor of subsequent clinical response in the same patient at week 12 (wald $\chi^2 = 6.87$, OR = 12.077, $P = 0.009$) (Table 2).

3.3.2. Week 24. As Figure 1 shows, ROC curve analysis indicated that baseline ASDAS (AUC = 0.696, $P = 0.040$) was also significant predictors of ASAS20 response at week 24. However, the baseline MRI sacroiliitis score, CRP, BASDAI, BASFI, and ESR could not predict the achievement of an ASAS20 response at week 24 after 6 infliximab infusions (MRI sacroiliitis score: AUC = 0.575; $P = 0.434$; CRP: AUC = 0.641; $P = 0.140$; BASDAI: AUC = 0.649; $P = 0.118$; BASFI: AUC = 0.625; $P = 0.191$; ESR: AUC = 0.558; $P = 0.545$) (Figures 1(d), 1(e), and 1(f)).

Likewise, considering CRP, ASDAS, SIJ MRI score at baseline, and age as corrected factors, we analyzed ASAS20 response at week 2 by stepwise regression method (logistic regression) after 24 weeks' treatment. The result showed that an ASAS20 response at week 2 was a significant predictor of subsequent clinical response in the same patient at week 24 (wald $\chi^2 = 5.171$, OR = 6.764, $P = 0.023$) (Table 2).

4. Discussion

Few studies reported the efficacy for active axial SpA with TNF-α blocker treatment. Sieper et al. reported that adalimumab has a positive benefit-risk profile in active nr-axSpA patients with inadequate response to NSAIDs [7]. However, it is rarely reported about the efficacy for active axial nr-SpA with infliximab treatment, and our results suggested infliximab may be an effective treatment for active axial nr-SpA. The results of our study confirmed that TNF-α blocker has a significant efficacy for early axial SpA patients, and, moreover, we detected four indexes for predicting clinical efficacy with infliximab therapy in active axial SpA patients, which included ASDAS score, MRI sacroiliitis score, CRP, and first dose response.

So far high CRP, high MRI spine score, and lower BASFI were reported to be predictors of major clinical responses to anti-TNF therapy in patients with active AS patients [19–21]. However, we firstly report that ASDAS score and first doses response are the predictors of clinical response to infliximab therapy in active axial SpA patients.

As is known to us all, ASDAS score is a good index for assessing the disease activity, but it is unknown whether it predicts the clinical response in active axial SpA patients [22]. This study firstly demonstrates the ability of the baseline ASDAS to predict the efficacy for active nonradiographic axial spondyloarthritis patients after 12 weeks' and 24 weeks' infliximab therapy. ASDAS is a highly discriminatory instrument for assessing disease activity in AS that includes an objective inflammatory marker (serum CRP concentration) and subjective assessments of disease activity. Based on these, the ASDAS score provides information regarding the overall state of inflammation in AS patients [16, 17, 22]. The results of this study showed that BASDAI at baseline could not predict the efficacy of infliximab therapy but ASDAS could. As we knew, the difference between ASDAS and BASDAI was that ASDAS included an objective inflammatory marker (serum CRP concentration). It may be a possible reason why ASDAS at baseline can predict the efficacy of infliximab therapy but BASDAI cannot. In consistence with this supposition, our result suggested that baseline serum CRP concentration was a significant predictor of clinical response to infliximab therapy at week 12, which was consistent with findings of Luc and colleagues [19]. Luc's study showed that baseline serum CRP concentration was the only factor out of several evaluated that predicted AS patient continuation of anti-TNF therapy, as opposed to discontinuing such therapy because of lack of efficacy [19].

Besides, ASDAS, MRI sacroiliitis score were another significant predictor of clinical response to infliximab therapy at week 12 in our study. MRI has emerged as a key tool in diagnosing axial SpA [23] based on its ability to detect the bone marrow edema that precedes structural changes in the spine, but the research on the predictive ability of MRI scores in AS and axial SpA is limited. Rudwaleit and colleagues reported that the MRI spine score was predictive of a major clinical response to anti-TNF therapy in patients with active AS [20]. In their study, Rudwaleit and colleagues defined a major clinical response to anti-TNF therapy as achievement of a BASDAI50 (at least 50% improvement in the BASDAI) response. In addition, their results indicated that inflammation of the spine, but not the sacroiliac joint, as detected by MRI significantly predicted clinical efficacy of anti-TNF therapy at week 12 [19]. Nevertheless, the MRI sacroiliitis score was a significant predictor of achievement of an ASAS20 response after 3 infliximab infusions at week 12 in our study. As a possible explanation for these differences between study results, we note that patients in the Rudwaleit study had AS for an average of 14 years, compared with 1.4 years in the our study. And during the initial stages of axial SpA, inflammation in the sacroiliac joints is typically evident within the first 2 years of disease, even when spinal mobility remains normal. Only 19 patients (27.14%) were detected in having inflammation of the spine by MRI in our study. Taken together, these MRI findings suggest that the sacroiliac joint is a more important location for MRI assessment in active nonradiographic axial spondyloarthritis patients, while the spine should be the focus of MRI assessments in AS patients with

much more established disease. In addition, results of these 2 studies suggest that MRI scores can help predict subsequent clinical response to anti-TNF therapy in axial SpA patients. And sacroiliac joint MRI plays a more important role in active nonradiographic axial spondyloarthritis patients.

In addition, the ability of active nonradiographic axial spondyloarthritis patients to achieve an ASAS20 response 2 weeks following an initial infliximab infusion was a significant predictor of the same patient's ability to also achieve this response at week 12 (i.e., following 3 infliximab infusions). It was an interesting and important finding. As we know, although infliximab efficacy has been documented in AS [21, 24–26], the cost of infliximab therapy can deter its use in the clinical setting. Having the ability to predict which patients might benefit the most from infliximab therapy could help guiding the clinician in appropriately allocating limited healthcare resources. Our results could be particularly valuable to the physicians when deciding whether to start or continue with a patient's infliximab therapy.

Moreover, previous studies suggested that lower BASFI scores were predictors of a major clinical response to TNF blockers in active AS [27]. However, in our study, BASFI at baseline cannot predict the efficacy in active nonradiographic axial spondyloarthritis patients after infliximab treatment. As the result shown, mean BASFI which only was 23.41 ± 19.46 mm in our study was significantly less than previous study (reach 54 mm) [27]. It may be the reason why our result was different from previous study. Therefore, these data need to be confirmed in further studies.

However, the baseline MRI sacroiliitis score and CRP could not predict the achievement of an ASAS20 response at week 24 after 6 infliximab infusions, although they were considered as predictors of clinical response at week 12 after 3 infliximab infusions in our study. But ASDAS still showed its superiority of predicting the clinical response after infliximab treatment at week 24. Taking the results above into consideration, ASDAS score may be a more sensitive indicator than other indicators for predicting the clinical response in axial SpA patients with infliximab treatment.

In conclusion, infliximab has a high efficiency in active nonradiographic axial spondyloarthritis patients. ASDAS score and first dose response could effectively help predict clinical efficacy with infliximab therapy in these patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Zhiming Lin and Zetao Liao contributed equally to this paper.

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Clinical Study

The Surgical Treatment Principles of Atlantoaxial Instability Focusing on Rheumatoid Arthritis

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Object. This retrospective review was conducted to determine the surgical treatment principle for rheumatoid arthritis (RA) patients with atlantoaxial instability (AAI). *Methods*. Thirteen patients with AAI, including 5 RA patients, received preoperative computed tomography- (CT-) based image-guided navigation system (IGS) in C1 lateral mass-C2 pedicle screw-rod system fixation (LC1-PC2 fixation). These 13 patients were analyzed for 52 screws inserted into C1 and C2. We defined these patients as non-RA group (8 patients, 32 screws) and RA group (5 patients, 20 screws). The neurological status for RA group was evaluated using the Ranawat classification. The causes of AAI, surgical indications, complications, surgical method revolution, and CT-based navigation application are discussed. *Results*. None of the 13 patients expressed neurological function deterioration. The non-RA group screw accuracy was 100%. In the RA group, 1 RA patient developed left C2 screw loosening at 1^+ months after operation due to screw malposition. The screw accuracy for this group was 95%. *Conclusions*. Higher intraoperative surgical complication rate was described in RA patients. Preoperative CT-based IGS in LC1-PC2 fixation can provide good neurological function and screw accuracy results. However, for higher screw accuracy in RA patients, intraoperative CT-based IGS application may be considered.

1. Background and Introduction

AAI is characterized by excessive movement between the atlas and axis. It is notorious for nuchal pain and neural compression. Early recognition of the progressive neurological symptoms for early surgical intervention is an important predictor for good recovery [1, 2]. Various surgical methods were applied in AAI.

RA patients may have bone erosion and osteoporosis due to rheumatoid synovitis and medication. Therefore, besides trauma, infection, congenital disease, and postirradiation status, RA is another important risk factor for AAI [3]. In addition, patients with comorbid rheumatoid and spine pathology have been shown to have higher wound and implant-related complications [4]. LC1-PC2 system was used in RA patients for higher fusion rate. In addition, more preop

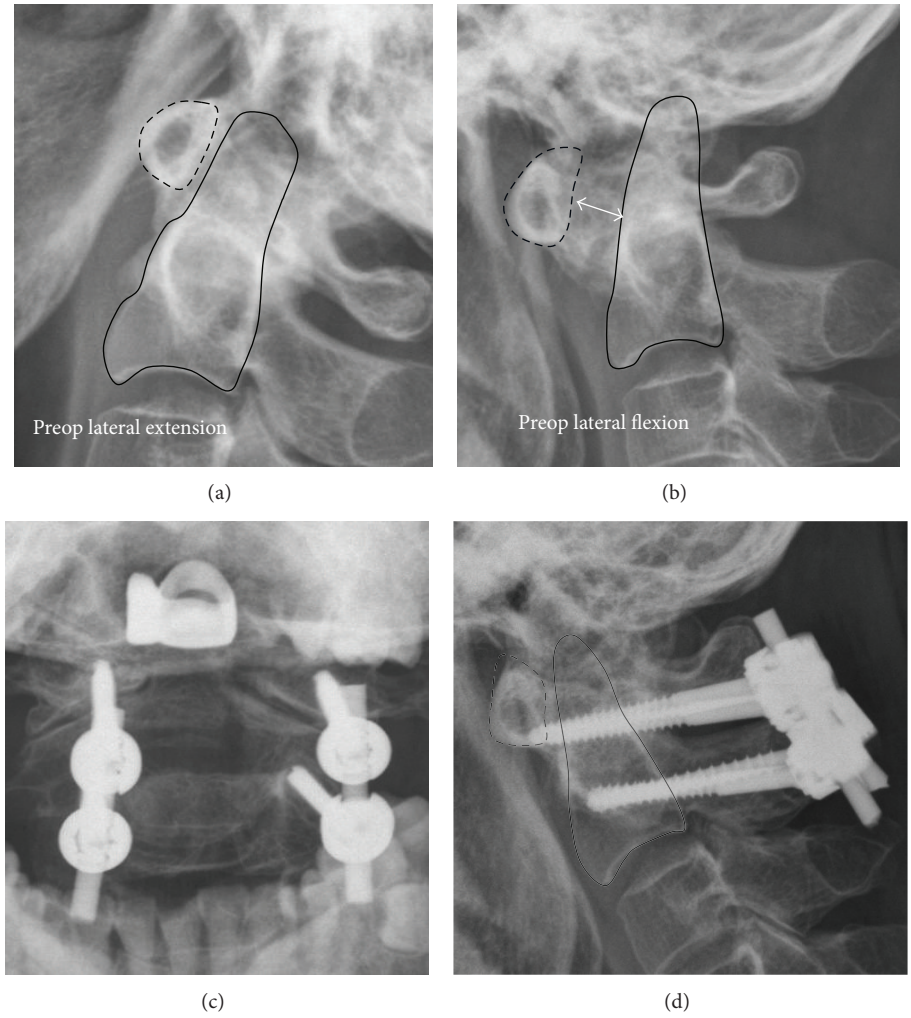

Figure 1: Preop dynamic lateral radiographs revealed AAI ((a) and (b)). The atlantodental interval 6 mm in flexion position (b) is measured (double-headed arrow). Postop radiographs revealed atlantoaxial fusion with LC1-PC2 system ((c) and (d)).

evaluation, surgical planning, and postop care should be considered in RA patients.

2. Methods

2.1. Patient Classification. Thirty-five patients with AAI were treated between April 2004 and September 2014 by one surgeon at one institute. Eighteen patients (51.4%) were female and 17 (48.6%) were male (mean age, 55.3 years, range 21–77 years). All patients were divided into trauma group (19 patients; 54.2%), RA group (6 patients; 17.1%), degenerative osteoarthritis (4 patients; 11.4%), movement disorder group (1 patient, 2.8%), symptomatic Os odontoideum (1 patient, 2.8%), osteomyelitis (1 patient, 2.8%), previous implant failure group (1 patient with previous titanium cable wire fixation and autogenous iliac bone fusion; 2.8%), and patients with unknown cause (2 patients, 5.7%) (Table 1).

Table 1: Indications for atlantoaxial instability surgery.

Indications	Patients ($n = 35$)
Fracture	19
Rheumatoid arthritis	6
Degenerative osteoarthritis	4
Movement disorder	1
Symptomatic Os odontoideum	1
Osteomyelitis	1
Previous implant failure	1
Unknown	2

2.2. Preop Survey. Radiographs in AP view, lateral flexion-extension view, and open-month view were checked for bone structure and stability (Figures 1(a) and 1(b)). Before surgery, 128-slice spiral CT scanning (Philips, iCT 256) was carried out. Patients were placed supine for spinal CT axial scanning, and the data were recorded in DICOM format in the computer. The scanning conditions were 140 kV voltage and 171 mA electric current. The scanning parameters included image matrix 512 × 512, slice thickness 0.9 mm, pitch 0.49, and

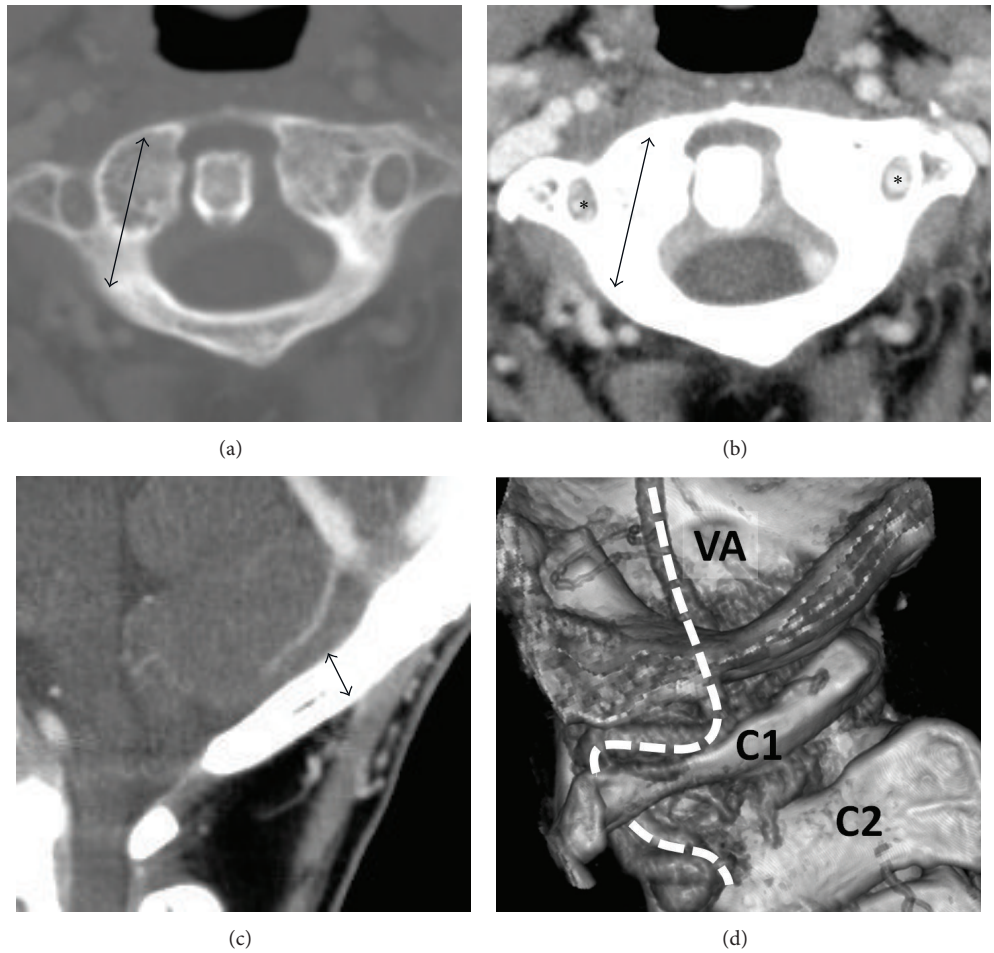

Figure 2: Preop reconstructive computed tomography for choosing pedicle screw length and diameter (a), knowing the screw and vertebral artery relationship (*asterisk*) (b), choosing occiput screw length (c), and the vertebral artery (*dashed line*) direction (d).

reconstruction slice thickness of 1 mm. Careful preoperative study of this CT scan with 3D reconstruction including the occiput was acquired to check occipital bone thickness, vertebral artery, and diameter and length estimation of the lateral mass or transpedicle screw before operation (Figure 2). In the cases with CT-based IGS, the images were then transferred into the navigation system (BrainLAB Vector Vision Navigation System).

2.3. Operation Methods. One patient received transoral partial odontoidectomy and decompression prior to posterior approach with LC1-PC2 fixation for chronic C1-2 subluxation with pseudotumor and spinal cord compression. One patient underwent transoral biopsy prior to LC1-PC2 fixation owing to difficult osteomyelitis or tumor differential diagnosis by neuroradiologist. One patient received C1-2 Halifax interlaminar clamp with autogenous iliac bone fusion. Six patients received anterior odontoid screw fixation. Five patients received occipitocervical fusion with screw-rod system (4 patients O-C2-C3, 1 patient O-3-4-5; one of these 4 patients received revision surgery as replacement for loosening occiput Y-plate with screw-rod system).

Twenty-three patients received LC1-PC2 fixation (Figures 1(c) and 1(d)) (1 of the 23 patients was in post-Gallie wiring and grafting techniques with cable wire breakage). Three-dimensional (3D) assessment with a preoperative CT-based IGS was applied in 13 of these 23 patients since February 2012 (Figures 3(e) and 3(f)). There were 6 RA patients enrolled in this study. The one who presented cranial settling and received occipitocervical junction fusion with a screw-rod system was excluded from the screw accuracy analysis. These 13 patients, including 5 RA patients, were analyzed for 52 screws inserted into C1 and C2. We defined these patients as non-RA group (8 patients, 32 screws) and RA group (5 patients, 20 screws).

3. Results

None of the 13 patients who received preoperative CT-based IGS in LC1-PC2 fixation expressed neurological function deterioration. Five patients had a history of RA. Their neurological status was evaluated using the Ranawat classification (Table 2). Two of these 5 patients were Ranawat class IIIA,

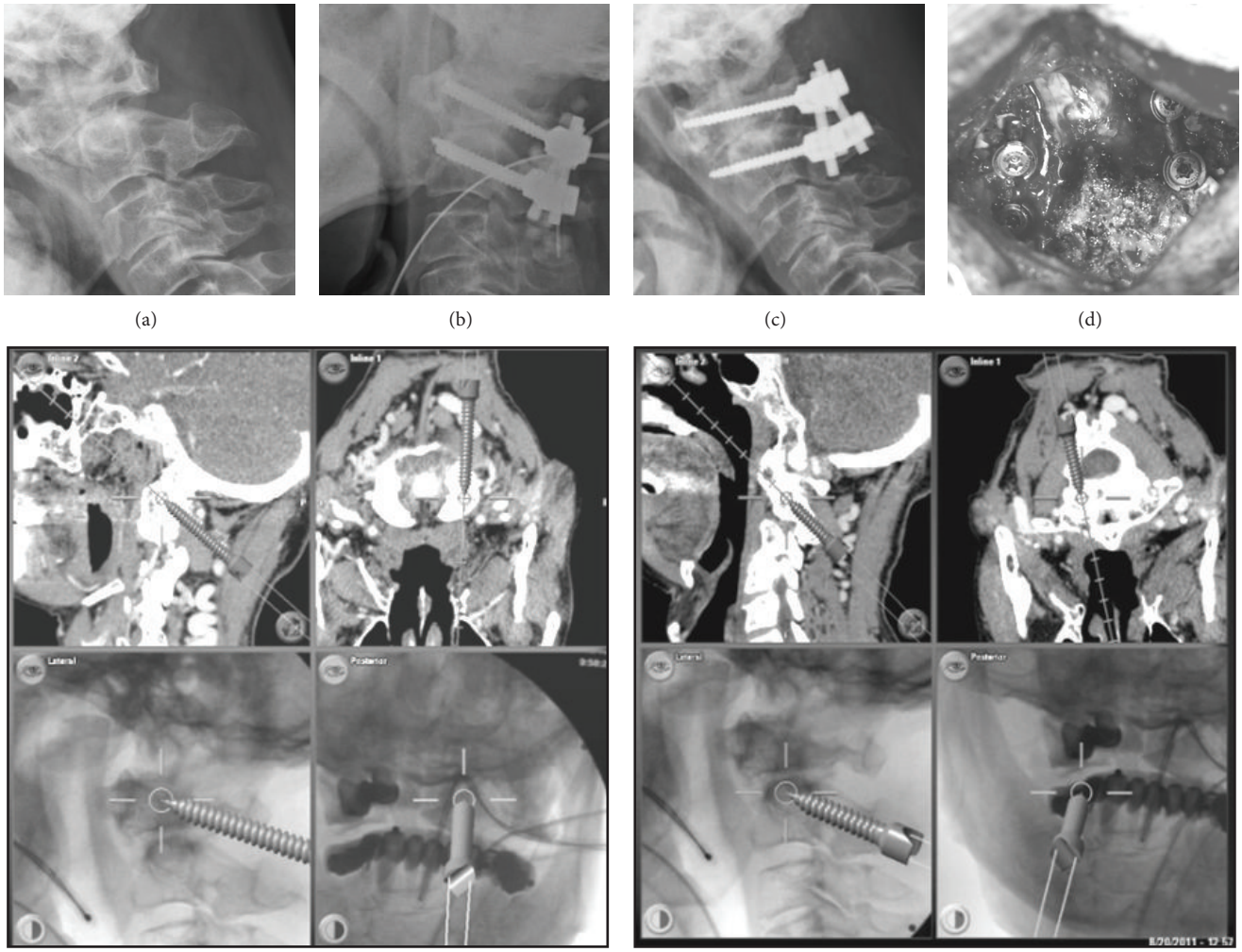

Figure 3: Preop lateral radiograph revealed AAI (a). Postop radiographs revealed fixation after operation ((b) and (c)). The operative photo showed LC1-PC2 system (d). The intraoperative CT navigation guided technique for placement screw for C1 (e) and C2 (f).

Table 2: Ranawat classification of neurological deficit.

Ranawat classification	
Class I	No neural deficit
Class II	Subjective weakness, dysesthesias, and hyperreflexia
Class IIIA	Objective weakness and long-tract signs; patient remains ambulatory
Class IIIB	Objective weakness and long-tract signs; patient no longer ambulatory

another patient was Ranawat class IIIB, and the remaining two patients were classified as Ranawat classes I and II.

Satisfactory C1-2 screw placement and atlantoaxial reduction were achieved in all patients except one RA patient with left C2 screw malposition. This patient developed left C2 screw loosening at 1^{+} months after operation due to screw malposition during surgery (Figure 4). The patient hesitated at reopening surgery owing to uneventful outcome from the screw loosening.

These 13 patients (52 screws for C1 and C2) received preoperative CT-based IGS for LC1-PC2 fixation. Of the 32 screws inserted in the non-RA group (8 patients), 32 screws were in the correct position. Of the 20 screws inserted in the RA group (5 patients), 19 screws were in the correct position. The non-RA group screw accuracy was 100%. The C1 and C2 screw accuracy in the RA group was 95%.

Two of these 23 patients who received LC1-PC2 fixation (including "virtual fluoroscopy" and navigation system) suffered from occipital neuralgia. There were no vertebral artery (VA) injuries during the operations and no neurological deterioration after surgery related to the procedure.

One patient received O-C2-C3 Y plate and pedicle screw-rod fixation system. Three years later occiput Y-shaped plate screw dislodgement was found in radiograph. A revision operation was performed.

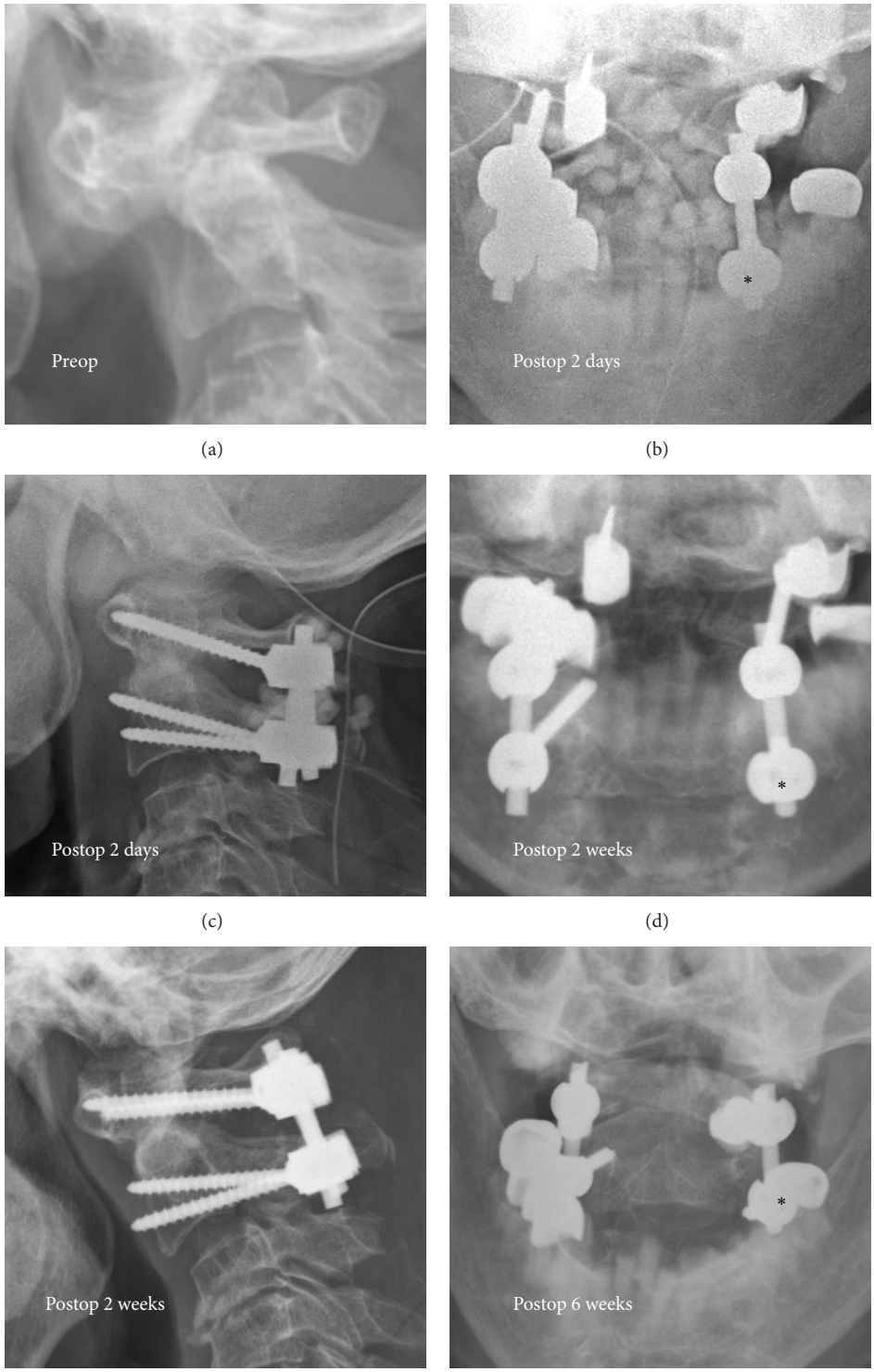

Figure 4: Preop lateral radiograph revealed AAI (a). Second day postop radiographs showed malposition of left C2 screw (*asterisk*) ((b) and (c)). Two weeks postop radiographs for follow-up of the fusion condition ((d) and (e)). Six weeks postop open mouth radiograph revealed loosening of left C2 screw (f).

One major complication occurred in one quadriparesis patient in trauma group due to chirotherapy who received an atlantoaxial fixation using a LC1-PC2 system. The pain was relieved and muscle power much improved in all four limbs after the operation. We weaned the patient from the ventilator 1 day after operation. However, suffocation and cardiac arrest occurred on the 6th day after operation. With emergency cardiopulmonary resuscitation the patient's vital signs recovered. However, dull consciousness with ventilator support persisted. Three years after operation she died due to cardiopulmonary failure.

4. Discussion

AAI is characterized by disproportionate movement between the atlas and axis due to either bony or ligamentous abnormality. AAI may occur after trauma, upper respiratory infection or infection following head and neck surgery, inflammatory disease as rheumatoid arthritis, or congenital disease. The most common cause for AAI is trauma. Tiu KL reported that irradiation-related delayed healing, higher infection risk, and osteonecrosis may result in atlantoaxial instability [5].

Cervical spine involvement occurs in over half of patients with RA. The atlantoaxial joint is often affected in patients with RA [6]. Atlantoaxial subluxation causes the odontoid process or the posterior arch of the atlas to impinge on the spinal cord. Spinal cord and C2 root compression can result in such symptoms as myelopathy, occipital pain, and nuchal pain in RA patients [7]. Medication, rehabilitation, and surgical intervention have their roles in different conditions. However, when it comes to intractable pain, progressive neurological deficits, or progressive instability, surgical intervention is indicated [8].

Rheumatoid cervical disease usually develops within 2–10 years of RA [9]. A cohort study with 161 patients described the natural course of cervical lesions in RA. Ninety-two patients (57%) had upper cervical involvement, which progressed into anterior atlantoaxial subluxation, vertical subluxation, and both. Neural involvement occurred in 10 patients. In 7 of these 10 patients vertical subluxation of the atlas was responsible for the neural deficit [10]. In a cohort of 55 rheumatoid cervical patients who received surgery after myelopathy deteriorated to Ranawat class IIIB patients. The early postoperative mortality rate was high (12.7%). Only 14 patients (25.5%) were judged to have had a favorable outcome as determined by an improvement to Ranawat class I or II [11]. Mikulowski et al. reported postmortem findings in 104 rheumatoid patients and found that 11 deaths were associated with cervicomedullary compression from atlantoaxial dislocation [12]. The cervicomedullary compression may cause serious sequelae, including paresis, hypertonia, delayed motor milestones, and respiratory compromise. Because of the poor natural history, it is believed that earlier surgical intervention, before the development of vertical translocation, permanent neurological damage, and spinal cord atrophy, is necessary in RA patients.

More than 80% of RA could be detected with cervical spine involvement by radiology modalities [13]. In basilar invagination cases with RA, alar ligament and tectorial membrane relaxation or disruption developed. The odontoid process then migrates upward. It is radiologically defined by the amount of protrusion of the tip of the odontoid process by more than 5 mm beyond McGregor's line.

AAI is defined if atlantodental interval is greater than 3 mm in adults and greater than 5 mm in children. The atlantodental interval is the distance between the posterior aspect of the anterior atlas ring and the anterior aspect of the odontoid process (Figures 1(a) and 1(b)). Other papers pointed that the posterior atlantodental interval has been found to be a more sensitive tool for evaluating neurologic injury because it reflects the potential spinal canal. The decrease in potential space for the spinal cord increases the risk for spinal cord compression. The posterior atlantodental interval, also known as space available for spinal cord (SAC), critical lower limit is 13 mm, which has a 97% sensitivity to predict paralysis [14, 15].

Furthermore, image studies for the preoperative survey include CT angiography and MRI of the cervical spine. Both of these studies are helpful for planning the diameter, length, and trajectory of screws to avoid vertebral arteries and neural structures injuries during screw insertion (Figure 2).

The cessation of various rheumatoid medications before the operation is another issue for reducing surgical complications. For example, nonsteroidal anti-inflammatory drugs should be discontinued 3 to 5 half-lives before surgery. Perioperative corticosteroids stress doses should be given. Methotrexate should be discontinued for 6 to 8 weeks because it will increase the infection rate and affect bone healing. Biological agents (tumor necrosis factor-α and interleukin-1 antagonists) should be stopped preoperatively and held until 14 days after surgery to avoid the risk of opportunistic infections [16].

Operating on the atlantoaxial complex has always posed a challenge to the surgeon because of the complex anatomy and biomechanics of this spine region. Historically, Gallie wiring and grafting techniques were used for AAI [17], which were further modified by Brooks and Jenkins [18]. Three variants of lateral mass screws have been described by An, Magerl, and Roy-Camille. Goel and Laheri described plate and screw fixation for atlantoaxial subluxation which was further modified by Harms and Melcher to posterior C1 lateral mass and C2 pedicle or pars screws [19, 20]. In addition, in case of AAI with basilar invagination, anterior decompression with occipitocervical fusion and decompressive laminectomy was suggested [21]. Due to the high biomechanical strength, posterior atlantoaxial fixation using lateral mass screws at C1 in combination with pedicle screws at C2 has become popular [19]. In the C2 pedicle screw insertion procedure, complications from screw penetration into spinal canal and VA injury can occur. If one VA is hypoplastic, it presents lethal complications when the dominant VA is ruptured. It is more risky to insert a pedicle screw in patients with narrow C2 pedicle. Therefore, even though the pedicle screws have been shown to have the highest pullout strength, some authors did not recommend routine use due to a higher risk for vertebral artery injury [22]. In addition, occipital neuralgia was a frequent complication related to posterior atlantoaxial

fixation. It is due to surgical manipulation during preparation of the C1 screw entry point and impingement of C2 root by C1 screw. Gautschi et al. reported that among the clinically relevant complications related to posterior atlantoaxial fixation, postoperative C2 neuralgia is the most frequent problem (9.8%) [23]. To avoid occipital neuralgia complications, C2 root sacrifice is performed by some surgeons. The C2 root resection is also believed to achieve safe and wide exposure in performing C1-2 instrumented fixation. However, numbness occurs in approximately 12% of patients who received C2 root resection; a result that may be intolerable to certain patient populations [24, 25].

The poor bone quality of RA patients makes both intra- and postoperative periods more complex. Gallie or Brook wiring and bone grafting methods were used to fuse the atlantoaxial joint. This method achieved a lower fusion rate than other fusion methods in the general population. Case reports on wire-graft fusion complications due to C1 posterior arch fracture were described in RA patients [26]. The Magerl technique, known as C1-C2 transarticular screw fixation with posterior wiring, developed for higher fusion rate. Kuroki and colleagues reported that LC1-PC2 fixation is more stable than transarticular screw fixation [27]. Therefore, we propose that LC1-PC2 fixation provides more stability and less late complication rate in RA patients. However, when performing the C2 pedicle screw fixation, the VA may be injured. This is a disastrous complication. Few studies have compared the risk for VA injury in patients with and without RA. Masahiko demonstrated that RA is a significant risk factor for a narrow C2 pedicle, and narrowing of the C2 pedicle is elevating the risk for VA injury in RA patients. Therefore, in patients with RA, thorough preoperative evaluation of the bone architecture is very important for avoiding inadvertent injury to the VA [28]. Another paper reported that the cervical pedicle screw perforation rate was higher in spine tumors (16.7%), RA (37.5%), and destructive spondyloarthropathy patients [29]. Therefore, closer attention should be paid to the atlantoaxial complex in patients with RA.

Preoperative cervical CT is useful for bone architecture and surgical planning. Preoperative screw trajectory could be evaluated for avoiding inadvertent VA injury. Using 3D assessment with a CT-based IGS, the axial cut planning for the instrumented levels presents extreme benefit in determining the proper screw trajectory for the safety of adjacent neural and vascular structures during the operation. A systematic review including 18 cohort studies and 2 randomized controlled trials revealed that there is a significantly lower risk of pedicle perforation for navigated screw insertion compared with nonnavigated insertion for all spinal regions [30]. Linhardt et al. demonstrated increased pullout strength in pedicle screws placed with computer-assisted techniques compared with screws placed with conventional techniques [31]. Nottmeier et al. believed that using the 3D image guidance system planning function allows larger diameter screws to be placed, resulting in screws being placed in a more medial trajectory than standard techniques [32]. It is believed that 3D assessment with a CT-based IGS can avoid vascular and neural injury and also can enhance the screw-rod system stability. However, severe cervical pedicle screw malposition can occur even with 3D navigation. Ishikawa et al. recorded that, even with 3D navigation, prevalence of cervical pedicle screw perforations was 18.7% in their study [33]. In our practical experience, the possibility of screw malposition by preoperative CT-based IGS may be related to a change in body posture from the supine position during preoperative CT scanning to the prone position during the operation. We adjusted the patient's neck posture using the Mayfield pin-fixation device for better atlantoaxial reduction in the operation room prior to the posterior approach. These are the reasons why inaccurate screw placement occurred in preoperative CT-based IGS, particularly in RA patients with severe AAI. A large retrospective comparative outcome analysis [34] revealed excellent accuracy in preoperative and intraoperative CT-based IGS; there was a statistically significant advantage for intraoperative group. Intraoperative CT-based IGS application may provide higher screw accuracy in this patient group.

5. Conclusion

Higher intraoperative surgical complication rate was described in RA patients. Preoperative CT-based IGS in LC1-PC2 fixation can provide good neurological function and screw accuracy results. However, for higher screw accuracy in RA patients, intraoperative CT-based IGS application may be considered. Although the CT-based IGS surgical technique was used to decrease the complication rate and improve instrument biomechanical stability, advanced techniques, surgical experience, and anatomy knowledge are required to decrease the screw malposition rate.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

Analysis of Erythrocyte C4d to Complement Receptor 1 Ratio: Use in Distinguishing between Infection and Flare-Up in Febrile Patients with Systemic Lupus Erythematosus

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Objective. Fever in systemic lupus erythematosus (SLE) can be caused by infection or flare-up of the disease. This study aimed to determine whether the ratio of the level of erythrocyte-bound C4d to that of complement receptor 1 (C4d/CR1) can serve as a useful biomarker in the differentiation between infection and flare-up in febrile SLE patients. *Methods.* We enrolled febrile SLE patients and determined the ratio on the day of admission. The patients were divided into 2 groups according to the subsequent clinical course. *Results.* Among the febrile SLE patients, those with flare-up had higher ratios and lower C-reactive protein (CRP) levels than those with infection. Cut-off values of <1.2447 and >4.67 for C4d/CR1 ratio and CRP, respectively, were 40.91% sensitive and 100.0% specific for the presence of infection in febrile SLE patients; similarly, cut-off values of >1.2447 and <2.2, respectively, were 80% sensitive and 100% specific for the absence of infection in febrile SLE patients. *Conclusion.* The C4d/CR1 ratio is a simple and quickly determinable biomarker that enables the differentiation between infection and flare-up in febrile SLE patients at initial evaluation. Further, when combined with the CRP level, it is useful to evaluate disease activity in SLE patients with infection.

1. Introduction

Systemic lupus erythematosus (SLE) is a common autoimmune disease. Fever in SLE patients can be caused by a number of reasons, with infection and flare-up being the most common. The clinical presentation of SLE flare-up may mimic that of infection coincident with SLE, and the two situations may be difficult to differentiate in febrile patients. Differential diagnosis of fever in SLE is crucial for the optimal management of these patients.

Traditional biomarkers for the survey of disease activity in SLE include anti-dsDNA antibodies and serum complement proteins C3 and C4. However, most SLE patients exhibit persistently high levels of anti-dsDNA antibodies or low levels of complement proteins C3 and C4. Therefore, these

biomarkers are insufficient for differentiating disease flares from infection. Several biomarkers can be used to survey susceptibility, establish diagnosis, evaluate disease activity, and assess specific organ involvement in SLE [1, 2]. Among them, the novel biomarkers to evaluate disease activity include serum cytokines, soluble cytokine receptors, soluble cell surface molecules (CD27, CD154, and BAFF) [3], endothelial activation markers (soluble vascular adhesion molecule [sVCAM], soluble intercellular adhesion molecule [sICAM], and thrombomodulin) [4], and cell markers (plasma cell CD27 and erythrocyte-C4d) [5–7]. However, these biomarkers are totally not reliable for practical application to distinguish between active disease and infection.

C-reactive protein (CRP) is a serological parameter conventionally used to distinguish SLE flare-up from infection. Although patients with SLE relapse have an increased erythrocyte sedimentation rate (ESR), their CRP level does not robustly increase, whereas SLE patients with infection exhibit increase in both ESR and the CRP level. However, the CRP level is not always elevated in SLE patients with infection at initial admission, and it may increase in SLE flare-up patients without infection. Therefore, CRP alone is not a reliable parameter to identify infection in patients with SLE [8]. Other soluble biomarkers that can be used to differentiate infectious disease from exacerbation of SLE include reduced expression of soluble Fc gamma receptor III; elevated levels of granulocyte colony-stimulating factor; and elevated levels of sCD14, sICAM-1, sE-selectin [9, 10], and procalcitonin (PCT) [11]. However, some of these tests are carried out only by some medical centers and turnaround times and accuracy of the results can widely vary. PCT is the precursor of the calcitonin, and it is synthesized in the parafollicular C-cells of the thyroid. Serum PCT level increases in severe bacterial and fungal infections, but it may not increase, or increase only slightly, in viral infections [11, 12]. The presence of elevated levels of PCT raises the suspicion of a concurrent bacterial or mycotic infection in patients with active autoimmune diseases. However, no association has been noted between the activity of SLE and PCT levels [13].

Recently our studies found that reduced levels of erythrocyte CR1 may reflect disease activity in lupus patients by using specific monoclonal antibody CR1-2B11 [14, 15]. From previous study reports, increased erythrocyte-bound C4d (E-C4d) was also a useful marker for lupus disease activity except in condition with haemolytic anemia (HA) and chronic renal failure (CRF) [6, 16]. Theoretically we can combine those two markers as indicator for lupus activity determination. In this study, we aimed to identify useful biomarkers for instantly differentiating between infection and flare-up in febrile SLE patients at initial admission. We sought to examine the clinical applicability of the expressions of complement splitting product C4d and complement receptor 1 (CR1) on erythrocytes as a convenient "real value" for clinical application. Our results indicate that the C4d/CR1 ratio can serve as a predictor of infection in febrile SLE patients, thereby enabling the differentiation between infection and flare-up in febrile SLE patients. Most importantly, in the presence of both infection and disease flare-up in febrile lupus patient, this indicator can help determine the appropriate therapy strategy.

2. Materials and Methods

2.1. Study Participants. All study participants were ≥18 years and provided written informed consent. None of the patients was excluded from participation on the basis of sex or ethnicity. The participants included febrile SLE patients, febrile patients without SLE, and healthy controls. The criteria for the classification of the patients are provided below. The study protocol was approved by the Tri-Service General Hospital Institutional Review Board.

2.2. Fever Definition. Fever, in this study, was defined as an ear temperature of over 37.8°C measured on the first hospital day, by using an electronic thermometer.

2.3. SLE Patients. Blood samples were collected on the first day of admission, from 47 febrile SLE patients who met the 1982 ACR revised criteria for the classification of definite SLE. This group of patients included 40 women and 7 men, with ages ranging from 18 to 89 y (mean age: 42.28 ± 3.4 y). Disease activity was evaluated in each of these patients in terms of the SLE disease activity index (SLEDAI) score. The E-C4d and E-CR1 levels were measured by flow cytometry. Serum level of anti-dsDNA was determined by enzyme-linked fluorescent immunoassay (Phadia ImmunoCAP System, Phadia GmbH, Freiburg, Germany). The sera C3, C4, and CRP levels were quantitated by means of immunonephelometry on the Behring nephelometer systems (BN II). The patients also underwent laboratory tests to measure the following parameters: erythrocyte sedimentation rate (ESR); white blood cell count; haemoglobin level; platelet count; and serum levels of blood urea nitrogen, creatinine, anti-dsDNA, anticardiolipin IgG/IgM, anti-Sm, anti-Ro, anti-La, anti-RNP, and rheumatoid factor. SLE flare-up was defined as the elevation of over 3 points at admission from the latest preadmission SLEDAI score, without evidence of infection.

2.4. Non-SLE Patients. Twenty febrile patients with diseases other than SLE were recruited. For the reason of E-C4d levels being of limited use in evaluation of disease activity in lupus patients with HA and in lupus patients with CRF, febrile non-SLE patients with HA or CRF were also not enrolled. All of those enrolled patients had fever associated with cellulitis, pulmonary tuberculosis, pneumonia, urinary tract infection, virus infection, and so forth.

2.5. Healthy Controls. Thirty healthy individuals were recruited as controls. These participants were required to complete a brief questionnaire regarding previous or current medical conditions.

2.6. Flow Cytometric Characterization of Erythrocytes. 3 mL blood sample was obtained from each study participant at the time of the study visit. Blood samples were placed in Vacutainer tubes (BD Pharmingen, Franklin Lakes, NJ, USA) containing ethylenediaminetetraacetic acid (EDTA) and then shifted to 3 other study tubes. In the first tube, 5 μL of whole blood was incubated with 50 μL of TS1/22, an IgG1 anti-CD11a antibody; this served as the isotype control. Similarly,

in the secondary tube, 5 μL of whole blood was incubated with 50 μL of mouse anti-human C4d monoclonal antibody (Quidel, San Diego, CA, USA; 1 mg/mL) at a dilution of 1 : 200. Again, 5 μL of whole blood was added to the third tube and incubated with 50 μL of anti-CR1 monoclonal antibody 2B11 (5 μg/mL) at a dilution of 1 : 250. CR1-2B11 was a kind gift from Dr. L. B. Klickstein (Boston, MA) [14]. After incubation for 30 min, the cells were washed twice with 1 mL of diluent buffer and centrifuged at 1500 ×g for 3 min at 4°C. Before flow cytometric characterization of erythrocytes, 1 μL of fluorescein isothiocyanate- (FITC-) conjugated goat anti-mouse immunoglobulin-specific polyclonal antibody (BD Pharmingen; 500 μg/mL) was added to the supernatant for 30 min at 4°C. The cells were then washed twice again, as described previously, and resuspended in 1 mL of phosphate-buffered saline (PBS). Finally, the samples were analysed by flow cytometry by using a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry System, San Jose, CA, USA). Erythrocytes were electronically gated for 30,000 cells on the basis of their forward- and side-scatter properties. Surface expression of E-CR1 and E-C4d on the gated erythrocytes was reported as specific mean fluorescence intensity (sMFI). The ratio of C4d expression to CR1 expression (C4d/CR1) was calculated as follows: (sMFI of C4d − sMFI of isotype control)/(sMFI of CR1 − sMFI of isotype control).

After determining the C4d/CR1 ratio for all patients on the first day of admission, the patients were divided into 3 groups according to their subsequent clinical course and laboratory test results: SLE with infection (n = 22), SLE with flare-up without infection (n = 25), and non-SLE with fever (n = 20).

2.7. Statistical Analysis. SPSS version 15.0 (SPSS, Inc., Chicago, IL) was used for the statistical analyses. Differences in the median values of the participating groups were compared by using the nonparametric Mann-Whitney test. Spearman's rank correlation was applied to detect correlations among the study parameters. A P value less than 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curve, a graphical plot of the sensitivity (true positive rate) and specificity (true negative rate) versus false positive rate (1 – specificity or 1 – true negative rate) and false negative rate, was used in our study to determine the cut-off points for CRP and C4d/CR1 ratio that afforded maximum sensitivity and specificity to distinguish between febrile SLE patients with infection and those without infection.

3. Results

The clinical and laboratory manifestations of febrile SLE patients with and without infection are shown in Table 1. Fourteen SLE patients with infection had flare-up at initial evaluation. However, their C4d/CR1 ratios were not elevated, which indicated the absence of disease flare-up (Table 2, Patients 1, 2, 3, 7, 9, 11, 13, 16, 18, and 20). Among the 8 patients without increased SLEDAI scores, elevated C4d/CR1 ratio (>0.8731) was noted in 2 patients (Table 2, Patients 5 and 6), which indicated that these 2 patients had disease flare-up concurrent with infection.

Table 1: Comparison of clinical and laboratory manifestations of the febrile SLE patients with and without infection.

Manifestation	Febrile SLE patients with infection (n = 22)		Febrile SLE patients without infection (n = 25)	
	n	%	n	%
Convulsion	0	0	0	0
Psychosis	1	5	2	8
Mental organic syndrome	0	0	0	0
Cerebrovascular event	1	5	1	4
Vasculitis	0	0	0	0
Arthritis	2	9	3	12
Myositis	1	5	0	0
Urinary casts	0	0	5	20
Haematuria	8	36	11	44
Proteinuria	6	27	13	52
Pyuria	9	41	12	48
Exanthema	0	0	1	4
Alopecia	0	0	0	0
Oral ulcers	3	14	4	16
Pleuritis	3	14	5	20
Pericarditis	2	9	5	20
Complement decrease	10	46	22	88
DNA increase	6	27	13	52
Thrombocytopenia	3	14	6	24
Leukopenia	1	5	7	28

Febrile SLE patients without infection had a higher C4d/CR1 ratio than those with infection (3.34 ± 2.17 versus 0.80 ± 0.91, $P < 0.001$). The range of the C4d/CR1 ratio in the febrile SLE patients without infection was 0.68–8.80 and that in the febrile SLE patients with infection was 0.03–3.51 (Table 3, Figure 1). Among the SLE patients, 25 (20 women and 5 men; mean age: 35.44 ± 9.24 y) did not have infection and did not receive any antibiotic therapy, while 22 (20 women and 2 men, mean age: 50.05 ± 16.88 years) did show evidence of viral or bacterial infection and received the therapy (Table 3). Representative flow cytometry staining for each group is shown in Figure 2.

SLE patients with flare-up had significantly higher serum anti-dsDNA and E-C4d levels and lower CRP levels than those with infection (Table 3). However, among the patients with SLE, the E-CR1 expression level slightly differs between those with infection and those with flare-up ($P = 0.037$). The C4d/CR1 ratio was the highest in febrile SLE patients without infection ($P < 0.001$). Further, the C4d/CR1 ratio was significantly different between febrile SLE patients with infection and febrile non-SLE patients and between the former and healthy controls ($P < 0.001$) (Figure 1).

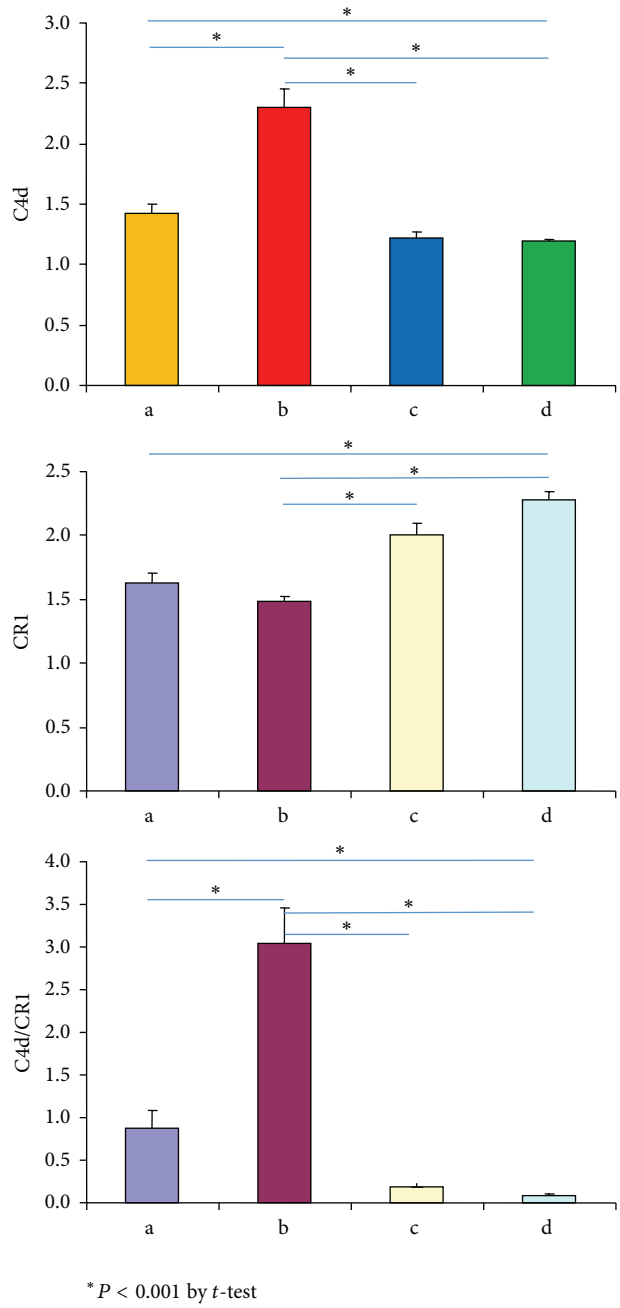

Figure 1: Comparison of the levels of E-C4d and E-CR1 expression and the C4d/CR1 ratio among groups a, b, c, and d. a: febrile SLE patients with infection, b: febrile SLE patients without infection, c: non-SLE febrile patients with infection, and d: healthy controls.

We used receiver operating characteristic (ROC) curve to assess the utility of the assessed parameters in differentiating between febrile SLE patients with infection and those without infection. Sensitivity of 40.91% and specificity of 100.0% were recorded for the presence of infection in febrile SLE patients when the cut-off values of <1.2447 and >4.67 were applied to the C4d/CR1 ratio and serum CRP level, respectively (Table 4, Figure 3); similarly, sensitivity of 80% and specificity of 100% were noted for cut-off values of >1.2447 and <2.2, respectively, for the absence of infection in febrile SLE patients (Table 5, Figure 3).

4. Discussion

C4d, a degradation product of C4, can bind with various cells, including reticulocytes and platelets, in the peripheral circulation, but they bind mostly with erythrocytes. Patients with SLE show increased expression of erythrocyte-bound C4d, which serves as a diagnostic tool and indicator of disease activity in SLE [6, 16–18]. CR1 (CD35)—a membrane receptor for C3b and C4b expressed on erythrocytes, leukocytes, and podocytes [14, 15, 19]—plays an important role in the removal of immune complexes and pathogens coated with C3b and

Table 2: Clinical pathogens and characteristics of patients with SLE and infection.

	Infectious disease	Pathogen	CRP	SLEDAI before admission	SLEDAI on admission	Proposed SLE flares (SLEDAI increased by ≧3)	C4d/CR1 ratio
1	UTI	*Escherichia coli*	10.4	1	5	Yes	0.4761
2		*E. coli*	13.1	2	8	Yes	0.0974
3		*E. coli*	5.16	1	8	Yes	0.0341
4		*Lactobacillus*	0.59	1	14	Yes	1.2286
5		*Candida*	2.56	4	5	No	3.5118
6		*Candida*	5.34	1	3	No	0.92
7		*Candida*	6.34	1	6	Yes	0.5254
8		*Klebsiella pneumoniae*	4.24	4	6	No	0.5143
9	Pneumonia	*Pseudomonas aeruginosa*	4.87	4	10	Yes	0.1613
10		*Streptococcus pneumoniae*	2.65	1	24	Yes	1.8182
11		*Mycoplasma*	0.1	1	5	Yes	0.1111
12		*Mycoplasma*	1.67	2	3	No	0.1
13		H1N1	10.6	6	14	Yes	0.4182
14	Peritonitis	*Klebsiella pneumoniae*	3.37	3	17	Yes	1.0384
15	Infectious diarrhoea	*Shigella sonnei*	7.88	1	1	No	0.054
16		*Shigella sonnei*	3.49	1	5	Yes	0.36
17	Sepsis	*Enterococcus faecalis*	5.76	1	2	No	0.175
18	Cellulitis	*Staphylococcus aureus*	5.24	1	14	Yes	2.1818
19		*Staphylococcus aureus*	0.42	1	1	No	0.25
20	Viral infection	CMV	4.47	2	5	Yes	0.5238
21		CMV	4.18	1	10	Yes	2.3636
22		Herpes simplex virus	1.83	1	2	No	0.7586

Table 3: Clinical characteristics of SLE patients with and without infection.

Variable	Infection ($n = 22$)	Noninfection ($n = 25$)	P value*
	Mean ± standard deviation		
Male (n, %)	2, 9.10%	5, 20.0%	0.423**
Age (y)	50.05 ± 16.88	35.44 ± 9.24	0.001
SLEDAI	7.41 ± 6.02	10.48 ± 5.67	0.079
C4d	1.40 ± 0.29	2.30 ± 0.66	<0.001
CR1	1.63 ± 0.31	1.48 ± 0.15	0.037
C4d/CR1	0.80 ± 0.91	3.34 ± 2.17	<0.001
CRP	4.71 ± 3.40	0.85 ± 1.16	<0.001
Anti-dsDNA	116.46 ± 155.21	182.77 ± 173.74	0.177
C3	81.98 ± 31.98	56.86 ± 29.40	0.007
C4	18.29 ± 9.93	13.00 ± 6.15	0.031

* P value by t-test, ** P value by Fisher's exact test.

C4b [20]. An abnormally low erythrocyte CR1 level is considered characteristic of SLE [15]. Although erythrocyte-bound C4d is a useful biomarker to predict and monitor SLE disease activity, the detected levels of C4d expression vary across laboratories because of the differences in the fluorescence-conjugated antibodies used; this reduces the utility of this marker in clinical settings. In this study, we combined those two markers which indicated the concomitant C4d deposition and CR1 consumption on erythrocyte to obtain a "ratio," and we sought to evaluate the usefulness of this ratio as a single indicator for differentiating between infection and flare-up in febrile SLE patients. The usefulness of this ratio is not influenced by the variation in the fluorescence-conjugated antibodies used by different laboratories.

Fever is usually caused by exogenic pyrogens; most often, they are infected by bacteria and their endotoxins, viruses, yeasts, spirochetes, and protozoa. Infection is a common problem and has become one of leading causes of mortality in SLE patients and fever is a common manifestation of SLE infection or flare-up. Therefore, differential diagnosis of several SLE flare-up syndromes from infection-related conditions is important [21]. We noted a significant difference of the C4d/CR1 ratios between groups: febrile SLE patients without infection had significantly higher C4d/CR1 ratios than those with infection at initial admission ($P < 0.001$, Table 3). Therefore, the C4d/CR1 ratio can serve as a useful marker to differentiate between fever caused by infection and that caused by flare-up in SLE patients.

Table 4: Receiver operating characteristic (ROC) curve analysis of the utility of the C4d/CR1 ratio and serum CRP level in febrile SLE patients with infection.

Rules			Number(s)	Sensitivity (%)	Specificity (%)
A	C4d/CR1 > 1.2447	CRP > 4.67	1	4.55	100.0
		CRP < 4.67	3	13.64	12.5
	C4d/CR1 < 1.2447	CRP > 4.67	9	40.91	100.0
		CRP < 4.67	9	40.91	69.2
Total			22		
B	C4d/CR1 > 1.2447	CRP > 2.2	4	18.18	80.0
		CRP < 2.2	0	0	0
	C4d/CR1 < 1.2447	CRP > 2.2	13	50.09	86.7
		CRP < 2.2	5	22.73	71.4
Total			22		

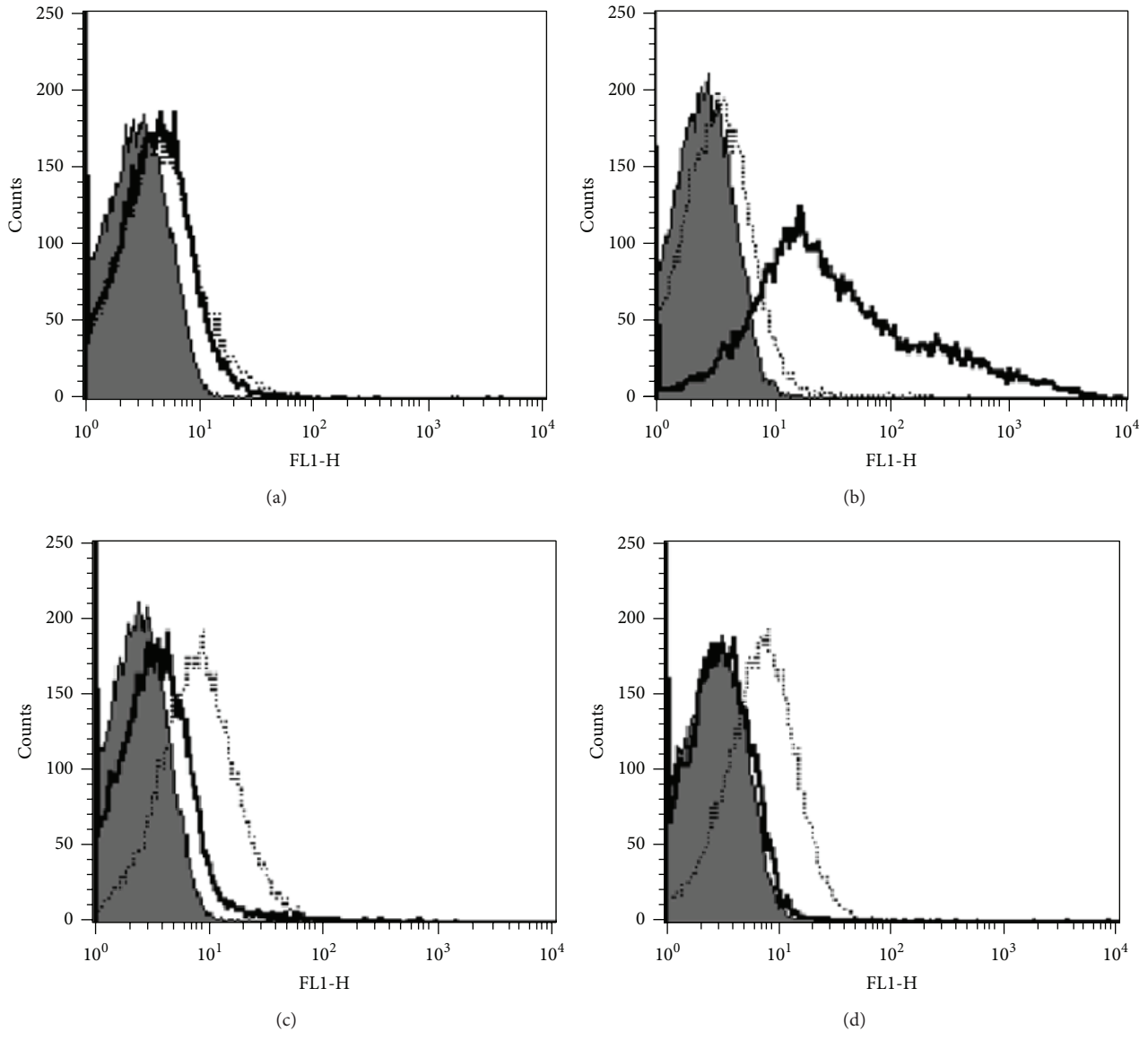

Figure 2: Flow cytometric analysis of E-C4d and E-CR1 expression in (a) febrile SLE patients with infection, (b) febrile SLE patients without infection, (c) non-SLE febrile patients with infection, and (d) healthy controls. Erythrocytes were stained with anti-C4d (black lines, open histogram), CR1-2B11 (dashed lines, open histogram), and isotype-matched control antibodies (solid gray histogram).

Table 5: Receiver operating characteristic (ROC) curve analysis of the utility of the C4d/CR1 ratio in febrile SLE patients without infection.

Rules			Number(s)	Sensitivity (%)	Specificity (%)
A	C4d/CR1 > 1.2447	CRP > 4.67	0	0	0
		CRP < 4.67	21	84	87.5
	C4d/CR1 < 1.2447	CRP > 4.67	0	0	0
		CRP < 4.67	4	16	30.8
	Total		25		
B	C4d/CR1 > 1.2447	CRP > 2.2	1	4	20.0
		CRP < 2.2	20	80	100.0
	C4d/CR1 < 1.2447	CRP > 2.2	2	8	13.3
		CRP < 2.2	2	8	28.6
	Total		25		

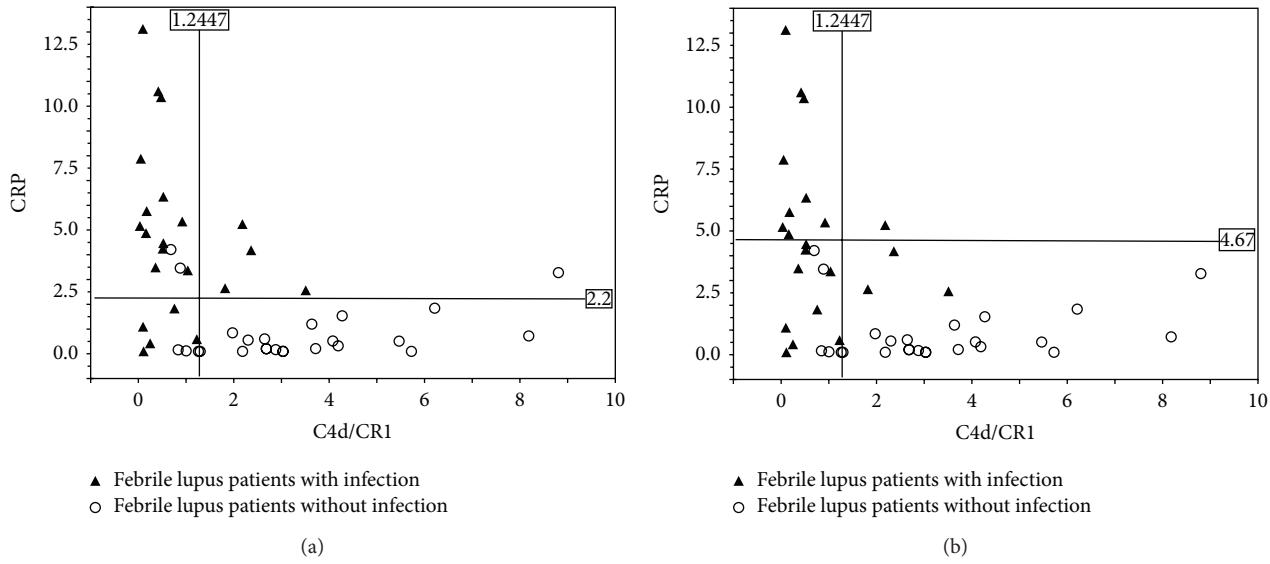

Figure 3: Detection of C4d to CR1 ratio and CRP on erythrocytes (E) from febrile SLE patients with infection and febrile SLE patients without infection. Cut-off points determined by receiver operating characteristic (ROC) curve are indicated by solid lines.

The pathogenesis of SLE involves a whole range of factors, including genetic and environmental factors [22]. Infections may play a pivotal role in the expression of the disease in genetically susceptible individuals and can serve as environmental triggers that induce or promote the development of SLE in such individuals [23]. In SLE patients, infection may trigger disease flare-up, and, sometimes, disease flare-up may be confused with infection. A broad spectrum of infections has been reported in SLE patients; these include bacterial, mycobacterial, viral, fungal, and parasitic infections, with the respiratory and urinary tracts being the most commonly involved sites [24]. Among the infections, urinary tract infection (UTI) has been reported as the most common primary or secondary infection in SLE patients, followed by respiratory tract infection. *Escherichia coli* is the most frequent organism identified in culture studies of the tissue samples of SLE patients. The clinical manifestations of UTI are variable, ranging from asymptomatic UTI to urosepsis [25]. In our study, 22 patients with SLE had infection, including urinary tract infection (UTI, $n = 8$), respiratory tract infection ($n = 5$), cutaneous and soft tissue infection ($n = 2$), gastrointestinal tract infection ($n = 2$), peritonitis ($n = 1$), sepsis ($n = 1$), and viral infection ($n = 3$).

The causative pathogens identified in our SLE patients included the following: *Escherichia coli*, *Lactobacillus* spp., *Candida* spp., *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Mycoplasma* spp., *Klebsiella pneumoniae*, *Shigella sonnei*, *Enterococcus faecalis*, *Staphylococcus aureus*, cytomegalovirus (CMV), and herpes simplex virus. In our study, the most frequently isolated pathogens were *Escherichia coli* and *Candida*. *Salmonella* spp. were also common pathogens identified. SLE patients with *Salmonella* infection are at high risk of mortality [26]. CMV infection has been associated with the exacerbation of SLE [27], and the mechanisms by which CMV may trigger autoimmunity have been proposed [28]. Of the 2 patients in our study who were infected by CMV, one had high C4d/CR1 ratio; the possible reason for this may be cooccurrence of CMV infection and SLE flare-up, which suggests that CMV infection can act as a potential trigger for SLE flare-up.

In clinical settings, it is difficult to ascertain whether fever in SLE patients is caused by infection combined with a flare-up. In this study, we used the SLEDAI as a tool to evaluate disease flare-up. The SLEDAI was developed in Canada and covers 24 items, including 16 clinical characteristics and 8 items based solely on laboratory results (urinary casts, haematuria, proteinuria, pyuria, hypocomplementemia, increased DNA binding, thrombocytopenia, and leukopenia). Unfortunately, this index focuses on new or recurrent manifestations and fails to account for clinically important manifestations of ongoing disease activity, such as haemolytic anaemia [29]. In our study, some febrile SLE patients had definitive evidence of infection and also elevated C4d/CR1 ratio, indicating the presence of flare-up with infection. However, their SLEDAI scores at initial assessment did not indicate SLE flare-up. In contrast, high SLEDAI scores were obtained in patients with low C4d/CR1 ratios in patients with definitive infection. According to the clinical presentation and posttreatment outcomes, the C4d/CR1 ratio appears to be more accurate than the SLEDAI score in evaluating disease activity in febrile SLE patients.

Thus, when SLE patients exhibit elevations of both serum CRP level and C4d/CR1 ratio on admission, the cooccurrence of infection and disease flare-up may be suspected. When serum CRP level increases in SLE patients without elevation of the C4d/CR1 ratio, it is likely that the patients have only infections and not flare-up. On the contrary, when only C4d/CR1 ratio is elevated in febrile SLE patients, the cause of fever is mostly SLE flare-up. We found that cut-off values of <1.2447 and >4.67 for C4d/CR1 and serum CRP level, respectively, were sufficient to distinguish febrile SLE patients with infection (40.91% sensitive and 100.0% specific) (Table 4) from febrile SLE patients without infection. Further, the cut-off values of >1.2447 and <2.2 for C4d/CR1 and serum CRP level, respectively, were 80% sensitive and 100% specific for the absence of infection in febrile SLE patients (Table 5).

There are some limitations to this study. In SLE patients with HA, the C4d/CR1 ratio may be higher than expected, which may lead to overestimation of disease activity; in these patients, the C4d/CR1 ratio is too high to differentiate between flares-up and infections. Oppositely, lower C4d/CR1 may be observed and lead to underestimation in patients with CRF. Thus, this novel biomarker may not be suitable to monitor disease activity in SLE patients with HA or CRF [6]. The mechanism underlying the increased levels of active complement fragment in SLE patients with HA remains unclear.

In conclusion, the C4d/CR1 ratio is a simple and quickly determinable biomarker to differentiate between infection and flare-up in febrile patients with SLE. Further, it is a useful marker for follow-up assessment of febrile SLE patients with infections who manifest disease flare-up later in the clinical course. Furthermore, regular monitoring of this ratio in SLE patients can facilitate the assessment of disease activity and recognize infection, in case it occurs subsequently.

Conflict of Interests

No potential conflict of interests relevant to this paper was reported.

Acknowledgments

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Research Article

Regulation of Cell Cycle Regulators by SIRT1 Contributes to Resveratrol-Mediated Prevention of Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a major cause of morbidity and mortality in rheumatic diseases. Vascular remodeling due to the proliferation of pulmonary arterial smooth muscle cells (PASMCs) is central to the development of PAH. To date, it is still unclear if Silence Information Regulator 1 (SIRT1) regulates cell cycle regulators in the proliferation of PASMCs and contributes to prevention of PAH by resveratrol. In this study, we found that a significant decrease of SIRT1 expression levels in platelet-derived growth factor BB (PDGF-BB) treated human PASMCs (HPASMCs) and in monocrotaline (MCT) induced PAH rat. Overexpression of SIRT1 induced G1 phase arrest and increased p21 expression but decreased cyclin D1 expression in PDGF-BB treated HPASMCs. Moreover, resveratrol attenuated pulmonary arterial remodeling, decreased pulmonary arterial pressure, and upregulated SIRT1 and p21 expression but downregulated cyclin D1 expression in MCT induced PAH rat. Notably, knockdown of SIRT1 eliminated the regulation of resveratrol on p21 and cyclin D1 expression in PDGF-BB treated HPASMCs. These results demonstrated that SIRT1 mediated the regulation of resveratrol on the expression of cell cycle regulatory molecules. It suggests that SIRT1 exerts a protective role in PAH associated with rheumatic diseases and can be a potential treatment target.

1. Introduction

Pulmonary arterial hypertension (PAH) is a progressive disease characterized by an increase in pulmonary vascular resistance leading to right ventricular (RV) failure and death [1]. PAH can occur in a variety of other conditions and circumstances including a number of rheumatic diseases. In this regard, systemic sclerosis (SSc), mixed connective tissue disease (MCTD), and systemic lupus erythematodes (SLE) are associated with PAH [2]. In community-based rheumatology practices in the US, the prevalence of PAH was 13.3% in patients with SSc and MCTD as analysed by echocardiography [3]. PAH is a major cause of morbidity and mortality in connective tissue diseases. While 3-year survival rates after diagnosis in idiopathic PAH (IPAH) are as low as 48%, these alarming numbers are even worse in PAH associated with SSc [2]. This survival rate highlights the need for early diagnosis and treatment of PAH associated with rheumatic diseases [2].

Despite its heterogeneous origin, it is generally accepted that the pathogenesis of PAH involves three major processes including vasoconstriction, vascular remodeling characterized by enhanced proliferation of pulmonary arterial smooth muscle cells (PASMCs) and endothelial cells and coagulation abnormalities [4]. The traditional methods such as treatment with calcium-channel blockers and anticoagulants have limited function, while current therapies such as endothelin

receptor antagonists [5, 6], phosphodiesterase 5 inhibitors [7–10], and prostacyclin analogs [11–13] are developed mainly as vasodilators. Although these therapies appear to somewhat improve the quality of life of PAH patients, the prognosis remains poor [14]. Novel treatments are required to prevent progression of pulmonary hypertension by interfering with the pathomechanisms of the disease at multiple levels [15]. For example, in a preclinical setting, experimental therapeutics that exert antimitogenic effects on proliferation of PASMCs [16–18], addition to promoting vasodilation, show promise in enhancing overall prognosis.

Resveratrol (3,5,4-trihydroxystilbene) is a dietary polyphenolic compound that exerts significant antioxidant, anti-inflammatory, and endothelial protective effects in the systemic circulation [19–22]. Resveratrol prevents MCT induced pulmonary hypertension in rats [15]. Resveratrol, a Silence Information Regulator 1 (SIRT1) activator, induces apoptosis MH7A human rheumatoid arthritis synovial cells in a SIRT1-dependent manner [23]. SIRT1, a NAD-dependent histone deacetylase, has been implicated in aging, metabolism, and tolerance to oxidative stress via its ability to deacetylate a variety of substrates, including histones, transcription factors, and coregulators [24]. SIRT1 attracts lots of interest in its cardiovascular protective role, which serves as a key regulator in vascular endothelial homeostasis by controlling angiogenesis, vascular tone, and endothelial dysfunction as well as by decreasing atherosclerosis [25–28]. Our previous study has shown that SIRT1 overexpression markedly inhibited vascular smooth muscle cell (VSMC) proliferation and migration and induced cell cycle arrest at G1/S transition *in vitro* [29]. Whether SIRT1 mediates the protective role of resveratrol on PAH and the mechanism that SIRT1 inhibits HPASMCs proliferation is still unknown. We therefore hypothesize that SIRT1 may inhibit HPASMCs proliferation through affecting the cell cycle regulator and contribute to prevention of PAH by resveratrol.

In this study, we found that SIRT1 regulated the expression of cell cycle regulatory molecules and arrested PDGF-BB-treated HPASMCs in G0/G1 phase. Decreased SIRT1 expression was found in an experimental rat PAH model induced by MCT. Resveratrol attenuated MCT induced rat pulmonary arterial remodeling and decreased pulmonary arterial pressure. Meanwhile, resveratrol increased SIRT1 and p21 expressions but decreased cyclin D1 expression in PAH rat model. Furthermore, resveratrol regulated the expression of cell cycle regulatory molecules in HPASMCs in a SIRT1-dependent manner.

2. Materials and Methods

2.1. Drugs. Recombinant human PDGF-BB was purchased from R&D systems. Sterile 4 mmol/L HCL containing 0.1% BSA was added to the vial to prepare a stock solution of 100 μg/mL of PDGF-BB. Resveratrol was purchased from Sigma.

2.2. Animals. All of the animal protocols were approved by the Animal Care and Use Committee at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, 8th Edition, 2011). Adult male Sprague-Dawley rats (280–300 g in body weight) were randomly assigned to the following groups ($n = 20$ animals in each group): (1) control animals (receiving a s.c. injection of saline) receiving vehicle treatment (saline, given orally) for 14 or 21 days; (2) MCT (receiving a s.c. injection of MCT with a dose of 60 mg·kg^{-1}) challenged animals receiving vehicle treatment (saline, given orally) for 14 or 21 days; (3) MCT challenged animals receiving resveratrol (2.5 mg·kg^{-1}d^{-1}, given orally) from day 1 to day 14 or day 21 after MCT injection; (4) MCT challenged animals receiving resveratrol (20 mg·kg^{-1}d^{-1}, given orally) from day 1 to day 14 or day 21 after MCT injection.

2.3. Assessment of Hemodynamics and Right Ventricle (RV) Hypertrophy. The animals were anesthetized with ketamine (60 mg·kg^{-1} IM) and xylazine (3 mg·kg^{-1} IM). Efficiency of anaesthesia was monitored by lack of withdrawal reflex upon hind toe pinching, and no reaction to skin pinch over the area to be incised. A right heart catheter was inserted into the right jugular vein then pushed through the right ventricle (RV) into the pulmonary artery for measurement of mean pulmonary arterial pressure (mPAP) and RV systolic pressure (RVSP) [30]. Another polyethylene catheter was inserted into the left carotid artery to measure the systemic arterial pressure (SAP). The heart was dissected and weighed. The right ventricular hypertrophy index (RVHI) was calculated as the ratio of RV free wall weight divided by the sum of the free left ventricle (LV) wall and ventricular septum [17].

2.4. Immunohistology. After measurement of hemodynamics, rats were perfused with phosphate-buffered saline and the thorax was opened and the left lung immediately removed and fixed in the distended state with formalin buffer. The media thickness was measured in Verhoeff Van Gieson (EVG) sections (5 μm thick). EVG staining was performed using a EVG staining kit (Baso) according to the manufacturer's instructions. 5-6 rats were used in each group and in each rat, 10 microscopic fields were chosen at random. The media thickness was expressed as the following formula: index% = (external diameter − internal diameter)/external diameter. The internal and external diameter were calculated according to the lamina elastic internal and the lamina elastic external indicated by EVG staining in a blinded manner by a single observer using Image Pro Plus 6.0 software (Media Cybernetics) [31]. According to the external diameter, arterials were categorized to three groups as diameter between 25–50 μm, 51–100 μm, and 101–500 μm. The muscularization degrees of pulmonary arteries were measured in α-smooth muscle actin (α-SMA) staining sections. For the analysis of pulmonary arteries muscularization degrees, slides were deparaffinized, and endogenous peroxidase activity was quenched with 3% hydrogen peroxide in 1 × PBS for 10 minutes. Nonspecific binding sites were blocked in 10% goat serum in PBS at room temperature for 1 hour. Slides were incubated at 4°C overnight

with anti-α-smooth muscle actin rabbit polyclonal antibody (Abcam) and then with biotinylated secondary antibody at 37°C for 30 minutes and subsequently with horseradish peroxidase-labeled streptavidin solution for 20 minutes at 37°C. Slides were then stained with diaminobenzidine and counterstained with hematoxylin. In each rat, 10 microscopic fields were chosen at random and a total of about 80 intraacinar arteries were categorized as muscular (i.e., with a complete medial coat of muscle), partially muscular (i.e., with only a crescent of muscle), or nonmuscular (i.e., no apparent muscle), as previously reported [17].

2.5. PASMCs Apoptosis Assessment. Apoptosis of rat PASMCs were evaluated with the TdT-mediated dUTP nick and labeling (TUNEL) assay. The TUNEL assay was performed using a DeadEnd Fluorometric TUNEL System (Promega) according to the manufacturer's instructions. Briefly, 5 μm-thick tissue sections were obtained from the samples. The slides were washed with 1 mL of the wash buffer provided in the kit and labeled with the DNA labeling solution at 37°C for 60 minutes. After the slides were rinsed and incubated with equilibration buffer, nucleotide mix, and rTdT enzyme at 37°C for 1 hour in the dark, they were counterstained with Hoechst and then examined using confocal fluorescence microscopy. TUNEL positive cells were identified by a green fluorescence.

2.6. Flow Cytometry Analysis. Adenovirus-infected HPASMCs were maintained in serum-free M231 medium for 24 hours and then stimulated with 10 ng/mL PDGF-BB for different periods. The cells were trypsinized, fixed in 70% ethanol at 4°C overnight, washed twice with ice-cold PBS, and incubated with RNase and propidium iodide. The cell-cycle phase was analyzed by flow cytometry using a Becton Dickinson FACStar flow cytometer and the Becton Dickinson CellFIT software.

2.7. Cell Culture, Adenovirus Generation, and Infection. HPASMCs (Invitrogen) were cultured in M231 medium (Invitrogen) with smooth muscle growth supplement. The replication-defective adenoviral vectors expressing SIRT1 (Ad-SIRT1) or control green fluorescent protein (Ad-GFP) and adenovirus-mediated knockdown of SIRT1 (Ad-SIRT1 RNAi) or control vector (Ad-U6) were generated as described previously [26, 32]. HPASMCs were infected for 2 hours with the above adenovirus using a multiplicity of infection (MOI) of 100, washed, and incubated in serum-free medium without virus for at least 24 hours before drugs challenge.

2.8. Reverse Transcription and Real-Time PCR. Total RNA was extracted from cells or lungs of rats using Trizol (Invitrogen) according to the manufacturer's instructions. Two micrograms of total RNA were used to synthesize first-strand cDNA with M-MuLV reverse transcriptase (New England BioLabs) using random primers. Real-time PCR was performed using the BioRad iCycler iQ5 Real-Time PCR Detection System with the Quantitect SYBR Green One-Step RT-PCR Kit (QIAGEN). Fluorescence curves were analyzed with iCycler iQ5 Optical System Software (Version 2.0).

2.9. Western Blotting. Cellular and rat tissue proteins were extracted using RIPA buffer (25 mM Tris–HCl pH 7.6, 150 mmol/L NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS). After complete homogenization on an ice rotator, samples were sonicated and centrifuged at 4°C. The supernatants were transferred into fresh tubes and protein concentrations were determined by the BCA method. Equal amounts of protein (20 μg/lane) were separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Millipore). After being blocked, the filters were incubated with the following primary antibodies: anti-SIRT1 (Santa Cruz Biotechnology), anti-p21 (Santa Cruz Biotechnology), anti-cyclin D1 (Santa Cruz Biotechnology), anticyclin E (Santa Cruz Biotechnology), anti-GAPDH (Santa Cruz Biotechnology), anti-CDK2 (Santa Cruz Biotechnology), and anti-CDK4 (Santa Cruz Biotechnology). After being washed and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology), the immune complexes were visualized with a chemiluminescence reagent. Western blots were quantified densitometrically with Quantity One software (Bio-Rad), and the intensity values were normalized to GAPDH.

2.10. Statistics. Data are expressed as means ± SEM. Statistical analyses were performed by a two-tailed unpaired student's t-test or a one-way ANOVA as appropriate to determine statistical significance between the groups. A P value less than 0.05 was considered significant.

3. Results

3.1. PDGF-BB Affects Cell Cycle Regulatory Molecules and SIRT1 Expression. Vascular remodeling is a critical step of PAH progression and is characterized by proliferation of PASMCs. The development of vascular remodeling requires cells arrested in the G0/G1 phase to enter the cell cycle [33]. p21, cyclin D1, and cyclin E are key regulators in the switch of G0/G1 phase to S phase and in VSMC proliferation [33–35]. PDGF-BB, a potent mitogen involving in proliferation and migration of PASMCs, has been proposed as a key mediator in the progression of PAH [17]. PDGF-BB (10 ng·mL^{-1}) treatment decreased expression of p21 but increased cyclin D1 and cyclin E expression in HPASMCs (Figure 1(a)), while the expression of CDK2 and CDK4 made no remarkable change (see Figure S1 in Supplementary Material available online at http://dx.doi.org/10.1155/2015/762349). We observed that PDGF-BB treatments significantly decreased SIRT1 mRNA (Figure 1(b)) and protein (Figure 1(c)) expression in HPASMCs.

3.2. SIRT1 Regulates Expression of Cell Cycle Regulatory Molecules and Arrests PDGF-BB-Treated HPASMCs in G0/G1 Phase. SIRT1 overexpression significantly increased p21 expression, however, decreased cyclin D1 and cyclin E expression in PDGF-BB treated HPAMSCs. (Figure 2(a)). Overexpression of SIRT1 decreased CDK2 expression in PDGF-BB treated HPAMSCs but had no effect on CDK4 expression (Figure S2A). Knockdown of SIRT1 showed a marked

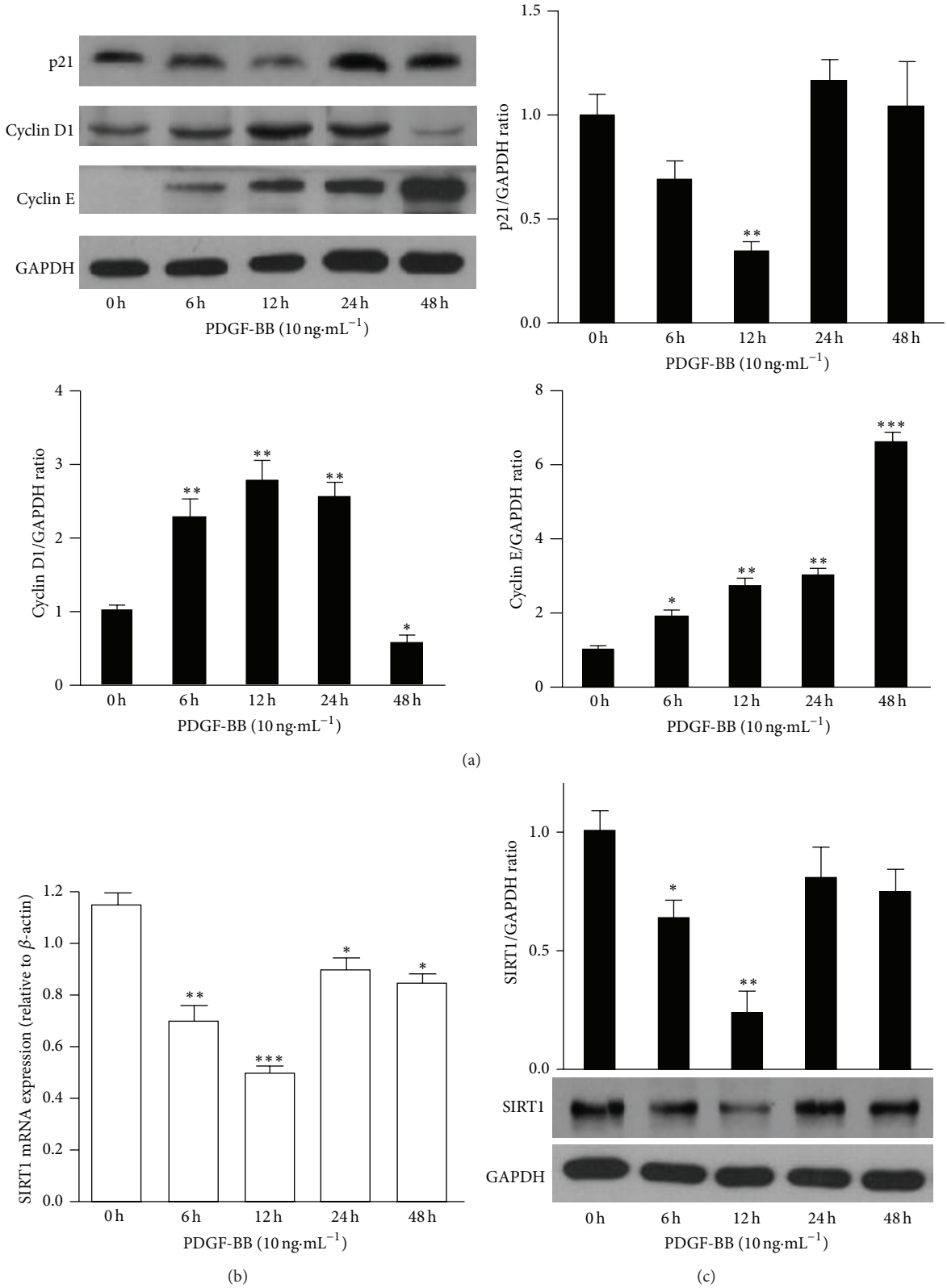
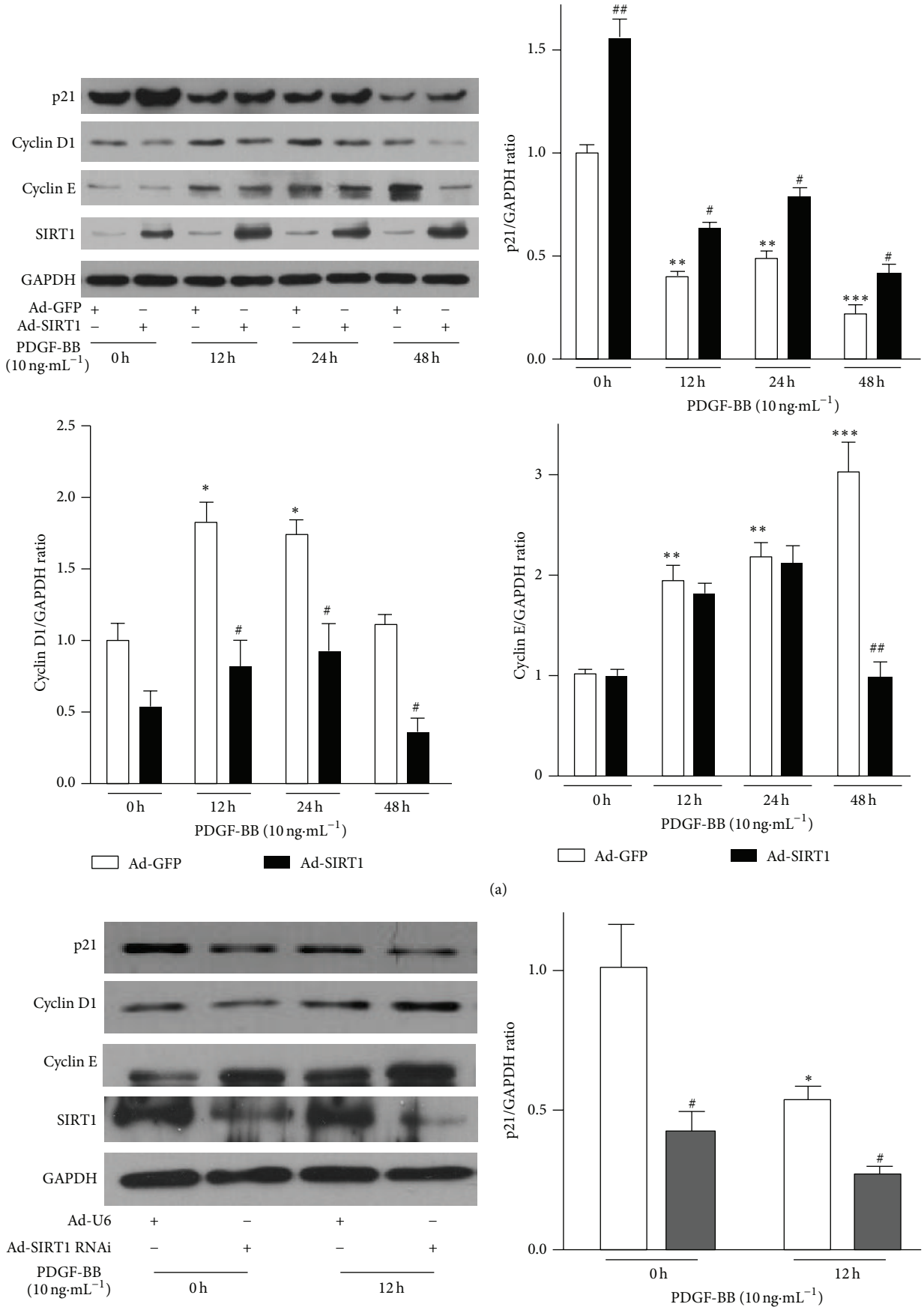


Figure 1: PDGF-BB affects cell cycle regulatory molecules and SIRT1 expressions. After 24-hour serum starvation, HPASMCs were treated with 10 ng·mL^{-1} PDGF-BB and harvested at the indicated time points. p21, cyclin D1, cyclin E (a), and SIRT1 (c) protein levels were analyzed by western blotting. Bar graphs show densitometric analysis of western blotting. The densitometric quantification was normalized to GAPDH. Data are shown as means ± SEM for three independent experiments. SIRT1 mRNA level was analyzed by real-time PCR (b) ($n = 3$). mRNA level was normalized to the internal control β-actin. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus 0 hour.



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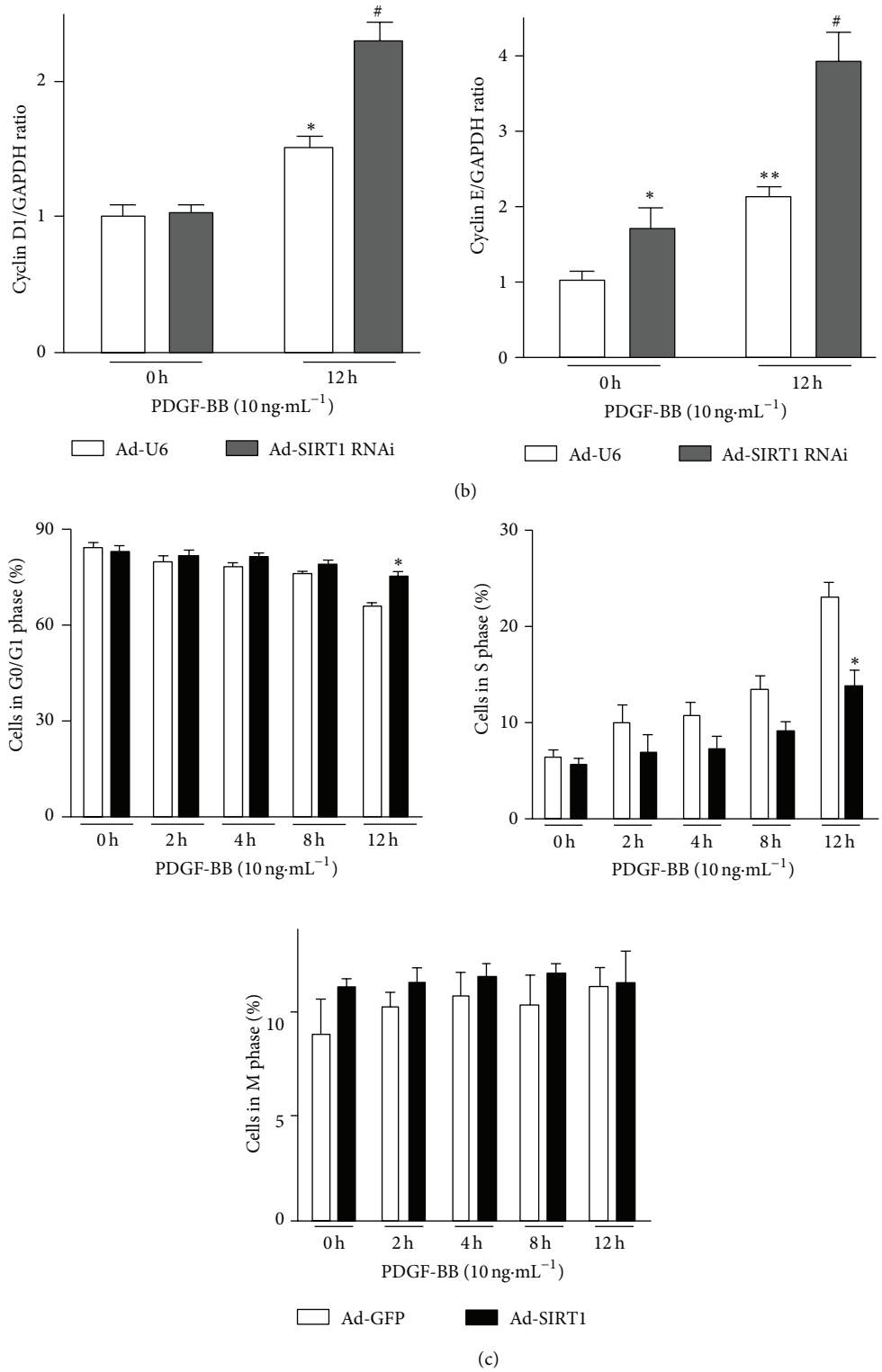


Figure 2: SIRT1 regulates expression of cell cycle regulatory molecules and arrests PDGF-BB-treated HPASMCs in G0/G1 phase. HPASMCs infected with adenoviral vectors encoding SIRT1 overexpression (a) or knockdown (b) were treated with PDGF-BB for indicated period. p21, cyclin D1, and cyclin E protein levels were analyzed by western blotting. Bar graphs show densitometric analysis of western blotting. The densitometric quantification was normalized to GAPDH. Data are shown as means ± SEM for three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus 0 hour Ad-GFP group (a) or 0 hour Ad-U6 group (b); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus corresponding Ad-GFP group (a) or Ad-U6 group (b) at the indicated time points. (c) Flow cytometry analysis of HPASMCs after SIRT1 overexpression and treatment with PDGF-BB ($n = 3$). $^{*}P < 0.05$ versus Ad-GFP at the indicated time points.

decrease in p21 expression and an increase in cyclin D1, cyclin E (Figure 2(b)), and CDK2 expression levels (Figure S2B) in PDGF-BB treated HPAMSCs. However, CDK4 expression remained unchanged (Figure S2B). Flow cytometry analysis showed significant G0/G1 accumulation of HPASMCs with SIRT1 overexpression 12 hours after PDGF-BB treatment (Figure 2(c)). These results indicated that SIRT1 reversed the regulation of PDGF-BB on cell cycle regulators, which likely leads to the G0/G1 accumulation of HPASMCs.

3.3. Resveratrol Plays an Antiremodeling Effect in Rat Pulmonary Arteries. To elucidate the role of SIRT1 in pulmonary arterial remodeling, we constructed MCT induced PAH rat model. Real-time PCR and western blotting showed that the mRNA and protein levels of SIRT1 in the lungs of PAH rats declined dramatically 14 and 21 days after MCT treatment (Figures S3A, S3B). We chose resveratrol, the SIRT1 activator, to intervene in the PAH rat model and observed the role of SIRT1 in pulmonary arterial remodeling. All the rats treated with MCT developed PAH within 14 days. Consequently, RVSP and mPAP levels increased significantly compared with the saline-treated group. Resveratrol significantly prevented mPAP from increasing in both the $2.5\,mg{\cdot}kg^{-1}d^{-1}$ group and $20\,mg{\cdot}kg^{-1}d^{-1}$ groups at both 14 and 21 days (Figure 3(a)). SAP was comparable in each group (Figure S3C). As a consequence of high pulmonary arterial pressure, in the MCT-treated group, the RVHI increased 14 and 21 days after MCT challenge. Addition of resveratrol decreased RVHI in a dose-dependent manner (Figure 3(a)). To examine whether a reduction in remodeling of pulmonary arteries contributed to the amelioration of PAH rats, the degree of muscularization and the medial wall thickness of pulmonary arteries were assessed by α-SMA and EVG staining, respectively. The muscularization degree of pulmonary arteries with a diameter between 25 and 50 μm was calculated. On day 21, with respect to controls, the percentages of both partially muscular and fully muscular pulmonary arteries increased significantly, but that of nonmuscular pulmonary arteries decreased significantly in the MCT-treated group. Resveratrol-treated groups had a markedly lower percentage of fully muscular pulmonary arteries but a higher percentage of partially muscular pulmonary arteries compared with MCT-treated vehicle treatment group (Figure 3(b) and Figure S3D). This result suggested that resveratrol attenuated the muscularization of intra-acinar arteries. Two different doses of resveratrol had comparable effects on muscularization of intra-acinar arteries (data not shown). Medial wall thickness of pulmonary arteries of diameter 25–50 μm, 51–100 μm, and 101–500 μm was significantly increased in the MCT-treated vehicle treatment groups, both on day 14 and on day 21. Both $2.5\,mg{\cdot}kg^{-1}d^{-1}$ and $20\,mg{\cdot}kg^{-1}d^{-1}$ resveratrol attenuated the increase of medial wall thickness (Figures 3(c)-3(d) and Figure S3E). Treatment with resveratrol at $2.5\,mg{\cdot}kg^{-1}d^{-1}$ had the comparable antithickening effect as resveratrol at $20\,mg{\cdot}kg^{-1}d^{-1}$ in pulmonary arteries sized 25–50 μm and 51–100 μm. However, in pulmonary arteries sized 101–500 μm, resveratrol at $20\,mg{\cdot}kg^{-1}d^{-1}$ had a more powerful antithickening effect than resveratrol at $2.5\,mg{\cdot}kg^{-1}d^{-1}$ (data not shown). No apoptosis of PASMCs was observed in any group (Figure S3F).

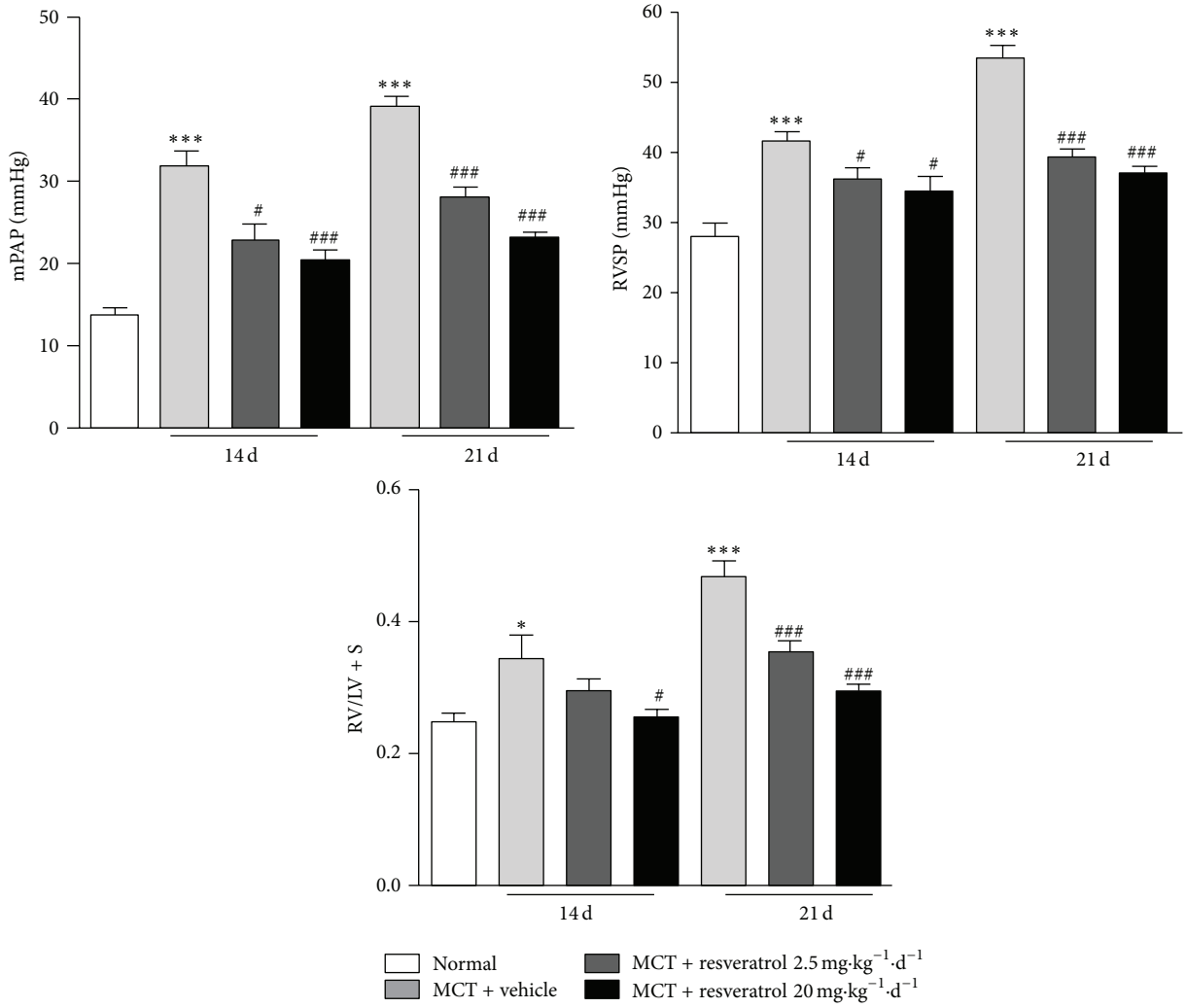
3.4. Resveratrol Increases SIRT1 and p21 Expression but Decreases Cyclin D1 Expression in Lungs of MCT-Induced PAH Rats. To investigate the mechanism of resveratrol on antagonizing pulmonary arterial remodeling, the expressions of SIRT1, p21, cyclin D1, and cyclin E were detected in lungs of MCT-induced PAH rats. On both day 14 and day 21, resveratrol treatment at doses of both $2.5\,mg{\cdot}kg^{-1}d^{-1}$ and $20\,mg{\cdot}kg^{-1}d^{-1}$ markedly increased SIRT1 expression as compared with vehicle treatment group. p21 expression decreased significantly compared with untreated controls; however, resveratrol increased its expression markedly. Cyclin D1 expression increased significantly compared with untreated controls (Figure 4); however, resveratrol decreased its expression dramatically. No significant change in cyclin E expression was observed upon MCT challenging and resveratrol treatment did not influence cyclin E expression either (Figure S4).

3.5. Resveratrol Regulates the Expression of Cell Cycle Regulatory Molecules through SIRT1. We found that resveratrol increased SIRT1 and p21 expression but decreased cyclin D1 and cyclin E expression in a concentration-dependent manner in HPASMCs (Figure 5(a)). The activity of SIRT1 correlates positively with its level of expression [36–38]. To further investigate whether SIRT1 was required for the regulatory effect of resveratrol onthe expression of cell cycle regulatory molecules, SIRT1 was knocked down by RNA interference (RNAi) before resveratrol treatment. We found that resveratrol increased p21 expression but decreased cyclin D1 and cyclin E expressions after PDGF-BB stimulation. However, SIRT1 knockdown eliminated the effects of resveratrol on p21, cyclin D1 and cyclin E expressions (Figure 5(b)). These results suggested that resveratrol regulated p21, cyclin D1, and cyclin E expressions through SIRT1 in PDGF-BB treated HPASMCs.

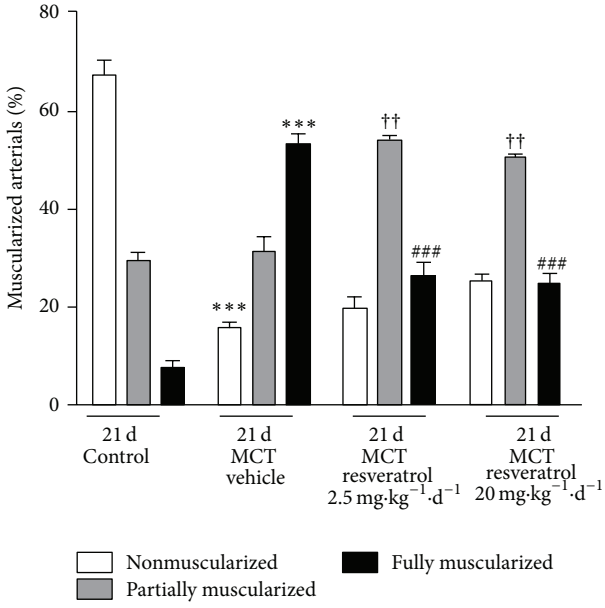
4. Discussion

In the present study, we observed the critical role of SIRT1 in the prevention of PAH by resveratrol and investigated the underlying mechanism. There are several major findings in this study. First, SIRT1 regulated the expression of cell cycle regulators such as p21, cyclin D1, and cyclin E and arrested HPASMCs in G0/G1 phase after PDGF-BB treatment. Second, resveratrol preserved SIRT1 and p21 expressions but decreased cyclin D1 expression and exhibited an antipulmonary arterial remodeling effect on MCT induced PAH rats. This protective effect was observed at both $2.5\,mg{\cdot}kg^{-1}d^{-1}$ and $20\,mg{\cdot}kg^{-1}d^{-1}$ doses. Third, resveratrol regulated p21, cyclin D1, and cyclin E expressions in a SIRT1-dependent manner in PDGF-BB induced HPASMCs.

Resveratrol was reported to be a SIRT1 activator and has been shown to protect against type 2 diabetes, cancer, heart disease, inflammation, and neurodegenerative diseases [39]. Resveratrol induces apoptosis MH7A human rheumatoid



(a)



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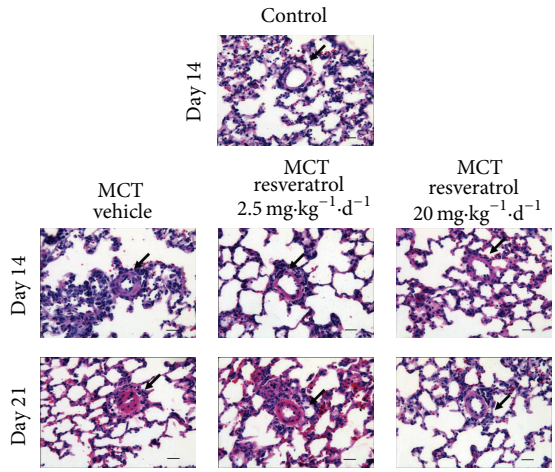

(c)

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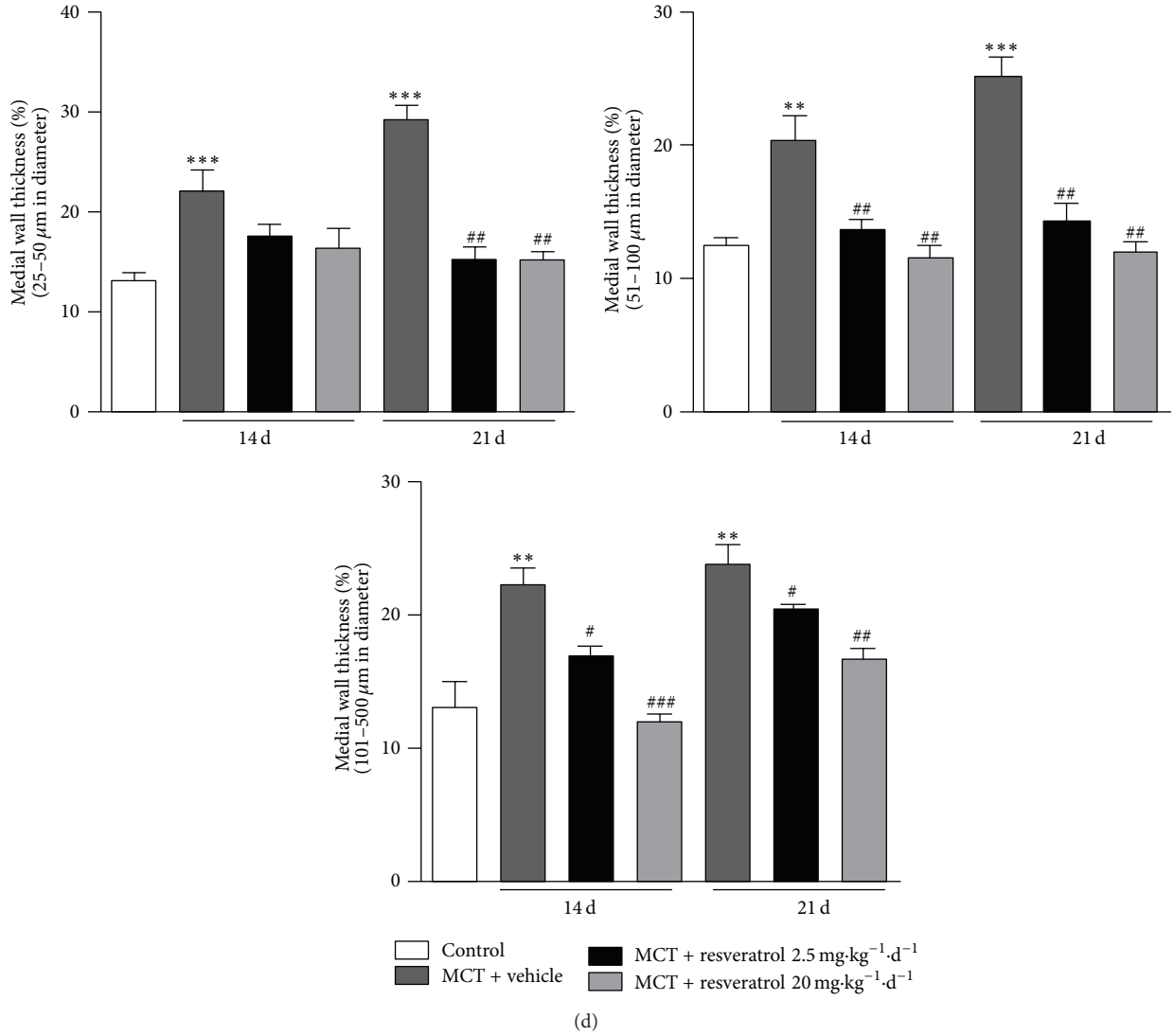


Figure 3: Resveratrol plays an antiremodeling effect in pulmonary arteries. (a) Hemodynamics examinations (n = 8–10). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus normal control; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus vehicle treatment group. (b) Pulmonary arteries muscularization degrees (n = 5-6). $^{*}P < 0.05$, $^{***}P < 0.001$ versus control; $^{\#\#\#}P < 0.001$ and $^{\dagger\dagger\dagger}P < 0.001$ versus corresponding vehicle group. (c) Representative H&E staining photomicrographs. (d) Pulmonary arteries medial wall thickness (n = 5-6). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ versus vehicle treatment group.

arthritis synovial cells in a sirtuin 1-dependent manner. Recently, Csiszar et al. have demonstrated that resveratrol prevented monocrotaline-induced pulmonary hypertension in rats [15]. Furthermore, resveratrol significantly inhibited PDGF-BB stimulated proliferation and cellular hypertrophy in HPASMCs [40]. However, our study focused on whether SIRT1 was involved in resveratrol-mediated prevention of pulmonary arterial hypertension and the underlying mechanism. We found that SIRT1 knockdown significantly eliminated the effects of resveratrol on expressions of cell regulatory molecules such as p21, cyclin D1, and cyclin E in PDGF-BB treated HPASMCs. Resveratrol preserved the expression of SIRT1 and p21 and prevented the increase of cyclin D1 expression in the lungs of MCT-induced PAH rats. *In vitro* and *in vivo* experiments suggested that SIRT1-mediated regulation of p21 and cyclin D1 expression contributed to the antipulmonary arterial remodeling effect of resveratrol. Other mechanisms such as endothelial dysfunction and activation of inflammation take part in the pathological process of PAH. A growing body of evidence has been implicated in the role of SIRT1 in protecting the endothelium from dysfunction, inhibiting inflammation and in antioxidative stress. Whether SIRT1 mediates other beneficial actions of resveratrol also requires further elucidations.

A wide range of resveratrol concentrations (ranging from ~32 nM to 100 μM *in vitro* and ~100 ng·kg^{-1} to 1500 mg·kg^{-1} body weight in animals) have been used in previous studies [41]. However, the relationship between the concentration and effects of resveratrol remains unclear. Both 2.5 mg·kg^{-1}d^{-1} and 20 mg·kg^{-1}d^{-1} are commonly used oral

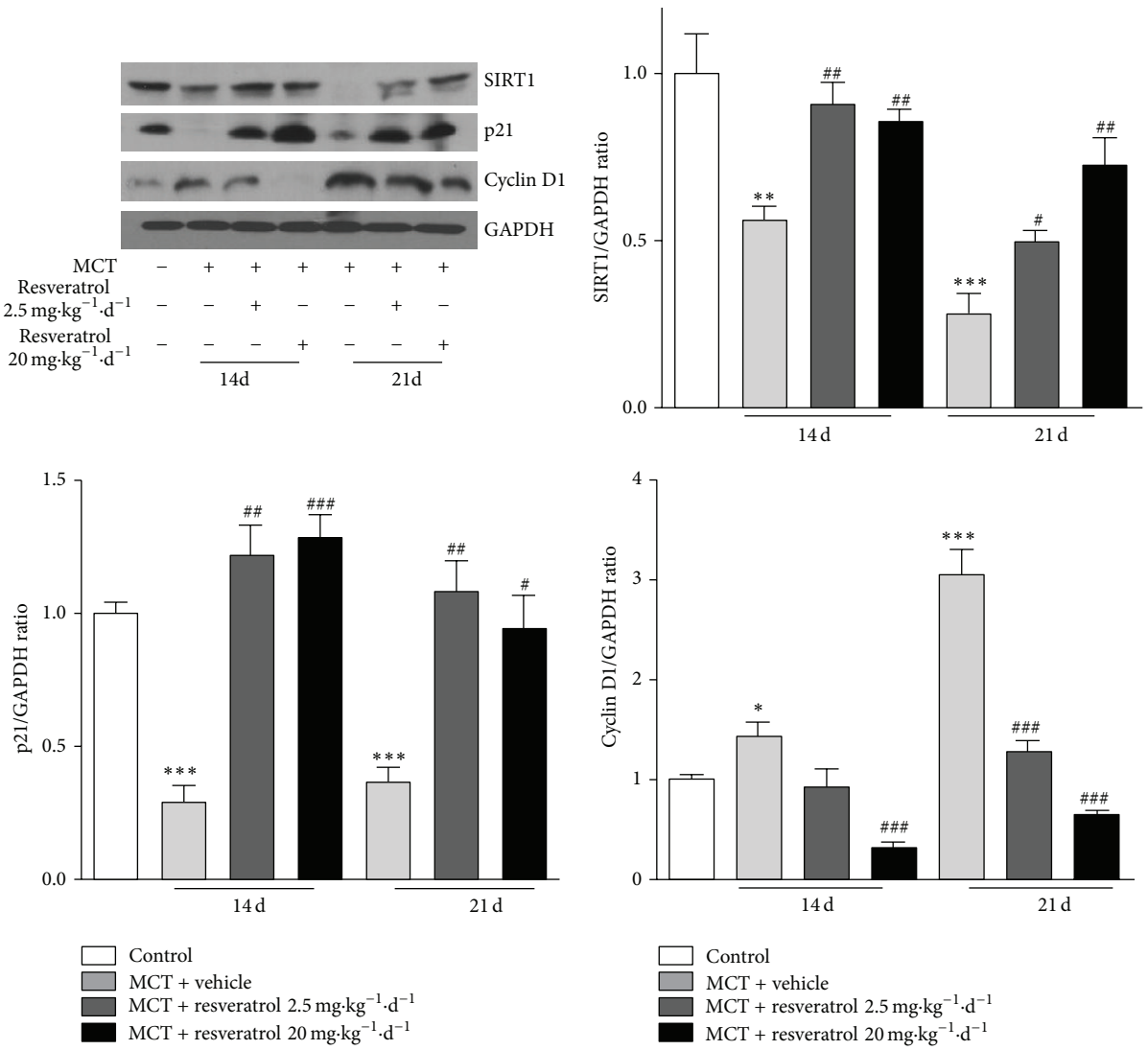
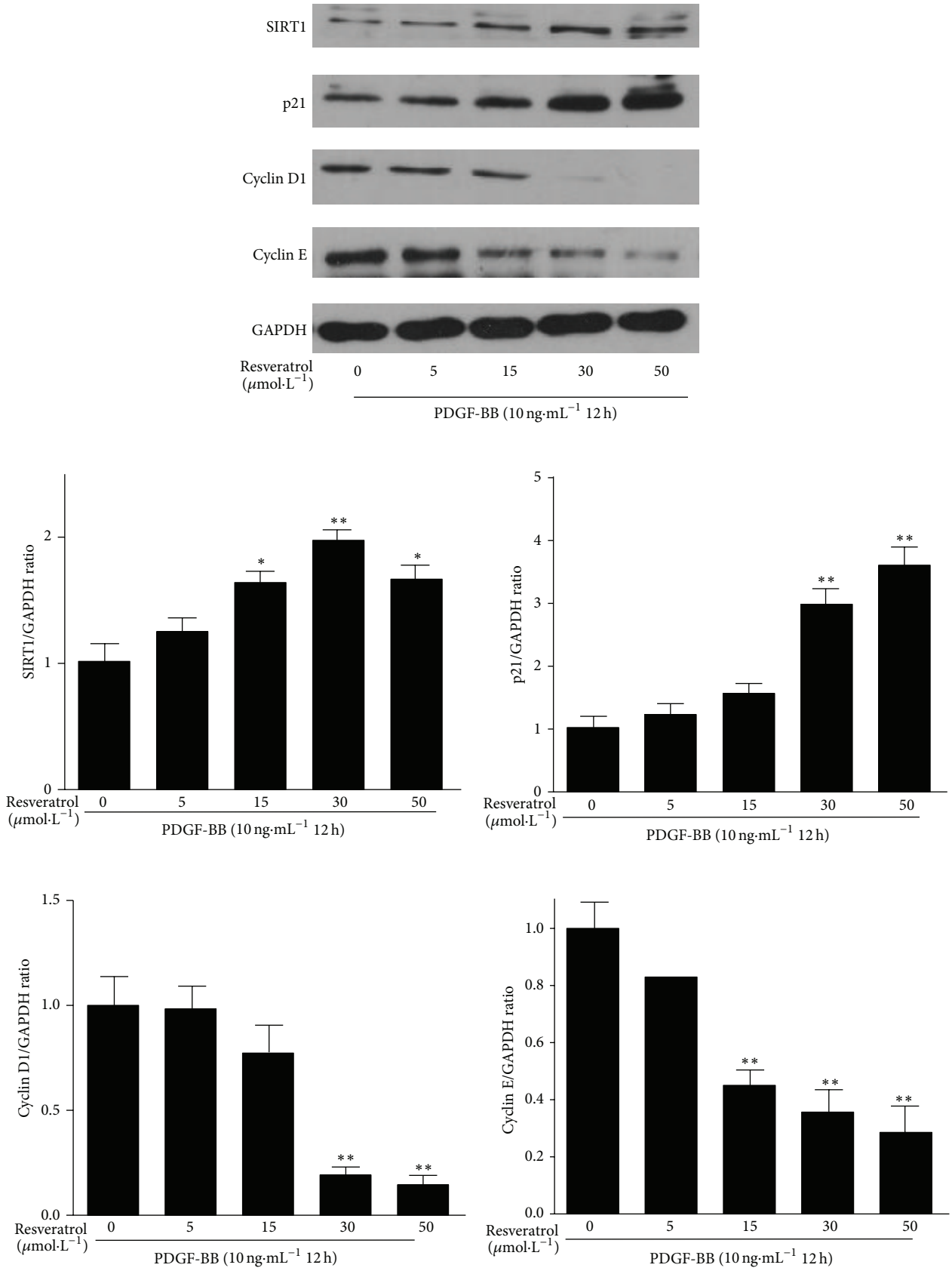


FIGURE 4: Resveratrol increases SIRT1 and p21 protein levels in PAH rats ($n = 5$). Bar graphs show densitometric analysis of western blotting. The densitometric quantification was normalized to GAPDH. Data are shown as means ± SEM for five independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ versus vehicle treatment group on day 14 or 21.

doses of resveratrol in studies of cardiovascular disease. Resveratrol at 2.5 mg·kg^{-1}d^{-1} has a cardioprotective effect in diabetic- and ischemia-reperfusion-induced heart damage in rats [42, 43]. Resveratrol at 20 mg·kg^{-1}d^{-1} induces myocardial angiogenesis in hypercholesterolemic rats [44]. Csiszar et al. have demonstrated the effect of 25 mg·kg^{-1}d^{-1} resveratrol treatment in anti-inflammatory, antioxidant, antiproliferative, and endothelial dysfunction in the rat pulmonary arteries. In our study, we compared the effects of 2.5 mg·kg^{-1}d^{-1} and 20 mg·kg^{-1}d^{-1} resveratrol in PAH rat model. We found that, in the aspect of pulmonary circulation hemodynamics, the high dose group had a significantly lower mPAP and RVHI compared with low dose group; however, there were no significant differences in the effect of antimedial wall thickening in pulmonary arteries sized 25–100 μm in diameter. There were no significant differences in ratios of nonmuscularized or fully muscularized intra-acinar arteries between 2.5 mg·kg^{-1}d^{-1} and 20 mg·kg^{-1}d^{-1} doses of resveratrol. We speculated that the differences in the antiremodeling effect between these two doses are likely to be significant if resveratrol is administered over extended periods of time. In addition to pulmonary arterial remodeling, the pathological process of PAH includes endothelial dysfunction and activation of inflammation. Whether the two doses of resveratrol have similar effects in other pathological process requires further investigation.

PASMC proliferation is central to pulmonary arterial remodeling, which requires PASMCs arrested in G0 or G1 to enter the cell cycle. p21, the cell cycle inhibitor, can cause cells in G1 arrest which contributes to the inhibition of PASMC proliferation by angiotensin converting enzyme inhibitors and the preservation of p21 is essential in suppressing MCT induced PAH in rats [45]. Cyclin D1 acts as a mitogenic signal sensor and promotes G1-to-S phase progression of cell



(a)

Figure 5: Continued.

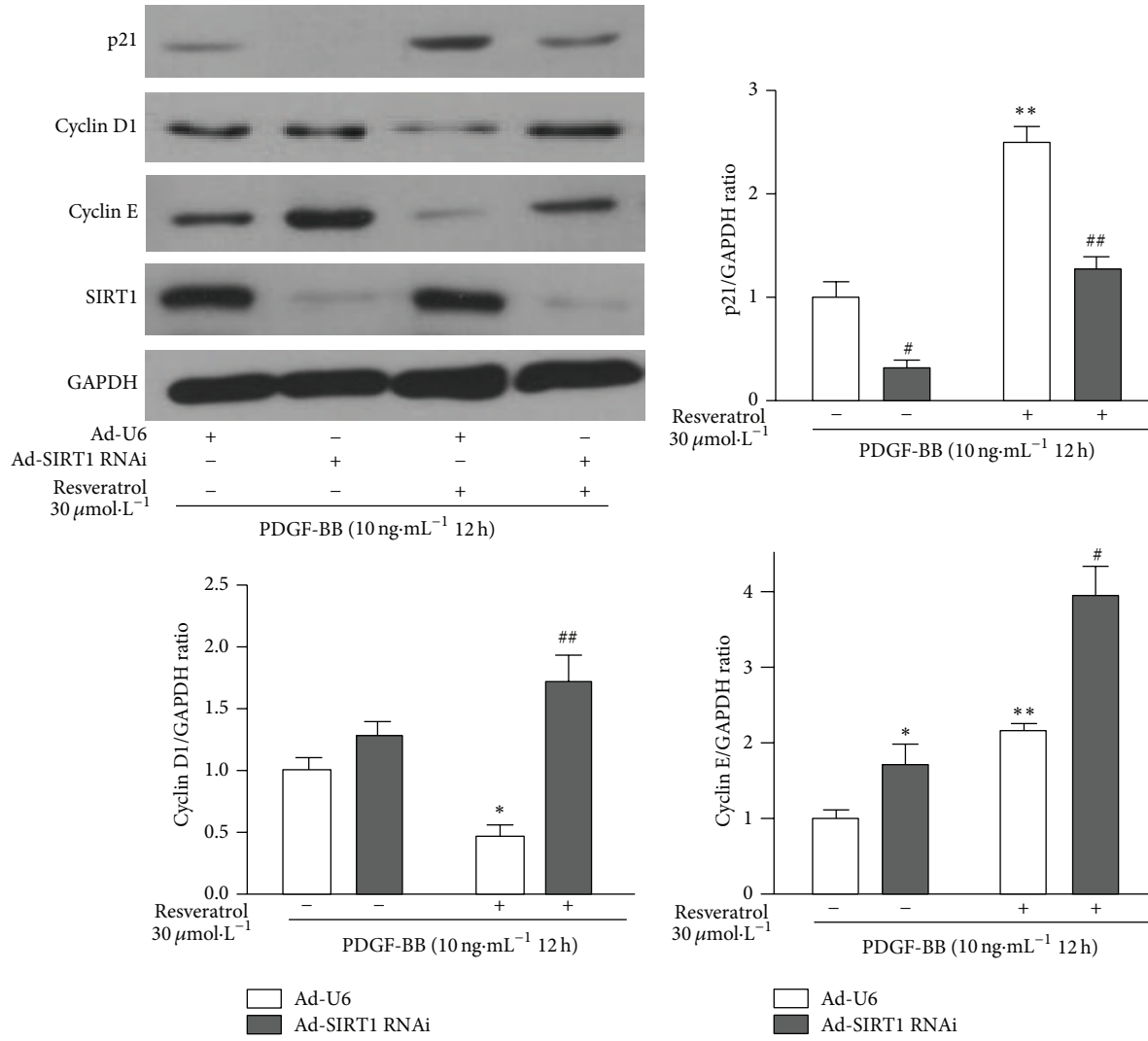


Figure 5: Resveratrol regulates the expression of cell cycle regulatory molecules through SIRT1. (a) Resveratrol increased SIRT1 and p21 expression but decreased cyclin D1 and cyclin E expression in HPASMCs ($n = 3$). The densitometric quantification was normalized to GAPDH. Data are shown as means ± SEM for three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ versus vehicle. (b) SIRT1 RNAi reversed the regulation of p21, cyclin D1, and cyclin E expression level by resveratrol ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$ versus vehicle pretreated Ad-U6 group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus resveratrol pretreated Ad-U6 group.

cycle [46]. Increased cyclin D1 expression and medial VSMC proliferation have been observed in rat carotid artery balloon injury model and mouse carotid artery ligation model [47]. In this study, we found that PDGF-BB decreased p21 expression but increased cyclin D1 expression in HPASMCs; however, resveratrol reversed the effects of PDGF-BB. In the lungs of MCT induced PAH rats, p21 expression was maintained and the increase in cyclin D1 expression level was prevented by resveratrol treatment. Therefore, the antiremodeling effect of resveratrol may at least partially due to the regulation of p21 and cyclin D1 expressions. We observed that resveratrol prevented the increase of cyclin E expression after PDGF-BB treatment; however, cyclin E expression did not change in our PAH rat model. The exact role of cyclin E in proliferation of PASMCs and pulmonary arterial remodeling remains unclear. Our results suggest that the role of cyclin E in pulmonary arterial remodeling is not as important as cyclin D1 and p21.

In conclusion, our findings demonstrated that resveratrol upregulated the expression of the SIRT1 expression in PDGF-BB treated HPASMCs. Furthermore, SIRT1 mediated the role of resveratrol in regulating expression of cell cycle regulatory molecules and arresting PDGF-BB treated HPASMCs in G0/G1 phase, which contributed to the attenuation of pulmonary arterial remodeling and the alleviation of pulmonary arterial hypertension in MCT-treated rats. It suggests that SIRT1 exerts a protective role in PAH associated with rheumatic diseases and can be a potential treatment target.

Nonstandard Abbreviations and Acronyms

PAH:	Pulmonary arterial hypertension
SSc:	Systemic sclerosis
MCTD:	Mixed connective tissue disease
SLE:	Systemic lupus erythematodes
PASMCs:	Pulmonary arterial smooth muscle cells
SIRT1:	Silence Information Regulator 1
PDGF-BB:	Platelet-derived growth factor BB
MCT:	Monocrotaline
RV:	Right ventricular
RVSP:	Right ventricular systolic pressure
RVHI:	Right ventricular hypertrophy index
LV:	Left ventricle
mPAP:	Mean pulmonary arterial pressure
SAP:	Systemic arterial pressure
α-SMA:	α-smooth muscle action.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Shuang Zhou, Meng-Tao Li, and Yu-Yan Jia contributed equally to this work.

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Clinical Study

Can Whole-Body Cryotherapy with Subsequent Kinesiotherapy Procedures in Closed Type Cryogenic Chamber Improve BASDAI, BASFI, and Some Spine Mobility Parameters and Decrease Pain Intensity in Patients with Ankylosing Spondylitis?

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The present study investigated whether whole-body cryotherapy (WBC) procedures could potentially have more beneficial effects on index of BASDAI and BASFI, pain intensity, and spine mobility parameters: Ott test, modified Schober test, chest expansion in ankylosing spondylitis (AS) patients, than kinesiotherapy procedures used separately. AS patients were exposed to a cycle of WBC procedures lasting 3 minutes a day, with a subsequent 60 minutes of kinesiotherapy or 60 minutes of kinesiotherapy only, for 10 consecutive days excluding weekend. After the completion of the cycle of WBC procedures with subsequent kinesiotherapy in the AS patients, BASDAI index decreased about 40% in comparison with the input value, whereas in the group of patients who received only kinesiotherapy it decreased only about 15% in comparison with the input value. After the completion of the treatment in the WBC group, BASFI index decreased about 30% in comparison with the input value, whereas in the kinesiotherapy group it only decreased about 16% in comparison with the input value. The important conclusion was that, in WBC group with subsequent kinesiotherapy, we observed on average about twice better results than in the group treated only by kinesiotherapy.

1. Introduction

Ankylosing spondylitis (AS) is a chronic, usually progressive inflammatory rheumatic disease affecting primarily the axial skeleton and sacroiliac joints. It usually begins in the second or third decade of life and tends to occur more often in males with a male to female ratio of roughly 2 to 1. The overall prevalence is between 0.1% and 1.4%. Chronic spinal inflammation can develop a complete fusion of the vertebrae, a process called ankylosis, which causes total loss of mobility of the spine. In addition, AS may affect peripheral joints, the skin, eyes, bowel, or lungs [1].

The main symptoms of the disease are pain and stiffness in the low back, upper buttock area, neck, and the remaining regions of the spine, which may lead to structural and functional impairments [2].

Although over the last years a revolution in the treatment of AS has taken place, in terms of improved understanding of disease pathophysiology, still in the 21st century, physiotherapy plays very important role and is recommended as a cornerstone in the management of AS, together with medication [3–5].

The primary goals of physiotherapy of the AS patient are to improve mobility and strength, prevent or decrease

Table 1: Demographic data of the study subjects.

Characteristic	WBC group with subsequent kinesiotherapy ($n = 32$)	Kinesiotherapy group ($n = 16$)	p value
Age, years, mean (SD)	46.03 ± 1.20	46.63 ± 1.50	0.095
Gender M/F	32/0	16/0	1.00
BMI, kg/m^2, mean (SD)	24.1 ± 4.2	23.9 ± 5.8	0.564
Smoking (yes/no)	0/32	0/16	1.00
BASDAI index	5.39 ± 1,64	5.28 ± 1.71	0.767
BASFI index	5.18 ± 2.25	5.01 ± 2.06	0.965
Medication			
DMARD (yes/no)	0/32	0/16	1.00
Biological agents (yes/no)	0/32	0/16	1.00
NSAID (yes/no)	32/0	16/0	1.00

SD: standard deviation; BMI: body mass index; BASDAI: the Bath Ankylosing Spondylitis Diseases Activity Index; BASFI: the Bath Ankylosing Spondylitis Functional Index; NSAID: nonsteroidal anti-inflammatory drug, DMARD: disease-modifying antirheumatic drug.

spinal deformity, improve chest expansion, reduce pain, and improve one's overall function and quality of life [6].

Recent studies have shown that a combination of pharmacological treatment and physical therapy gave synergetic effects and produced positive benefits on pain, function, and health-related quality of life in the AS patients [7–9].

One of the most efficient methods of physical medicine used in the treatment of many diseases of the locomotor system is cryotherapy using extremely low temperatures (below −100°C) applied for a short time (2-3 minutes) to stimulate physiological reactions of the human organism, in order to make more effective pharmacological treatment and kinesiotherapy [10]. Cryotherapy can be used as local cryotherapy and whole-body cryotherapy. The action of cryogenic temperatures causes in human organism several favorable and physiological reactions such as analgesic effect, neuromuscular effect, anti-inflammatory and antioedematous effect, and circulatory effect [11, 12].

Cryogenic temperatures applied for whole-body apart from aforementioned effects have also significant influence on psyche and endocrine and immune system [13–15].

The studies show that WBC procedures in AS patients do not influence ejection fraction, late ventricular potentials, and QT dispersion in patients without significant pathology of circulatory system [16, 17].

In addition cryogenic temperatures applied for whole body of AS patients have beneficial influence on adaptive processes of vegetative nervous system [18], profile lipid [19] as well as antioxidant status [20, 21], decrease of inflammatory process [22], and improvement of rheological blood properties [23].

2. Materials and Methods

2.1. Participants. The study protocol has been reviewed and approved by the Bioethical Committee of the Medical University of Silesia in Katowice (permission no. NN-6501-93/I/07), and all analyzed patients were informed about trial and gave written consent for inclusion in the study. All clinical investigations have been conducted according to the principles expressed in the Declaration of Helsinki (1964).

The study involved 48 nonsmoking male patients with ankylosing spondylitis who were divided by the physician into two groups with allocation ratio 2 : 1: 32 patients exposed to whole-body cryotherapy procedures with subsequent kinesiotherapy (WBC group, mean age 46.03 ± 1.20 years) and 16 men exposed only to kinesiotherapy procedures (kinesiotherapy group, mean age 46.63 ± 1.50 years), with no significant difference in mean age and body mass index between them.

The enrolment to the study was performed in the group of succeeding male patients, with definite diagnosis of AS who did not suffer from any other diseases, had no associated pathologies, and whose attending physician did not apply disease modifying antirheumatic drugs (DMARDs), biologic agents, or steroids. The AS patients were treated with NSAIDs, whose doses were not altered in the research course. All patients included in the trial fulfilled the modified New York Criteria for definite diagnosis of AS, which serve as the basis for the ASAS/EULAR recommendations [24]. Final selection to the study included only patients HLA B27 positive, who exhibited II and III radiographic grade of sacroiliac joint disease, who attended consulting unit in Health Resort in the period of subsidence of acute clinical symptoms, in order to qualify for sanatorium treatment (physiotherapy). The demographic data of the study subjects are shown in Table 1.

Before the study each patient was examined by a physician to exclude any coexisting diseases as well as any contraindications for whole-body cryotherapy procedures. Prior to the study a resting electrocardiogram was performed on all patients, and before each session of cryotherapy the blood pressure was measured in all the patients.

2.1.1. Whole-Body Cryotherapy and Kinesiotherapy Procedures. Depending on the group, the AS patients were exposed to a cycle of whole-body cryotherapy (WBC) procedures lasting 3 minutes a day, with a subsequent 60 minutes of kinesiotherapy, or 60 minutes of kinesiotherapy only, for 10 consecutive days excluding weekend.

The whole-body cryotherapy procedures were performed in a liquid nitrogen cooled cryogenic chamber (CR 2000)

(produced by Creator, Poland), which consists of two compartments: the antechamber and the proper chamber connected by an internal door. In the trial the temperature in the antechamber was −60°C, whereas in the proper chamber it reached −120°C. After a 30-second adaptation process in the antechamber, the subjects were exposed to cryogenic temperatures for 3 minutes in the proper chamber. During the WBC procedure, all patients were dressed in swimsuits, cotton socks and gloves, and wooden shoes, whereas their mouths and noses were protected by surgical masks and their ears by ear-protectors. All jewellery, glasses, and contact lenses were removed before entry into the chamber. During the WBC procedure the subjects were walking round the chamber without touching each other.

Immediately after leaving the cryogenic chamber and changing clothes and shoes (for track-suits and trainers), the AS patients underwent 1-hour long kinesiotherapy. The program of kinesiotherapy was the same for all patients in both groups. Kinesiotherapy procedures included range-of-motion exercise of the spine and major joints (including the ankle, knee, hip, wrist, elbow, and shoulder). Chest expansion and breathing exercises were also included. Apart from range-of-motion exercise, the AS patients received strengthening exercise of the muscles of the major joints (including the ankle, knee, hip, wrist, elbow, shoulder, thoracolumbar spine, and cervical spine) as well as aerobic exercise (including cycling and fast walking).

All patients completed the study and no complications or side effects related to the WBC procedures were observed.

2.2. Assessments. The primary outcome measures were BASDAI and BASFI index. Secondary measures included pain intensity and chosen spine mobility parameters.

On the first day before the treatment cycle of WBC and/or kinesiotherapy and on the first day after treatment completion, the following parameters were estimated: the Bath Ankylosing Spondylitis Diseases Activity Index (BASDAI), the Bath Ankylosing Spondylitis Functional Index (BASFI), and pain intensity by means of 10-point visual analogue scale (VAS). Participants' spinal mobility was also examined through Ott test, modified Schober test, and chest expansion.

The BASDAI has six questions related to fatigue, back pain, peripheral pain, peripheral swelling, local tenderness, and morning stiffness (degree and length). Other than the item relating to morning stiffness, all questions are scored from 0 (none) to 10 (very severe) using a visual analogue scale (VAS). A sum score was calculated as mean of two morning stiffness items and the four remaining items [25].

The BASFI is the mean score of ten questions addressing functional limitations and the level of physical activity at home and work, assessed on VAS scales (0 = easy, 10 = impossible) [26].

Ott test measures the range of motion of the thoracic spine. Patient standing and measurement made 30 cm inferior to the C7 spinous process. The measurement was repeated with patient in full forward flexion. An increase of less than 2 cm suggests decreased thoracic spinal mobility [27].

Modified Schober test measures the range of motion of the lumbar spine. Patient standing and measurements made 10 cm above and 5 cm below the lumbosacral junction (iliac crest line). The measurement was repeated with patient in full forward flexion. An increase of less than 6 cm suggests decreased lumbar spinal mobility [27].

The subject's chest expansion was taken in standing position with the feet 5 cm apart and arms elevated. The upper limbs at the sides with the shoulder abducted the elbow in semiflexion, the wrist extended, and the thumb abducted with the web between the thumb and the first fingers placed on the level of the iliac crest. The chest expansion was taken as the change in circumference of the patient's chest at the level of the 4th intercostal space at the end of forced inspiration minus thoracic circumference at the end of forced expiration. Limitation of chest expansion is where the patient's measure recorded in centimeters is less than the average normal value by a minimum of 2.5 cm correcting for age and gender [28].

2.3. Statistical Analysis. Statistical analysis was undertaken using the statistical package of Statistica 10 Pl software. For each parameter the indicators of the descriptive statistics were determined (mean value and standard deviation SD). The normality of the data distribution was checked using Shapiro-Wilk's test, while the homogeneity of the variance was verified with the use of Levene's test. In order to compare the differences between the initial values of particular laboratory parameters and values after the end of a cycle of treatment procedures in both groups of subjects, a dependent sample Student's t-test when homogeneity of variances and normality of distribution have been fulfilled, otherwise Wilcoxon signed-rank test was used. On the other hand in the case of independent (unpaired) samples the t-test or Mann-Whitney U test has been used which was also dependent on homogeneity of variances and normality of distribution.

Differences at a significance level of $p < 0.05$ were considered as statistically significant.

3. Results

The obtained results are shown in Figures 1–6.

After the completion of the treatment in both studied groups, statistically significant improvement in the values of all examined parameters was noted.

After the completion of treatment in AS patients who underwent whole body cryotherapy procedures with subsequent kinesiotherapy (WBC group), a statistically significant decrease in BASDAI index (5.39 ± 1.64 and 3.24 ± 0.90 before and after therapy, resp., $p < 0.001$) (Figure 1(a)), BASFI index (5.18 ± 2.25 and 3.86 ± 2.1790 before and after therapy, resp., $p < 0.001$) (Figure 2(a)), and pain VAS score (5.34 ± 1.58 and 2.86 ± 1.28 before and after therapy, resp., $p < 0.001$) (Figure 3(a)) and a statistically significant increase in Ott test (1.11 ± 0.47 and 1.59 ± 0.45 before and after therapy, resp., $p < 0.001$) (Figure 4(a)), modified Schober test (1.47 ± 0.99 and 2.23 ± 1.00 before and after therapy, resp., $p < 0.001$) (Figure 5(a)), and chest expansion (1.92 ± 0.98 and 2.63 ± 1.00

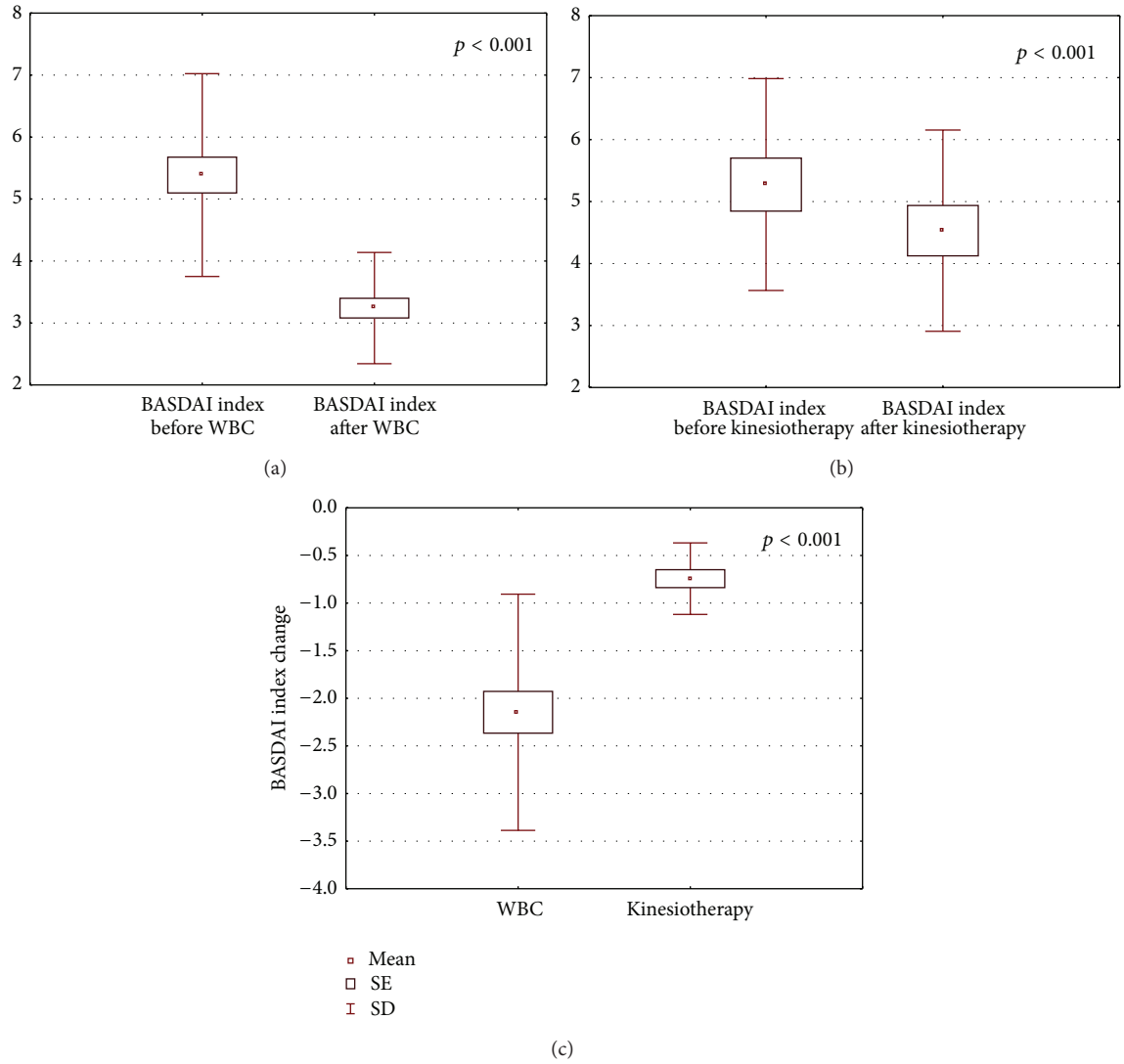

Figure 1: Comparison of BASDAI index before and after a cycle of whole-body cryotherapy (WBC) with subsequent kinesiotherapy procedures (a), before and after only a cycle of kinesiotherapy procedures (b), and BASDAI index change in whole-body cryotherapy and kinesiotherapy groups (c) obtained for patients suffering from AS.

before and after therapy, resp., $p < 0.001$) (Figure 6(a)) were obtained.

After the completion of treatment in AS patients who underwent only the kinesiotherapy procedures (kinesiotherapy group), a statistically significant decrease in BASDAI index (5.28 ± 1.71 and 4.53 ± 1.62 before and after therapy, resp., $p < 0.001$) (Figure 1(b)), BASFI index (5.01 ± 2.06 and 4.35 ± 2.23 before and after therapy, resp., $p < 0.001$) (Figure 2(b)), and pain VAS score (5.00 ± 1.63 and 4.09 ± 1.90 before and after therapy, resp., $p < 0.01$) (Figure 3(b)) and a statistically significant increase in Ott test (1.16 ± 0.57 and 1.38 ± 0.62 before and after therapy, resp., $p < 0.05$) (Figure 4(b)), modified Schober test (1.49±0.95 and 1.71±0.98 before and after therapy, resp., $p < 0.01$) (Figure 5(b)), and chest expansion (2.34±1.26 and 2.75±1.21 before and after therapy, resp., $p < 0.001$) (Figure 6(b)) were also observed.

However, in WBC group the examined parameter changes were significantly higher than in kinesiotherapy group: BASDAI change (−2.15 ± 1.24 in WBC group versus −0.74±0.34 in kinesiotherapy group, $p < 0.001$) (Figure 1(c)), BASFI change (−1.38±1.03 in WBC group versus −0.66±0.39 in kinesiotherapy group, $p < 0.001$) (Figure 2(c)), pain VAS score change (−2.48 ± 1.79 in WBC group versus −0.91 ± 0.74 in kinesiotherapy group, $p < 0.001$) (Figure 3(c)), Ott test change (0.48 ± 0.24 in WBC group versus 0.22 ± 0.31 in kinesiotherapy group, $p < 0.01$) (Figure 4(c)), modified Schober test change (0.77 ± 0.31 in WBC group versus 0.21 ± 0.24 in kinesiotherapy group, $p < 0.001$) (Figure 5(c)), and chest expansion change (0.70 ± 0.25 in WBC group versus 0.41 ± 0.27 in kinesiotherapy group, $p < 0.01$) (Figure 6(c)).

4. Discussion

The available professional literature lacks studies assessing the influence of whole-body cryotherapy on AS patients, besides our own studies.

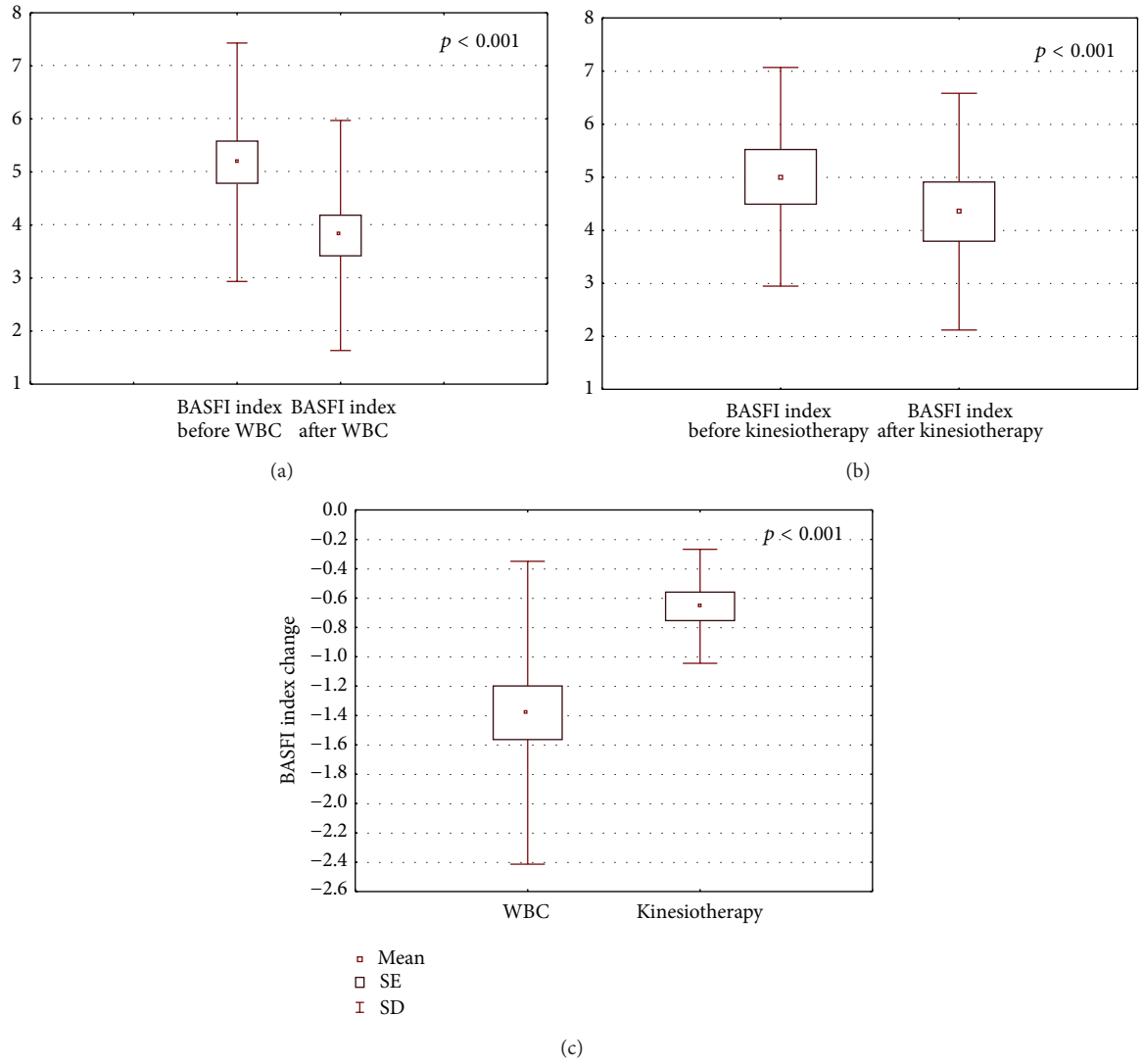

Figure 2: Comparison of BASFI index before and after a cycle of whole-body cryotherapy (WBC) with subsequent kinesiotherapy procedures (a), before and after only a cycle of kinesiotherapy procedures (b), and BASFI index change in whole-body cryotherapy and kinesiotherapy groups (c) obtained for patients suffering from AS.

One of the most significant effects of cryogenic temperatures is the analgesic effect connected with influence of low temperatures on endocrine system (increased secretion of β-endorphins), nervous system (functional disconnection of sensory receptors and their connections with proprioreceptors, release of conductivity in slowly conductive fibers, and selecting impulses coming to nervous system, mechanism of "control gates"), and metabolic action (among others decreased concentrations of histamine and lactate in inflammatory changed tissues) [11, 29, 30].

In the study [31] AS patients assessed subjectively the WBC procedures effects by means of a questionnaire. The WBC procedures were performed in cryogenic chamber with cold retention. In cited study the significant improvement concerned mainly the reduction of the intensity and frequency of pain occurring as well as relaxation and improvement in the quality of falling asleep and sleep.

In our own study [32] we showed that in AS patients WBC procedures performed in cryogenic chamber with cold retention caused the reduction of pain intensity by 46% in comparison with the input value, whereas in the group of the patients who received only kinesiotherapy the pain intensity reduction amounted only to 18%.

In the presented study we observed similar results. The pain intensity decreased about 43% in the WBC group, whereas in kinesiotherapy group the decrease was only about 21%.

BASDAI index estimates the disease activity by evaluation of fatigue, axial pain, peripheral pain, stiffness, and enthesopathy in the AS patients. It is a quick and simple index and demonstrates a sensitivity to change within a short period of time as well as statistically significant ($p < 0.001$) reliability. The value of BASDAI index above 4 means that the disease is in an active period [25]. Before the trial in

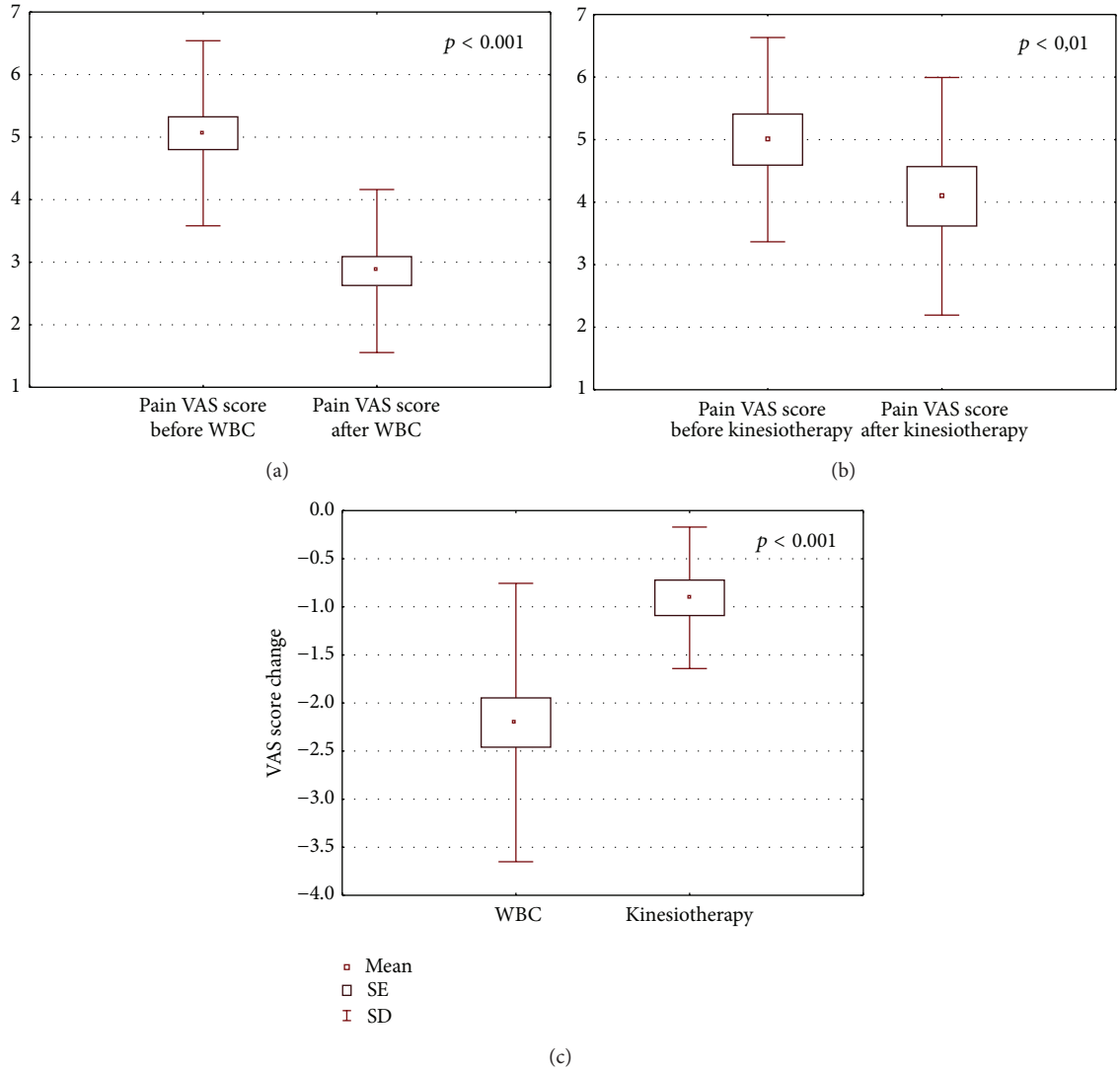

Figure 3: Comparison of pain VAS score before and after a cycle of whole-body cryotherapy (WBC) with subsequent kinesiotherapy procedures (a), before and after only a cycle of kinesiotherapy procedures (b), and VAS score change in whole-body cryotherapy and kinesiotherapy groups (c) obtained for patients suffering from AS.

both examined groups the value of BASDAI index was above 4. After the completion of cycle of whole-body cryotherapy procedures with subsequent kinesiotherapy in the AS patients it decreased about 40% in comparison with the input value, whereas in the group of the patients who received only kinesiotherapy the BASDAI index decreased about 15% in comparison with the input value.

Moreover, after the completion of the treatment only in the WBC group its value was below 4 (inactive disease).

After pain and stiffness, one of the most important complaints of patients with AS is disability. The BASFI index determines the degree of functional limitation in the AS patients. The first 8 questions evaluate activities related to functional anatomical limitations due to the course of this inflammatory disease. The final 2 questions evaluate the patients' ability to cope with everyday life. Its sensitivity and reliability are similar to the BASDAI index [26]. As in the case of BASDAI index, the BASFI index value was above 4 in both studied groups. After completion of the treatment in the WBC group the value of BASFI index decreased about 30% in comparison with the input value, whereas in the kinesiotherapy group it only decreased about 16% in comparison with the input value. Moreover, similar to the BASDAI index, after the completion of the treatment only in the WBC group its value was below 4 (inactive disease).

In WBC group Ott test, modified Schober test, and chest expansion increased about 60%, 83%, and 53% in comparison to the input value, whereas in the kinesiotherapy group Ott test, modified Schober test, and chest expansion increased only about 26%, 16%, and 26% in comparison to the input value. We observed similar results in AS patients who underwent WBC procedures in the cryogenic chamber with cold retention [33].

Whole-body cryotherapy with subsequent kinesiotherapy effects in AS patients was explicitly better compared to the group of patients who received only kinesiotherapy.

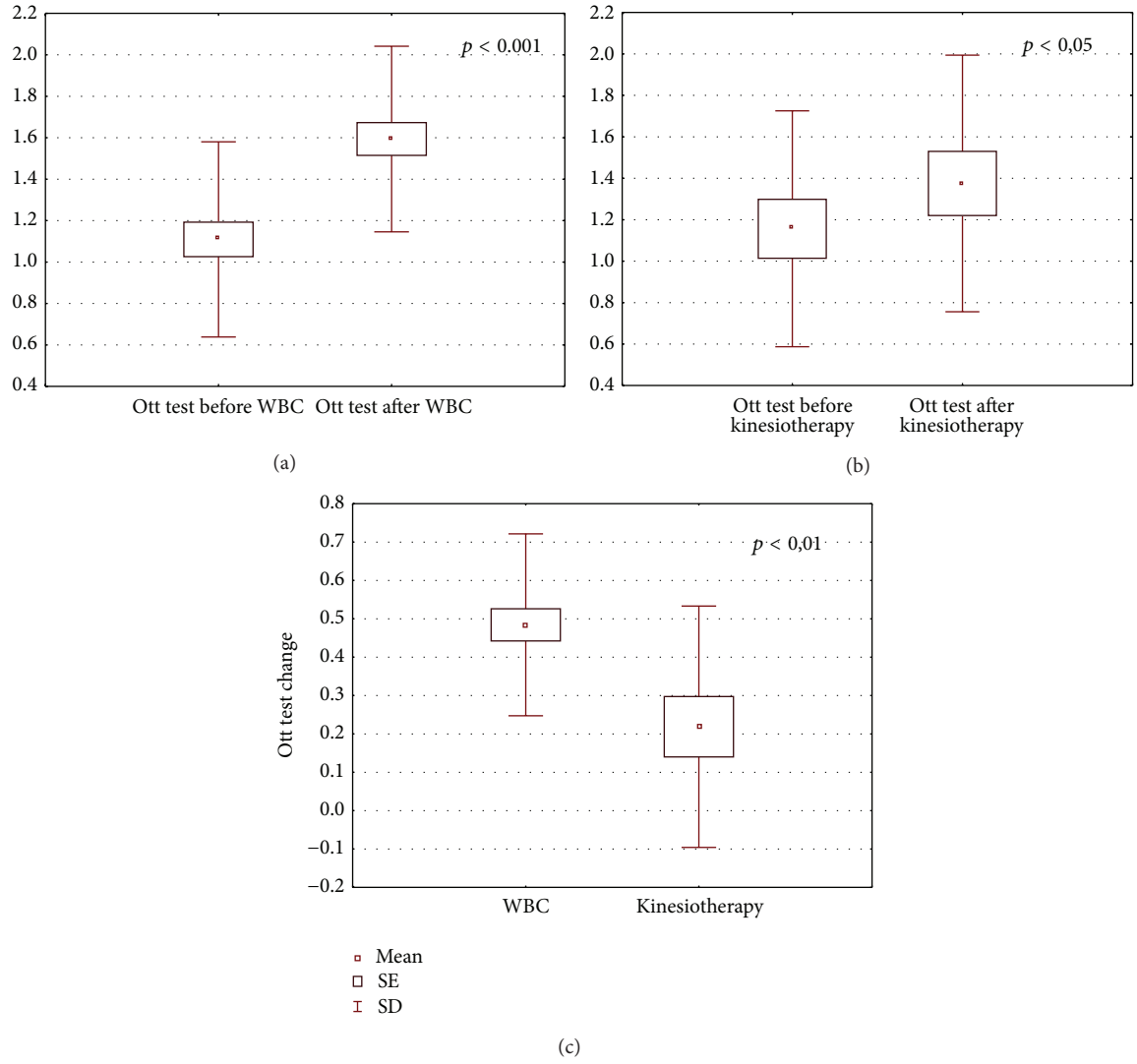

Figure 4: Comparison of Ott test (cm) before and after a cycle of whole-body cryotherapy (WBC) with subsequent kinesiotherapy procedures (a), before and after only a cycle of kinesiotherapy procedures (b), and Ott test change in whole-body cryotherapy and kinesiotherapy groups (c) obtained for patients suffering from AS.

Beneficial effect of whole-body cryotherapy procedures on the value of the index BASDAI and BASFI and parameters of the spine mobility in the AS patients is mainly due to its anti-inflammatory, neuromuscular, and antioedematous as well as aforementioned analgesic action.

Anti-inflammatory effect of WBC procedures may arise both from the impact of cryogenic temperatures on secretion of mediators of inflammation and on the prooxidant-antioxidant balance and stabilization of lysosome membranes and subsequent inhibition of release of active enzymes from lysosomes.

WBC procedures cause the decrease in level of inflammatory state parameters; among others are erythrocyte sedimentation rate (ESR) value, serum concentration of C-reactive protein (CRP), fibrinogen, seromucoid, sICAM (soluble intercellular adhesion molecule), proinflammatory cytokines IL-1, IL-2, IL-6, and IL-8 levels, and increase in anti-inflammatory cytokine IL-10 level [22, 34–36].

The other mechanism of anti-inflammatory action of WBC procedures could be related to its beneficial influence on prooxidant-antioxidant balance. It is suggested that repeated exposures to cryogenic temperatures may cause adaptative changes in the form of an increase in antioxidant status and antioxidant enzyme activity, resulting in the formation of a prooxidant-antioxidant balance at a higher level, assisting in an anti-inflammatory effect and protecting tissues against an increased generation of reactive oxygen species and oxidative stress caused by training [37–39]. In our own study in AS patients after ten treatments we also observed the beneficial influence of WBC procedures on antioxidant status (increased activity of superoxide dismutase, level of total antioxidant status, and decreased level of malondialdehyde) [20].

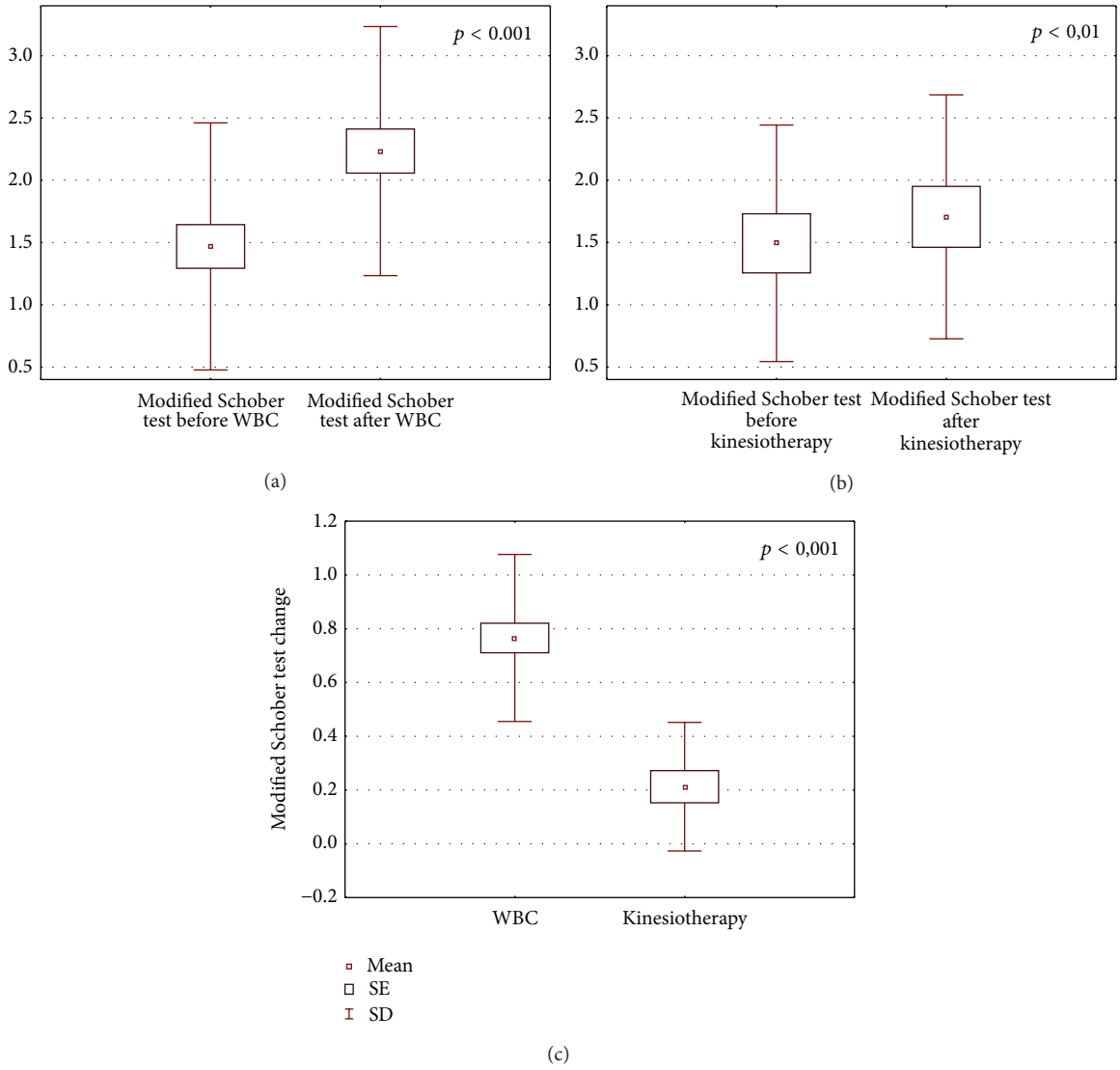

Figure 5: Comparison of modified Schober test (cm) before and after a cycle of whole-body cryotherapy (WBC) with subsequent kinesiotherapy procedures (a), before and after only a cycle of kinesiotherapy procedures (b), and Schober test change in whole-body cryotherapy and kinesiotherapy groups (c) obtained for patients suffering from AS.

The other mechanisms of anti-inflammatory action of whole-body cryotherapy may be linked to stabilization of lysosome membranes and subsequent inhibition of release of active enzymes from lysosomes [40]. It seems that this effect could be related to increased ACTH and cortisone blood concentrations, due to both WBC and physical training [41, 42].

The obtained results may be also connected with neuromuscular effect of cryotherapy which results in reduction of muscular tone (reduction of nerve transmission and reactivity of peripheral nerve endings) and increase of muscle strength [10, 29, 30].

The other effect of WBC which may have influence on achieved results is antioedematous action. This mechanism results in improvement in patency of lymphatic vessels draining intercellular space and increase in capillary filtration and acceleration of lymph flow [11].

Through these aforementioned mechanisms of WBC procedures, we can observe much better results compared to kinesiotherapy procedures used separately. WBC preceding kinesiotherapy allows intensification of the training and extension of the time of its duration several times [30]. Therefore, procedures of WBC and therapeutic exercises are altogether main components of the so-called cryorehabilitation [43–45].

In addition, observed calming and nervous tension reduction in AS patients after WBC treatment may increase the mobilization of patients to exercise and improve cooperation with the doctor and physiotherapeutist [31].

As authors' own experience and literature data show, WBC is well tolerated by patients, including children and the elderly. During the first days of procedure, there may occur slight aggravation of disease symptoms which is a generally promising prognosis. Patients admitted for the whole-body

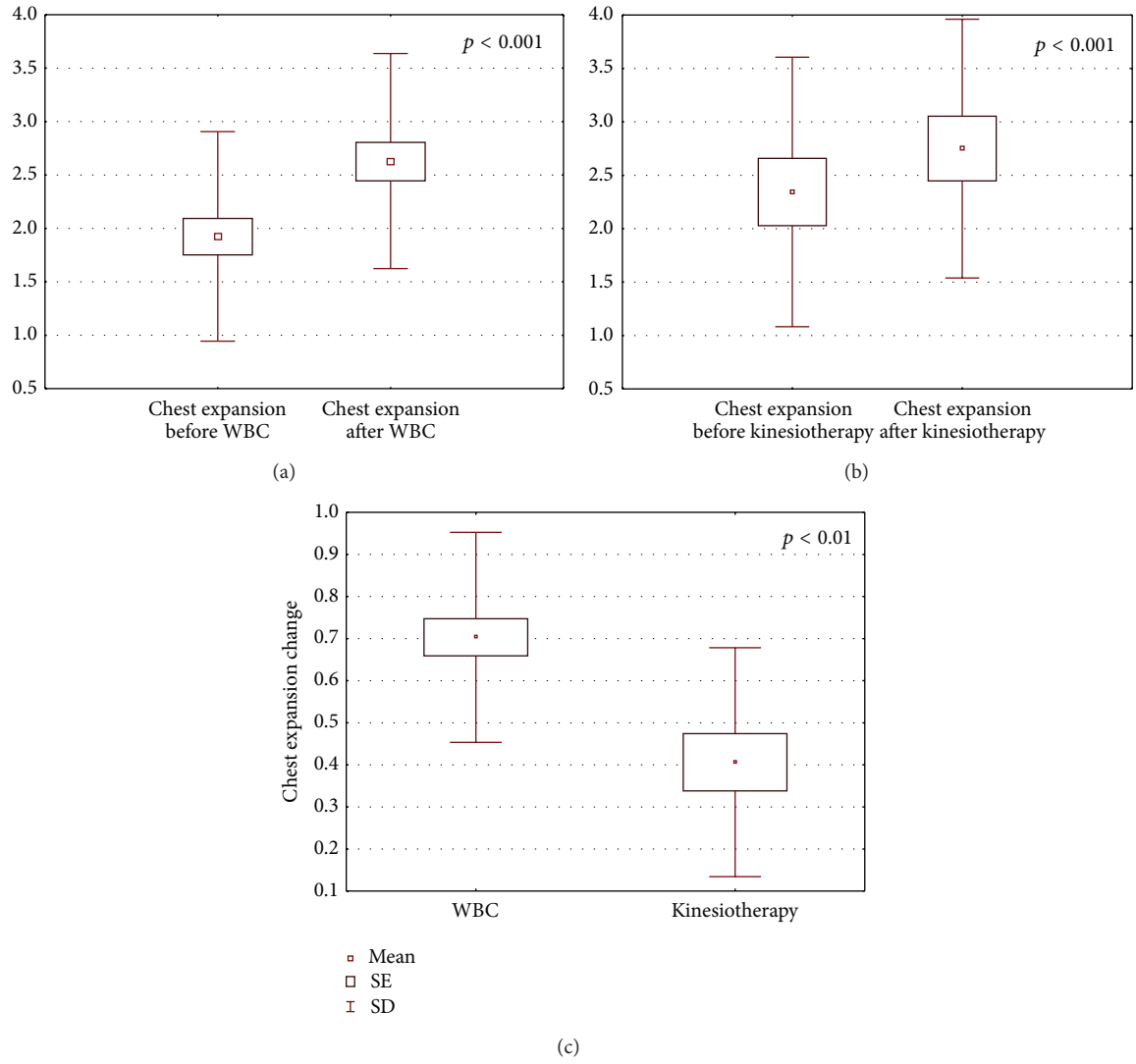

Figure 6: Comparison of chest expansion test (cm) before and after a cycle of whole-body cryotherapy with subsequent kinesiotherapy procedures (a), before and after only a cycle of kinesiotherapy procedures (b), and chest expansion test change in whole-body cryotherapy and kinesiotherapy groups (c) obtained for patients suffering from AS.

cryotherapy are instructed how to behave during the procedure. Special attention is paid to the way of breathing in the proper chamber during the procedure. Inhaling should be two times shorter than exhaling due to decompression of cooled air in lungs. Noncompliance with the recommendation may lead to serious breathing depression. Moreover, it is forbidden to touch other patients or rub own skin. In addition each time before WBC procedure patients should dry their skin with towel in order to remove sweat, as sweat drops turn into ice crystals in a cryochamber and it may lead to frostbite [11].

Our study has some limitations. First, the study did not provide long-term followup (e.g., 3 months), and thus we do not know how long the effect of whole-body cryotherapy with subsequent kinesiotherapy would be maintained after the end of study. Secondly, a cycle of whole-body cryotherapy (WBC) with a subsequent kinesiotherapy consisted of ten procedures only. A greater number of procedures (e.g., 20–30) might increase a treatment effect.

5. General Conclusion

In the present report, we demonstrated that whole-body cryotherapy procedures have beneficial influence on AS patients through decrease of BASDAI and BASFI index, pain intensity, and improvement of some spinal mobility parameters.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

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Research Article

Elevated Serum Levels of Soluble CD30 in Ankylosing Spondylitis Patients and Its Association with Disease Severity-Related Parameters

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Soluble CD30 (sCD30), a transmembrane glycoprotein that belongs to the tumor necrosis factor receptor (TNFR) superfamily, has been shown to be associated with various pathological conditions. This study was designed to measure the levels of serum sCD30 in patients with ankylosing spondylitis (AS) and to evaluate the relationships between serum sCD30 levels and other disease severity-related indexes, including bath ankylosing spondylitis disease activity index (BASDAI), ankylosing spondylitis disease activity score (ASDAS), and bath ankylosing spondylitis functional index (BASFI). Our results demonstrated significantly elevated sCD30 levels in AS patients compared to healthy controls (HCs) with mean values of 32.0 ± 12.2 and 24.9 ± 8.0 ng/mL, respectively ($P^{**} = 0.007$), suggesting a potential role of sCD30 in the pathogenesis of AS. However, no significant correlations of sCD30 with BASDAI, ASDAS, or BASFI were detected in our study ($P > 0.05$). Therefore, sCD30 cannot be used as a reliable marker for reflecting disease activity and functional ability of AS patients.

1. Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disorder that mainly affects the sacroiliac joints and axial skeleton of young males. The condition can lead to new bone formation and even disability if early diagnosis and treatment do not occur. The effects of environmental factors on genetically susceptible individuals are considered to be one possible mechanism for the pathogenesis of AS. It is known to be a highly genetic disease and there is a strong genetic association of MHC molecules with AS, especially human leukocyte antigen-B27 (HLA-B27) [1]. HLA-B27 is an MHC class I molecule that is highly expressed on antigen presenting cells. However, the exact roles of HLA-B27 in the pathogenesis of AS remain unclear, and several hypotheses have been proposed. For example, self or bacterial antigen peptides might be presented by HLA-B27 to CD8+ T cells, which would consequently lead to an aberrant immunological response. In addition, other non-HLA-B27 and non-MHC genes have also been shown to be associated with AS, including HLA-B60, HLA-B61, and the IL-1 gene cluster. Moreover, a recent study found that imbalances in subsets of T cell populations might be responsible for the pathogenesis of AS, including increased ratios of Th1/Th2 and Th17/Treg cells [2]. Abnormal functions of immune cells in patients with AS can lead to the upregulation of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), IL-6, soluble IL-2 receptor, IL-17, and IL-23 [1–3].

CD30 is a member of the TNF receptor superfamily and was originally recognized as a marker of Hodgkin and Reed-Sternberg cells in Hodgkin's lymphoma [4]. The protein is predominantly expressed on the surface of activated and memory T helper (Th) cells, rather than resting T and B cells. Subsequent studies have discovered that it is also present on other types of cells, including activated B cells, natural killer (NK) cells, dendritic cells [5], and neoplastic cells (such as myeloma or solid carcinoma cells) [6]. The CD30 ligand (CD30L) is also a member of the TNF superfamily and is

expressed on activated T cells, resting B cells, monocytes, and granulocytes. Pleiotropic biological effects are induced on CD30+ cells after the ligation of CD30 with CD30L, including activation, proliferation, differentiation, and cell death in a cell type-dependent manner, respectively [7, 8]. It has been shown that the extramembranous region of CD30 is cleaved by a metalloprotease and released into the bloodstream after cell activation as a soluble protein (sCD30), and recent studies have explored the roles of the protein in various diseases, including malignant disorders, infectious diseases [4], rejection after organic transplantation [9], and autoimmune diseases [10–14]. Moreover, it has been proposed that sCD30 may serve as a diagnostic marker in various autoimmune disorders. However, the changes in sCD30 occurring in AS patients remain poorly understood. In this study, we assessed sCD30 serum levels in AS patients and assessed whether there is an association with severity-related indicators.

2. Material and Methods

2.1. Patient Characteristics. Thirty-five patients who fulfilled the modified New York criteria for AS [15] and had visited our department between 2012 and 2013 participated in this study. A questionnaire was used to record the basic and clinical information of patients, including age, sex, bath ankylosing spondylitis disease activity index (BASDAI), ankylosing spondylitis disease activity score (ASDAS), bath ankylosing spondylitis functional index (BASFI), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). The scores of each scoring system ranged from 0 to 10. BASDAI and ASDAS were employed to evaluate the activity of AS, both of which are considered to be reliable indicators [16, 17]. Physical functions were assessed through BASFI, which is widely recognized for its strong reliability and construct validity [18]. The ESR was detected using the Westergren method while CRP measurements were taken by immunonephelometry using CRP reagents (BioSystems S.A, Spanish). Thirty-two age-matched healthy volunteers served as healthy controls (HCs). The exclusive criteria for AS patients were as follows: history of malignant cancers, infection, rheumatic diseases, or common diseases that presented obvious laboratory abnormalities. All AS patients enrolled in this study had not received biological agent therapies, such as a TNF-α inhibitor. HCs were volunteers who had no evidence of acute or chronic infectious disorders, autoimmune disease, or any other systemic condition. Written, informed consent was provided by each participant and the study was approved by the ethics committee of our university.

2.2. Determination of Serum sCD30 Levels. Peripheral blood was collected and processed by centrifugation. Serum was stored at −80°C until it was analyzed. Titers of sCD30 were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (eBioscience).

2.3. Statistical Analysis. Data in this paper were expressed as mean value ± standard deviation (SD). For statistical analysis, an independent samples t-test was used to compare the sCD30 levels between the two groups in this study. Correlation analyses were carried out using Pearson's rank correlation test and $P < 0.05$ was considered statistically significant. All statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) software for Windows.

Table 1: Clinical and demographic information of AS patients and HCs.

	AS ($n = 35$)	HC ($n = 32$)	P
Age (mean ± SD year)	27.5 ± 8.7	27.9 ± 3.0	0.76
Male/female	33/2	12/20	0.007*
NSAIDs user (%)	0	—	
Glucocorticoid users (%)	2.9	—	
DMARDs user (%)	8.6		
BASDAI (mean ± SD cm)	4.2 ± 1.8	—	
ASDAS (mean ± SD cm)	3.3 ± 1.2	—	
BASFI (mean ± SD cm)	2.4 ± 2.1	—	
ESR (mm/h)	36.4 ± 33.7	—	
CRP (mg/L)	39.7 ± 40.7	—	

AS: ankylosing spondylitis; HCs: healthy controls; NSAIDs: nonsteroidal anti-inflammatory drugs; DMARDs: disease modifying antirheumatic drugs; BASDAI: bath AS disease activity index; ASDAS: ankylosing spondylitis disease activity score; BASFI: bath AS functional index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.
*$P < 0.05$.

3. Results and Discussion

3.1. Serum sCD30 Levels Are Notably Elevated in AS Patients but Do Not Correlate with Disease Severity-Related Parameters. Our results revealed a statistically significant increase of serum sCD30 levels in AS patients compared to HCs (32.0 ± 12.2 and 24.9 ± 8.0 ng/mL, resp.; Figure 1(a)). We excluded patients who had previously undergone or were currently receiving treatment with biological agents; however, 3 cases (8.6%) stated that they had taken sulfasalazine for a short period of time (less than 3 months) and 1 case (2.9%) received glucocorticoid therapy 1 day prior to going on study (Table 1). The average serum sCD30 level for these 4 cases was 38.91 ± 8.87 ng/mL, hardly revealing a significant decrease. Moreover, there were no statistically significant differences between the 2 groups when these 4 cases were excluded from the analysis (data not shown). The small number of patients treated with disease modifying antirheumatic drugs (DMARDs) or glucocorticoids made it impossible to assess the effects of the drugs on serum sCD30 levels, and, therefore, a future prospective study is needed. Furthermore, serum sCD30 levels in male and female healthy controls were not statistically different (22.5 ± 8.3 and 26.4 ± 7.7 ng/mL, resp.; $P > 0.05$; Figure 1(b)). Unlike other studies showing significant correlations of sCD30 with disease severity indexes in several autoimmune disorders, our data indicated that serum sCD30 levels did not correlate with BASDAI ($r = -0.11$, $P = 0.54$), ASDAS ($r = 0.02$, $P = 0.89$), BASFI ($r = -0.21$, $P = 0.23$), ESR ($r = 0.17$, $P = 0.33$), or CRP ($r = 0.15$,

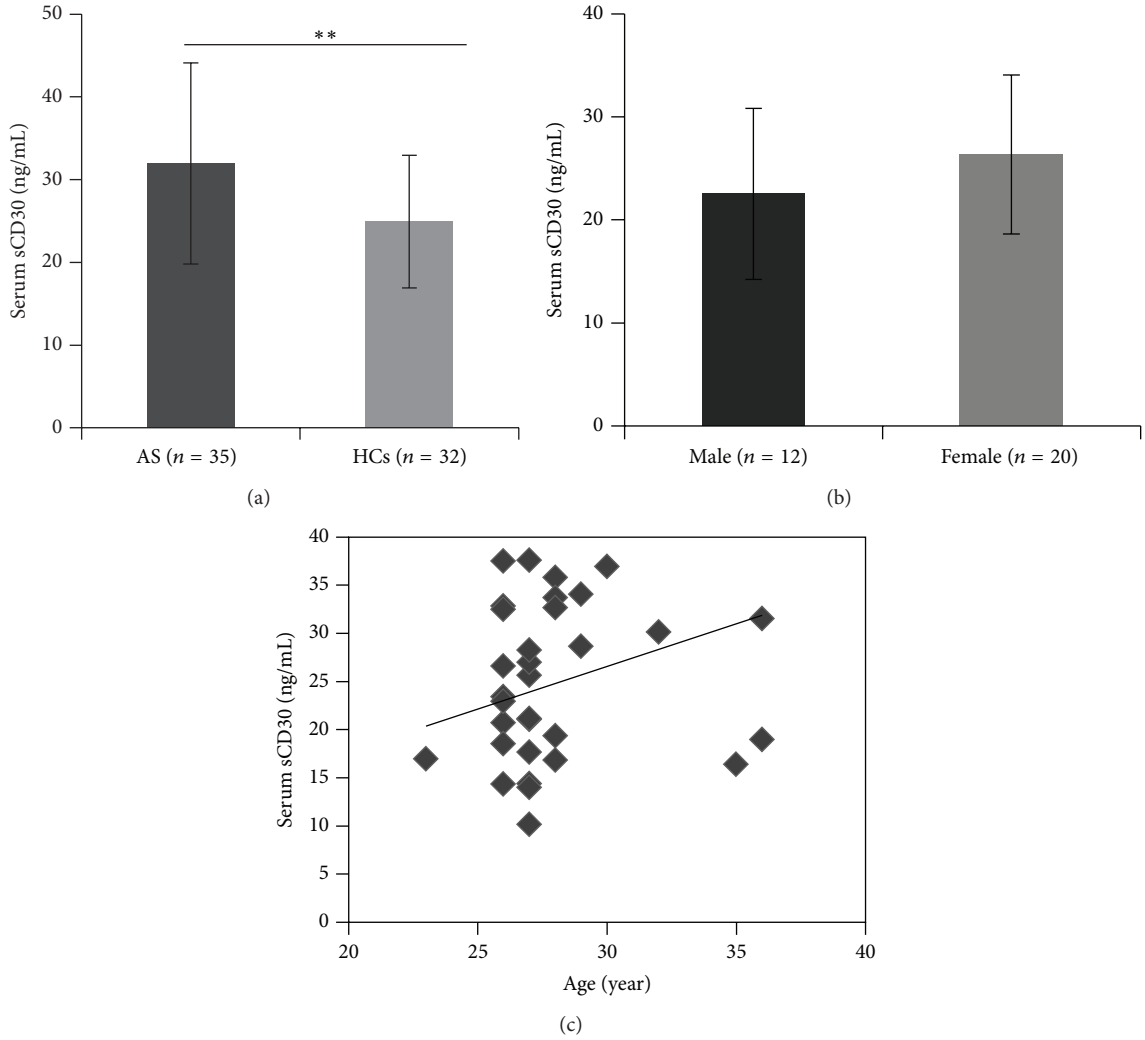

Figure 1: Serum levels of sCD30 in AS patients and healthy controls (HCs) were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (eBioscience). (a) Serum sCD30 levels in 35 AS patients and 32 healthy controls; (b) serum sCD30 concentrations in healthy male and female individuals; (c) the correlation of serum sCD30 levels with age in HCs ($r = 0.09$, $P = 0.63$). $^{**}P < 0.01$.

$P = 0.40$). However, positive correlations of ESR and CRP with BASDAI, ASDAS, and BASFI were observed (Table 2). In addition, no significant correlation was found between age and serum sCD30 levels in healthy individuals ($r = 0.09$, $P = 0.63$; Figure 1(c)).

3.2. Discussion. AS is a chronic inflammatory disease that has a poor understood etiology and pathogenesis of disease. Due to the inconspicuous symptoms of AS, patients are often diagnosed many years after the original onset. The delay may be between 5 and 7 years, and as a result patients often experience irreversible damage of joints [19]. Therefore, there is an urgent need for early diagnosis and proper treatment of AS. However, the poor understanding of its cellular and molecular mechanisms strongly limit improvements in the clinical strategies for treating AS. Evidence to date suggests that imbalances of immune cells and the resulting aberrant cytokine profiles are involved in the pathogenesis of AS [1–3].

Several studies have demonstrated the potential value of measuring serum sCD30 levels in diagnosing and monitoring serious pathological conditions, including diseases due to malignancy, autoimmunity, and viruses [4, 9–14, 20]. However, only one clinical trial to date has assessed serum sCD30 levels in AS patients [21]. Based on the results from other institutions as well as our previous data in systemic lupus erythematosus (SLE) [4, 9–14, 19], a similar phenomenon was expected in AS patients. Our data showed a statistically significant increase in serum sCD30 levels in AS patients compared to age-matched healthy controls (Figure 1(a)). However, in a previous clinical study by Østensen et al., no significant difference in serum sCD30 levels was observed between AS patients and healthy controls (AS: $n = 11$; HC: $n = 10$) [21]. Compared to that study, the numbers of AS

Table 2: Correlations between clinical and laboratory values in AS patients.

	BASDAI (r)	ASDAS (r)	BASFI (r)	ESR (mm/h) (r)	CRP (mg/L) (r)
Serum sCD30 (ng/mL)	−0.11	0.02	−0.21	0.17	0.15
ESR (mm/h)	0.35*	0.68**	0.39*	—	0.79**
CRP (mg/L)	0.41*	0.81**	0.42*	0.79**	—

AS: ankylosing spondylitis; BASDAI: bath AS disease activity index; ASDAS: ankylosing spondylitis disease activity score; BASFI: bath AS functional index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; *r:* correlation coefficient.
* $P < 0.05$; ** $P < 0.01$.

patients and HCs in our study were relatively higher, although only female participants were recruited in the previous study. In view of the fact that the number of males in the AS group of our study was notably higher than in the HCs group (Table 1) due to the male propensity of AS, we postulated whether this gave rise to the difference observed in the Østensen's study. Therefore, we compared serum sCD30 levels between healthy males ($n = 12$) and females ($n = 20$). We found no significant difference in serum sCD30 levels in the male and female HCs from our study ($P > 0.05$, Figure 1(b)), which was consistent with previous studies [22–24]. Furthermore, we found that serum sCD30 levels in healthy adults did not correlate with age (Figure 1(c)). Therefore, the evidence of a correlation between age and sCD30 levels remains controversial [23–26].

sCD30 was once identified as a marker of a T cell subtype that can produce Th2-type cytokines [23, 27, 28]. However, controversial results gradually emerged upon functional investigation of purified CD30+ T cells. For example, Pellegrini et al. reported that instead of a physiological marker of Th2 cells CD30 plays important roles in the regulation of the balance between Th1/Th2 cells by integrating Th1 and Th2 cell-specific cytokines and Bcl-2 expression. Inhibition of the CD30/CD30L interaction was proposed as a cause of equilibrium in the differentiation of Th0 to Th1 or Th2 cells. Moreover, studies have suggested that an abnormal increase of sCD30 levels can inhibit CD30 signals by blunting the CD30/CD30L interaction, which would subsequently lead to the Th1/Th2 imbalance [9, 23, 29–32]. Elevated sCD30 and soluble CD26 (sCD26) levels were previously shown by Mahmoudi et al. in patients with common variable immunodeficiency (CVID) and the authors proposed that the Th1/Th2 cell balance was impaired towards a Th1-like phenotype [32]. However, to the best of our knowledge, no study to date has investigated the Th1/Th2 cell balance induced by abnormal CD30 signaling in AS. Our present study did not investigate the numbers of CD30+ cells, other T cell subtypes, or their cytokines in both serum and involved tissues. Therefore, no conclusion can be drawn about whether sCD30 interferes with the Th1/Th2 cell balance and further studies are needed in this area.

Although significant correlations regarding serum sCD30 levels and disease severity-related indexes of various clinical disorders have been presented elsewhere [11, 12, 14], our results failed to find such a relationship in AS patients. Briefly, serum sCD30 levels did not correlate with BASDAI ($r = -0.11$, $P = 0.54$), ASDAS ($r = 0.02$, $P = 0.89$), BASFI ($r = -0.21$, $P = 0.23$), ESR ($r = 0.17$, $P = 0.33$), or CRP ($r = 0.15$, $P = 0.40$). However, all of the disease severity-related indexes showed remarkable positive correlations with ESR and CRP (Table 2), both of which are regarded as common markers of disease activity of AS [33, 34]. The most important limitation of the present study was the small sample size, which may have substantially attributed to the bias of the results. Moreover, the different disease stages of AS patients at the time of enrollment may have also led to unpredictable results. Nevertheless, a promising biomarker may yield important outcomes, even in a small cohort. Therefore, we propose that serum sCD30 is not a reliable biomarker in assessing disease activity of AS patients.

4. Conclusion

In summary, our results revealed an increase of sCD30 levels in AS patients, suggesting a potential role in the pathogenesis of AS. No correlation of sCD30 with BASDAI, ASDAS, or BASFI was observed in our study. Therefore, sCD30 cannot be used as a biomarker of disease severity and functional ability. A large prospective study is urgently needed to thoroughly investigate the precise roles of sCD30 in the pathogenesis of AS and the relationship among sCD30 and other indexes of disease severity.

Conflict of Interests

The authors declared that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Rongfen Gao and Wei Sun contributed equally to this work.

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Clinical Study

A Minimally Invasive Endoscopic Surgery for Infectious Spondylodiscitis of the Thoracic and Upper Lumbar Spine in Immunocompromised Patients

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This study evaluates the safety and effectiveness of computed tomography- (CT-) assisted endoscopic surgery in the treatment of infectious spondylodiscitis of the thoracic and upper lumbar spine in immunocompromised patients. From October 2006 to March 2014, a total of 41 patients with infectious spondylodiscitis underwent percutaneous endoscopic surgery under local anesthesia, and 13 lesions from 13 patients on the thoracic or upper lumbar spine were selected for evaluation. A CT-guided catheter was placed before percutaneous endoscopic surgery as a guide to avoid injury to visceral organs, major vessels, and the spinal cord. All 13 patients had quick pain relief after endoscopic surgery without complications. The bacterial culture rate was 77%. Inflammatory parameters returned to normal after adequate antibiotic treatment. Postoperative radiographs showed no significant kyphotic deformity when compared with preoperative films. As of the last follow-up visit, no recurrent infections were noted. Traditional transthoracic or diaphragmatic surgery with or without posterior instrumentation is associated with high rates of morbidity and mortality, especially in elderly patients, patients with multiple comorbidities, or immunocompromised patients. Percutaneous endoscopic surgery assisted by a CT-guided catheter provides a safe and effective alternative treatment for infectious spondylodiscitis of the thoracic and upper lumbar spine.

1. Introduction

In recent years, the incidence of infectious spondylodiscitis has increased due to vast improvements in medical care and prolonged life expectancies. The condition is associated with advanced age, intravenous drug use, immunocompromised status, and significant medical comorbidities [1]. Identifying the causative pathogen is the key to treatment. Computed tomography- (CT-) guided biopsy and drainage are the standard procedure for identifying causative pathogens. However, the pathogen-identification rate varies among studies [2–4]. Surgical intervention is indicated if neurological deficit, progressive deformity, failure to respond to conservative treatment, or the need to obtain specimens to identify causative pathogens is present. However, traditional anterior debridement and reconstruction with or without posterior instrumentation are associated with high rates of morbidity and mortality, especially in elderly immunocompromised patients and patients with multiple comorbidities. Percutaneous endoscopic discectomy, debridement, and drainage provide a minimally invasive surgical choice for the treatment of infectious spondylodiscitis [4–6]. This method provides adequate debridement and fast pain relief and has a relatively high pathogen-identification rate [4, 7, 8]. However, in the upper lumbar and thoracic spine, percutaneous endoscopy is associated with visceral organ damage, major vessel injury, and the spinal cord injury, which limits the use of this procedure in these areas [9].

A CT-guided catheter was placed before percutaneous endoscopic surgery as a guide to avoid injury to visceral organs, major vessels, and the spinal cord. We analyzed the clinical outcomes, inflammatory parameters, and radiographic findings for 13 lesions that occurred on thoracic or upper lumbar spine.

2. Materials and Methods

2.1. Patient Population. From October 2006 to March 2014, a total of 41 patients with infective spondylodiscitis underwent percutaneous endoscopic surgery with local anesthesia and 13 patients' lesions on the thoracic or upper lumbar spine were selected for evaluation (Table 1). A CT-guided angiographic catheter was placed before percutaneous endoscopic surgery as a guide to avoid injury to visceral organs, major vessels, and the spinal cord. Of the 13 patients evaluated, 5 were men and 8 were women. Their mean age was 65.6 years (range, 49–84 years). The affected level ranged from T11-T12 to L1-L2 in 11 cases, T8-T9 in 1 case, and T9-T10 in the other case. All patients underwent plain film radiography and enhanced magnetic resonance imaging (MRI) of the involved spine, which revealed evidence of infectious spondylodiscitis (Figure 1). Most patients had high inflammatory markers (C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)) and complained of severe back pain. They also had a variety of comorbidities, including renal failure, heart failure, rheumatic arthritis, liver cirrhosis, polycystic liver posttransplantation, and diabetes (Table 1).

2.2. Surgical Procedures. The CT-guided biopsy and catheter placement were performed by an experienced radiologist on the day of or the day before the scheduled percutaneous endoscopic surgery (Figure 2). The patient was positioned prone, and local anesthesia with 2% lidocaine was injected into the area of needle insertion. A 6-in-long number 11G-wide multiple side hole bone puncture needle was inserted into the lesion site with CT guidance. A J guidewire was then inserted via the bone biopsy needle. Finally, a number 5 Fr C1 angiographic catheter (Cook, Bloomington, USA) was inserted along the J guidewire and left in the infective area after guidewire removal. The specimen obtained during the procedure was sent for bacterial, tuberculosis (TB), and fungal cultures and pathologic analysis.

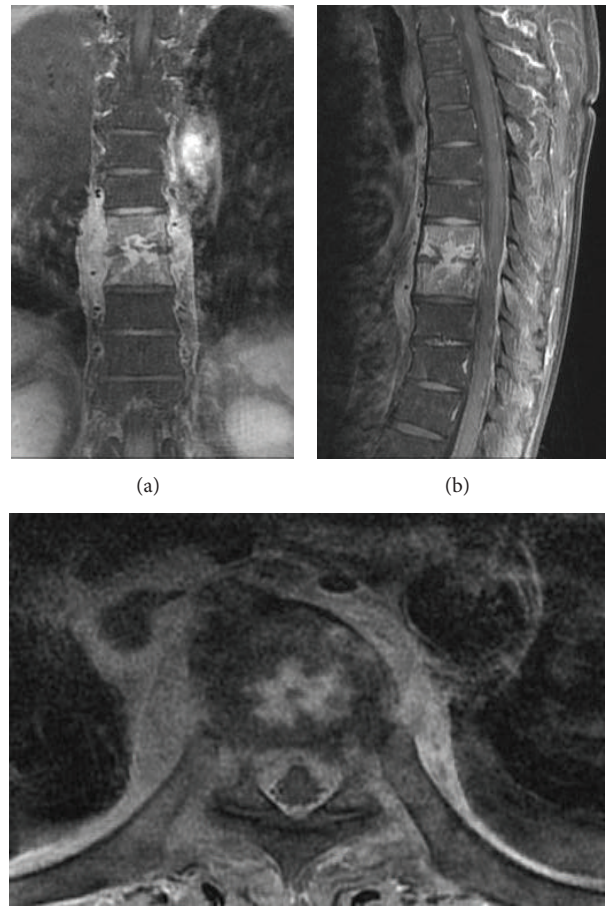
Percutaneous endoscopic surgery was then performed after placement of the CT-guided angiographic catheter. The patient was positioned prone on a spine-operating table with the abdomen hanging free. The patient was under intravenous pain control but was kept awake during the endoscopic surgery so that he or she could respond well when the dura or nerve roots were irritated. Local anesthesia was also performed with 2% lidocaine around the area of endoscopic insertion. A percutaneous endoscopic guidewire was inserted directly through the CT-guided number 5 Fr C1 angiographic catheter and advanced slowly with the assistance of fluoroscopy to ensure that the wire was targeting the infective area without penetrating the angiographic catheter wall and injuring any related structures. After the guidewire was set in the infected area, the number 5 Fr C1 angiographic catheter was then removed. A dilator was inserted along the endoscopic guidewire, and the position was again checked under fluoroscopy. The infected disc and vertebra were harvested for bacterial, fungal, and TB cultures by using endoscopic microrongeurs and microscissors before starting irrigation. After adequate tissue sampling for culture, radical debridement, sequestrectomy, and irrigation could be performed with the aid of direct endoscope vision and fluoroscopy. The Surgitron, a high-voltage bipolar probe (Ellman Innovations, New York, USA), was used for thermocoagulation of infected tissue and bleeders. All the operating instruments and endoscopic systems were supplied by Richard and Wolf (Knittlingen, Germany). The high-resolution endoscope has a diameter of 8 mm with a 4.1 mm intraendoscopic working channel. The angle of vision is 25°. The working sheath has an 8.0 mm outer diameter and a beveled opening, both of which enable the creation of visual and working fields in an area without a clear, anatomically preformed cavity. More than 4 L normal saline with 1 g cefazolin in each liter was used for pressured irrigation and drainage of infected materials and pus. A 1/4 in drainage tube was left in the infected area at the end of surgery for further drainage of infective materials, pus, and exudates (Figure 3).

2.3. Postoperative Care. The 1/4 in drainage tube was left in place for at least 7 days until the daily drainage amount was less than 5 mL. Effective antibiotics were administered intravenously for patients with known causative pathogens before surgery. For patients with unknown pathogens, empirical antibiotics were administered immediately after surgery.

Table 1: Patient demographic data.

Patient number	Age	Gender	Level	Neurological deficit	Associated medical illness
1	71	F	L1-2	Frankle D	Uremia, CHF, RHD
2	60	M	T11-12	Frankle D	CAD, DM, CHF, asthma
3	67	F	T12	Nil	Uremia, DM
4	65	F	L1-2	Nil	HTN, RA
5	55	F	T11-12	Nil	DM, HTN
6	73	F	T12-L1	Mild sensory deficit	DM
7	71	F	T11-T12	Nil	DM, liver cirrhosis, burst fracture T11 vertebra
8	71	M	L1	Frankle D	None
9	84	F	T9-10	Frankle D	HTN, CHF, PSVT
10	63	M	T12-L1-2	Mild sensory deficit	Liver cirrhosis, asthma
11	52	M	L1-2	Nil	BPH
12	49	F	L1-2	Nil	Polycystic liver, kidney s/p liver transplantation
13	72	M	T8-9	Mild sensory deficit	DM
Average	**65.6**				
Standard deviation	**9.73**				

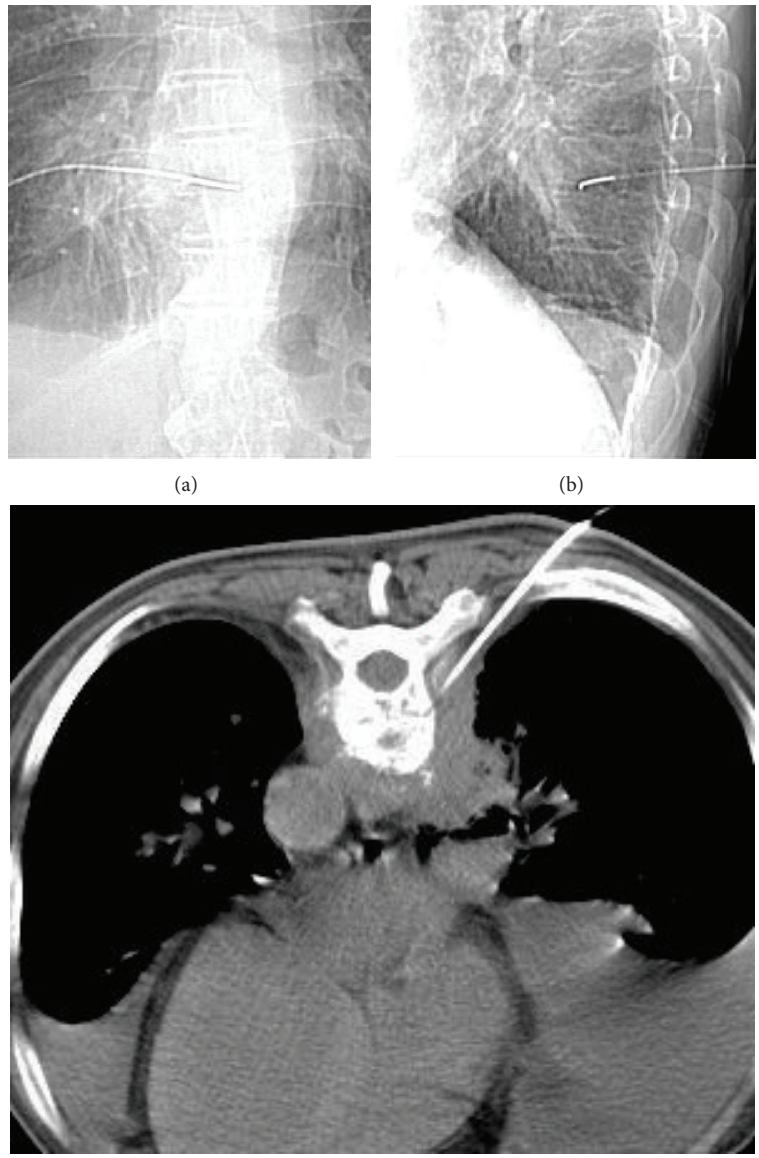
CHF: congestive heart failure, RHD: rheumatic heart disease, CAD: coronary artery disease, DM: diabetes mellitus, HTN: hypertension, RA: rheumatoid arthritis, PSVT: paroxysmal supraventricular tachycardia, and BPH: benign prostate hypertrophy.



(a) (b)

(c)

Figure 1: MRI evidence of T8-T9 infectious spondylodiscitis in patient number 13 in (a) coronal, (b) sagittal, and (c) axial view. T1-weighted signal with gadolinium contrast of spine MRI showed increased signal at the T8 and T9 vertebral bodies and also ring enhancement of the destroyed T8-T9 intervertebral disc.



(a) (b)

(c)

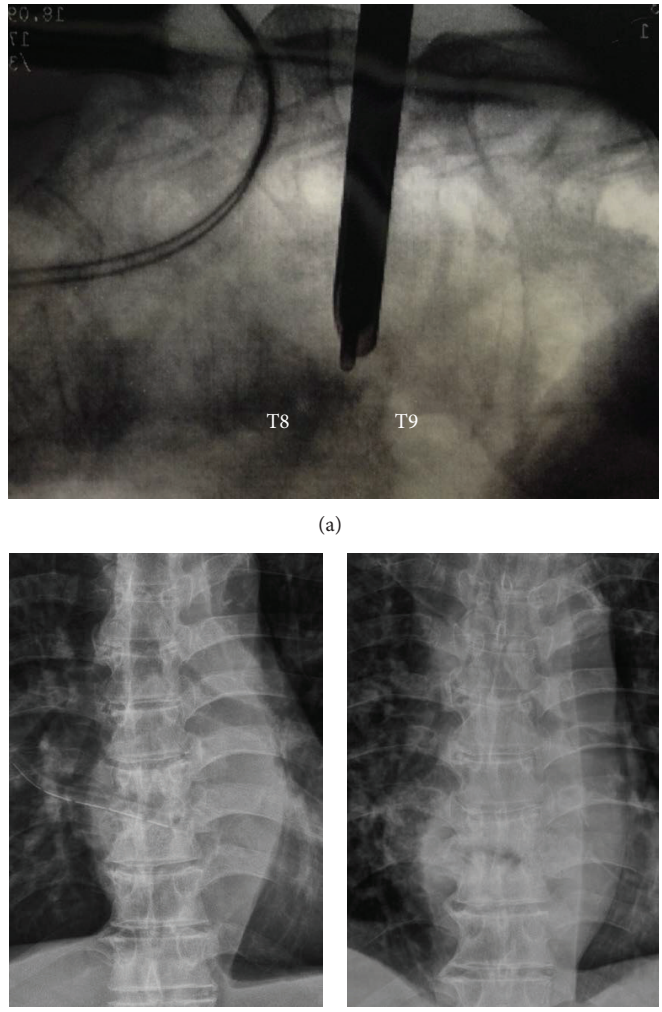
Figure 2: CT-guided catheterization in T8-T9 intervertebral disc: (a) anteroposterior, (b) lateral projection of the catheter during the procedure, and (c) axial illustration of the catheter in the intervertebral disc with CT-guided procedure.

These were switched to specific antibiotics after identification of the causative pathogen was made from intraoperative tissue or pus culture. Intravenous antibiotics were used for 4 to 6 weeks according to follow-up inflammatory markers. The patients were then switched to oral antibiotics and discontinued when the inflammatory markers were within a normal range. The patient remained in bed for 2 weeks after surgery. A rigid thoracolumbar spinal orthosis was then used for ambulation. The orthosis was used until radiographs showed evidence of bone union or prominent syndesmophyte formation along the anterior lateral aspect of the infected level.

2.4. Clinical Evaluation. Preoperative clinical symptoms and signs were recorded. Pain was evaluated with a visual analogue scale (VAS; 0–10) before and after surgery at 1 month, 3 months, and 6 months. The ESR and CRP levels were checked before surgery and every week after surgery. Spine plain radiography was performed immediately after surgery and at 1 month, 3 months, 6 months, and 1 year after surgery. Any evidence of spinal kyphotic deformity due to infectious spondylodiscitis was recorded.

3. Results

All patients had prominent back pain (VAS = 9.23) before surgery, and all reported quick pain relief (VAS = 2.31) after endoscopic surgery and antibiotic treatment (Table 4). The causative pathogens were identified in 10 patients (77% culture rate) postoperatively (Table 2), and effective



(a)

(b) (c)

Figure 3: (a) Intraoperative fluoroscopic image demonstrated the working sheath and endoscope within the intervertebral disc space, (b) immediate postoperative AP view demonstrated a 1/4 inch drainage tube in the intervertebral space, and (c) postoperative 3-month AP view revealed partially united T8-T9 vertebral body.

Table 2: Surgical procedures.

Patient	Level	Procedures	Bacteria culture
1*	L1-L2	CT-guided catheter + PEDD	*Delftia acidovorans*
2*	T11-T12	CT-guided catheter + PEDD	No growth
3	T12	CT-guided catheter + PEDD	No growth
4	L1-L2	CT-guided catheter + PEDD	*Escherichia coli* (ESBL)
5	T11-T12	CT-guided catheter + PEDD	*Staphylococcus aureus*
6	T12-L1	CT-guided catheter + PEDD	*Staphylococcus aureus* (MRSA)
7	T11-T12	CT-guided catheter + PEDD	No growth
8*	L1	CT-guided catheter + PEDD	*Streptococcus anginosus*
9	T9-10	CT-guided catheter + PEDD	*Mycobacteria tuberculosis* complex
10	T12-L1-2	CT-guided catheter + PEDD	*Klebsiella pneumoniae*
11	L1-L2	CT-guided catheter + PEDD	*Staphylococcus aureus*
12	L1-L2	CT-guided catheter + PEDD	*Candida albicans, Ecoli*
13	T8-9	CT-guided catheter + PEDD	*Klebsiella pneumoniae*

PEDD: percutaneous endoscopic discectomy drainage.
*Dying of other medical problems during next few years.

Table 3: ESR and CRP levels before and after surgery.

Patient number	ESR (mm/1 hr)				CRP (mg/dL)		
	Preop	Postop 1 m	Postop 3 m	Last F/U	Preop	Postop 1 m	Postop 3 m
1	51	67	20	37	6.62	0.38	2.84
2	76	18	21	13	22.16	0.15	0.18
3	115	86	78	72	32.11	2.70	0.60
4	79	41	9	8	4.96	0.45	0.03
5	108	71	25	21	6.33	0.31	0.08
6	123	115	64	36	23.72	1.24	0.55
7	112	93	86	84	1.44	0.18	0.29
8	75	42	14	7	18.19	2.80	0.95
9	53	28	18	28	6.61	1.94	0.75
10	125	85	37	30	0.19	0.07	0.06
11	92	16	2	2	7.45	0.23	0.12
12	108	74	50	9	4.84	0.70	0.11
13	104	12	11	11	16.53	0.25	0.35
Average	93.02	57.53	33.46	27.53	11.63	0.88	0.53
Standard deviation	25.00	33.44	27.51	25.26	9.85	0.98	0.75

antibiotics were administered according to the sensitivity test of isolated pathogens. The blood culture result of patient 7 was methicillin-sensitive *Staphylococcus aureus* infection, but the results of the endoscopic specimen were negative. Oxacillin was used for infection control according to the sensitivity test report of blood culture. The other 2 patients had no known culture results, and empirical antibiotics were maintained until the inflammatory parameters returned to normal ranges. In patient 9, anti-TB medication was maintained for 9 months according to TB treatment protocol. The ESR and CRP levels of all patients decreased significantly after endoscopic surgery and 3 months of intravenous and oral antibiotic treatment. No patient had relapse of spinal infection at the treated level during the follow-up period (Table 3).

No surgery-related complications were noted during the follow-up period (average, 42.46 months; range, 8–70 months). No recurrence of infection developed in these patients, and no open spinal surgery was needed. Deformity of the spine was evaluated from plain film radiographs obtained before and after surgery and at the last follow-up. The kyphotic angle was measured as the angle between the upper end plate of the first vertebral body above the involved level and the lower end plate of the first vertebral body below the involved level. Only 1 patient had a kyphotic change greater than 10°. The other 12 patients had no significant changes in spinal deformity (Table 5; Figure 4).

Table 4: Back pain level before and after surgery.

Patient number	Visual analog scale (0–10)			Complication
	Preop	Postop 1 m	Postop 3 m	
1	10	5	2	Nil
2	9	4	2	Nil
3	10	5	3	Nil
4	10	4	1	Nil
5	10	5	4	Nil
6	10	5	3	Nil
7	10	4	3	Nil
8	9	5	1	Nil
9	9	4	3	Nil
10	10	5	4	Nil
11	8	1	1	Nil
12	8	3	2	Nil
13	7	3	1	Nil
Average	9.23	4.08	2.31	
Standard deviation	1.01	1.19	1.11	

4. Discussion

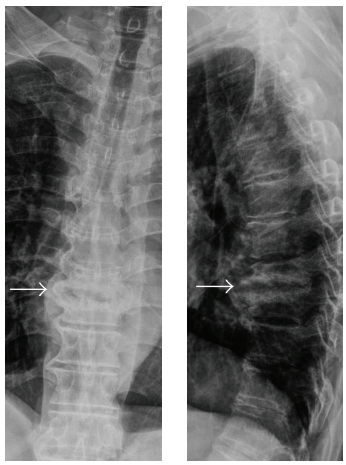
Infectious spondylodiscitis is an increasingly prevalent disease, especially in elderly or immunocompromised patients or those with medical comorbidities. Conservative treatment consisting of antibiotic administration and bed rest is the standard choice in cases of mild infection. CT-guided biopsy is a less invasive procedure used to obtain specimens for pathogen identification. Drainage function also contributes to infection control. However, the rate of pathogen identification varies widely, from 36% to 91% [2–4, 10–12], and the rate of successful infection control is also unsatisfactory. When conservative treatment fails, the treatment approach shifts to open surgery. However, traditional anterior debridement and reconstruction with or without posterior instrumentation are a major operation with a high rate of postoperative complications, especially in immunocompromised patients with many comorbid diseases or in the elderly.

Percutaneous endoscopic discectomy was first applied for lumbar disc herniation in the 1980s and now is a well-established surgical procedure. This procedure has also been used to treat infectious spondylodiscitis in recent years and

Table 5: Changes in kyphosis angle (°).

Patient number	Level	Preoperative	Postoperative	Last F/U
1	L1-2	−6°	−2°	−1°
2	T11-12	10°	8°	10°
3	T12	16°	19°	19°
4	L1-2	2°	−2°	−1°
5	T11-12	6°	4°	1°
6	T12-L1	13°	13°	24°
7	T11-12	11°	14°	12°
8	L1	12°	5°	4°
9	T9-10	0°	3°	3°
10	T12-L1-2	−13°	−12°	−12°
11	L1-2	7°	8°	12°
12	L1-2	17°	16°	16°
13	T8-9	12°	14°	15°
Average		8.69	6.77	7.85
Standard deviation		6.68	8.72	9.91

Kyphosis angle was obtained by measuring the sagittal angles with the Cobb method.



(a) (b)

Figure 4: The plain films 6 months after operation. (a) AP view and (b) lateral view revealed prominent syndesmophyte formation at anterior and lateral aspects of infective area without significant kyphotic deformity.

has proved to be as effective as open surgery for infection control [4, 6, 8, 13]. However, percutaneous endoscopic surgery for infectious spondylodiscitis has been limited to cases with lower lumbar spine infection for reasons of operative safety [7]. Percutaneous endoscopic surgery carries risks of injury to the surrounding visceral organs, the spinal cord, and major vessels of the thoracic and upper lumbar spine when used to treat lesions above the L2 level. In this study, a combination of a CT-guided angiographic catheter and percutaneous endoscopic surgery was found to be effective and safe for the treatment of infectious spondylodiscitis of the upper lumbar and thoracic spine. CT provides real-time images that can be used to insert the guidewire and catheter, which helps prevent injury to the spinal cord, major vessels, or visceral organs. Once the endoscope is inserted safely along the CT-guided catheter, percutaneous endoscopic surgery can be performed safely to carry out radical debridement or obtain a sufficient specimen culture, and a large-diameter drainage tube can be left in the infected level. Infection was well controlled with antibiotics in all patients, regardless of the culture results. No complications occurred during the operation or follow-up period. Moreover, no open surgery was needed in these patients, whereas anterior debridement and fusion surgery was previously performed in 19% to 25% of patients [4, 6].

In view of the risks of general anesthesia and traditional major surgery for infectious spondylodiscitis of the thoracic and upper lumbar spine, minimally invasive endoscopic surgery represents a good alternative treatment for immunocompromised patients. Percutaneous endoscopic surgery assisted by a CT-guided catheter is a safe and effective operation using local anesthesia and a small incision wound (<1 cm).

5. Conclusion

Percutaneous endoscopic discectomy, debridement, irrigation, and drainage with the assistance of a CT-guided catheter are a safe and effective alternative treatment for infectious spondylodiscitis of the thoracic and upper lumbar spine. It prevents many complications normally associated with percutaneous endoscopic surgery in the thoracic and upper lumbar area. Endoscopic surgery not only has a high rate of the causative-pathogen identification but also provides good infection control and pain relief, even if causative pathogen cannot be identified. The safe and minimally invasive nature of this procedure broadens the application of operative treatment for infectious spondylodiscitis, even in the thoracic and upper lumbar level, and prevents progressive deformity when prolonged antibiotic treatment is used. We have more confidence when treating patients in poor health, immunocompromised or elderly patients, and can now provide a more promising and safe treatment strategy. Motor weakness of the lower limbs may be the only contraindication for this procedure.

Conflict of Interests

The authors declare no conflict of interests regarding the publication of this paper.

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Research Article

Combined Home Exercise Is More Effective Than Range-of-Motion Home Exercise in Patients with Ankylosing Spondylitis: A Randomized Controlled Trial

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Home exercise is often recommended for management of patients with ankylosing spondylitis (AS); however, what kind of home exercise is more beneficial for patients with AS has not been determined yet. We aimed to compare the effectiveness of combined home exercise (COMB) and range-of-motion home exercise (ROM) in patients with AS. Nineteen subjects with AS completed either COMB ($n = 9$) or ROM ($n = 10$) program. The COMB program included range-of-motion, strengthening, and aerobic exercise while the ROM program consisted of daily range-of-motion exercise only. After exercise instruction, subjects in each group performed home exercise for 3 months. Assessment included cardiopulmonary exercise test, pulmonary function test, spinal mobility measurement, chest expansion, Bath Ankylosing Spondylitis Functional Index (BASFI), and other functional ability and laboratory tests. After exercise, the COMB group showed significant improvement in peak oxygen uptake (12.3%, $P = 0.008$) and BASFI ($P = 0.028$), and the changed score between pre- and postexercise data was significantly greater in the COMB group regarding peak oxygen uptake and BASFI. Significant improvement in finger-to-floor distance after 3-month exercise was found only in the COMB group ($P = 0.033$). This study demonstrates that a combined home exercise is more effective than range-of-motion home exercise alone in aerobic capacity and functional ability.

1. Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disorder mainly involving the sacroiliac joints and spine, although peripheral joints may also be involved. Inflammation of ligament or tendon insertion at the bone (enthesopathy) is also a characteristic finding. The disease can be accompanied by extraskeletal manifestations, such as acute anterior uveitis, aortic incompetence, cardiac conduction defects, fibrosis of the upper lobes of the lungs, neurological involvement, or renal amyloidosis [1]. In a recent report, patients with AS were at increased risk for cardiac morbidity including coronary artery disease [2]. The prevalence of AS is 0.15% to 0.86% [1].

The main biomechanical problems in patients with AS include limitations in spinal and peripheral joint mobility, restriction of chest expansion [3], reduction of vital capacity

[4, 5], and deterioration of aerobic capacity [6]. Carter et al. showed that peak oxygen uptake ($\dot{V}O_{2\,peak}$) was significantly lower (75% of normal) in patients with AS [6].

The main treatment for AS since the 1960s has been medications and exercise to maintain spinal mobility and function [1]. It has been shown that 2–4 weeks of intense inpatient treatment yields significant improvement of mobility and pain and that the benefit may persist for months or years [7, 8]. Because many patients with AS cannot receive inpatient exercise training, many exercises therapies via outpatient department or even home exercise have been conducted. van Tubergen et al. found that patients with AS receiving spa and weekly group therapy (including physical exercise, sports, and hydrotherapy) for 40 weeks showed improvement in functional ability and quality of life [9]. Ince et al. also reported the benefit of multimodal supervised exercise programs [10]. Helliwell et al. randomized 44 patients with AS to receive (a) intensive inpatient physiotherapy, (b) outpatient hydrotherapy and home exercise, or (c) home exercise alone. Both inpatient and hydrotherapy patients reported more subjective improvement; however, at six months, there were no differences in outcomes between the three groups [8]. In 2000, Uhrin et al. also showed that even unsupervised recreational exercise improves pain and stiffness [11]. ASAS/EULAR also suggested that optimal management requires a combination of nonpharmacological and pharmacological treatments, and home exercise was listed in category IIa in evidence of efficacy [12].

Because most patients with AS in our country are employed, inpatient or regular outpatient exercise program may be not feasible for many patients with AS. For most patients with AS, home exercise is more convenient and more likely to be continued for a long period of time. In addition, for patients with AS, previous studies emphasized range-of-motion exercise, and aerobic exercise was often neglected [13]. Literature review also showed that comparing between different home exercise programs in patients with AS has rarely been reported and, as far as we know, has never been reported in oriental population.

The purpose of this study was to compare the effectiveness of combined home exercise (COMB, including range-of-motion, strengthening, and aerobic exercise) and range-of-motion home exercise (ROM) in Taiwanese patients with AS.

2. Methods

2.1. Participants. Forty-four adult subjects with AS were recruited from the outpatient clinics of allergy-immunology-rheumatology (AIR) and physical medicine and rehabilitation (PM and R) in a private teaching hospital and an AS care group (a society organized by patients with AS in Taiwan). Inclusion criteria were as follows: (1) fulfilling the 1984 modified New York criteria for AS [14]; (2) age between 20 and 65 years; (3) disease in well-controlled condition; and (4) disease lasting for at least 6 months. Exclusion criteria included (1) presence of serious medical conditions or acute febrile disorders; (2) history of arthroplasties or major operations in the knee or hip joints; and (3) severe arthritis or contracture of knee or hip joints which preclude exercise testing with a bicycle. Use of concomitant medications was allowed, and no instructions were given to subjects to alter their daily activity except regarding the prescribed home exercise program.

Table 1: Demographic data of the study subjects.

Characteristic	COMB group ($n = 9$)	ROM group ($n = 10$)	P value*
Age, years, mean (SD)	36.2 ± 11.7	42.1 ± 8.8	0.219
Gender (M/F)	6/3	7/3	1.000
BW, kg, mean (SD)	64.0 ± 12.0	63.5 ± 9.9	0.935
BH, cm, mean (SD)	164.1 ± 7.8	160.5 ± 8.4	0.461
BMI, kg/m^2, mean (SD)	23.8 ± 7.8	24.7 ± 3.7	0.462
Disease duration, years, mean (SD)	11.1 ± 6.8	17.3 ± 10.7	0.164
Smoking (yes/no)	1/8	3/7	0.582
Marriage (yes/no)	5/4	5/5	1.00
Regular exercise (yes/no)	0/9	0/10	1.00
Medication			
NSAID (yes/no)	9/0	10/0	1.00
DMARD (yes/no)	6/3	7/3	1.00

COMB: combined home exercise; ROM: range-of-motion home exercise; BW: body weight; BH: body height; BMI: body mass index; NSAID: nonsteroidal anti-inflammatory drug; DMARD: disease-modifying agent. *P values for differences in the baseline data between the COMB and the ROM groups.

Participants who met the inclusion criteria were then scheduled for interviews and testing. Before study enrollment, all participants signed a consent form approved by the hospital ethics committee.

Of the 44 subjects with AS screened for the study, 3 had coronary artery disease, 2 had received total hip replacement, 5 refused to sign informed consent, 9 were busy in working or had home problems, and 3 were excluded due to illness or other causes, so that a total of 22 patients were randomized to the 2 home exercise programs. However, 2 in the COMB group and 1 in the ROM group did not complete the study due to personal reasons. Totally, 19 subjects with AS completed the study. Randomization was performed by a computer-generated random-number list. The allocation of the groups was initially concealed in an envelope, which was opened for each consecutive patient to reveal his or her group assignment at the time he or she was recruited into the study. A group of 9 subjects (mean age 36.2 years, standard deviation (SD) 11.7 years) served as the COMB group, and another 10 subjects (mean age 42.1 years, SD 8.8 years) comprised the ROM group (Figure 1).

The demographic data of the study subjects are shown in Table 1. There was no significant statistical difference between the COMB and ROM groups with regard to age, gender, body weight, body height, disease duration, smoking, marriage, exercise habit, and use of medications. None of the subjects participated in regular exercise prior to the study. All subjects were taking nonsteroidal anti-inflammatory drugs

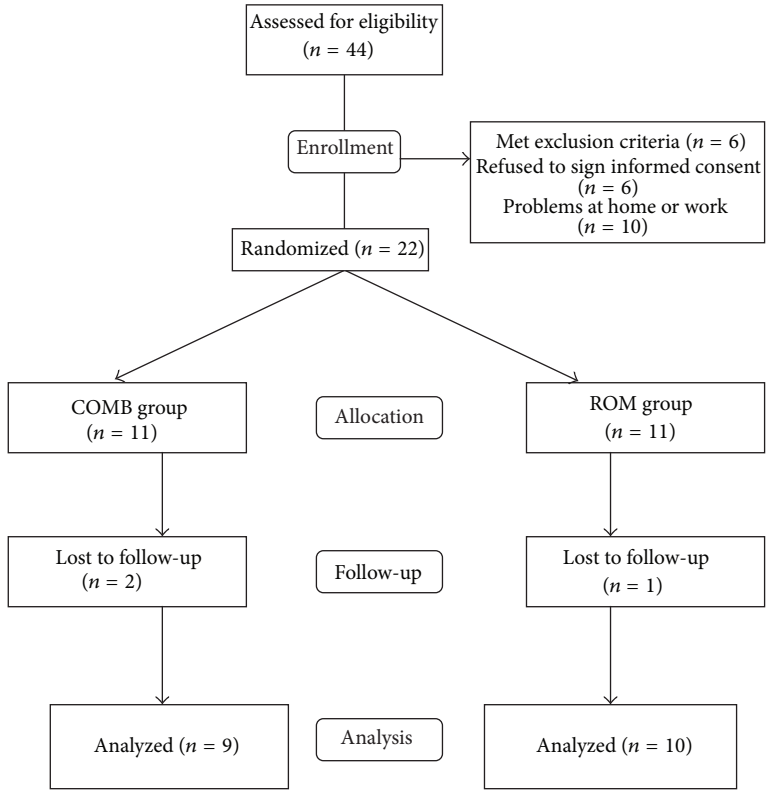

Figure 1: Flowchart for randomization procedure.

(NSAIDs), and most of them were also taking remittive agents. None of the subjects were treated with biologic agents.

2.2. Intervention. Subjects in the ROM group received instruction in range-of-motion exercise of the spine and major joints (including the shoulder, elbow, wrist, hip, knee, and ankle) from a senior physical therapist. Chest expansion and breathing exercise were also included. An exercise booklet was also given to each subject. After participants learned how to perform the range-of-motion exercise, they are instructed to conduct exercise at home daily for 3 months. Each range-of-motion exercise was repeated 5 times. Each subject was instructed to perform gentle stretch to tightness at end of the range-of-motion but not to pain.

The COMB group received not only range-of-motion exercise, but also strengthening of the muscles of the major joints (including the cervical spine, thoracolumbar spine, shoulder, elbow, wrist, hip, knee, and ankle) and aerobic exercise (including fast walking, cycling, and swimming as suggested). Each set of strengthening exercises consisted of 10 repetitions, and the intensity was set at 60% to 80% of one repetition maximum [15]. Each subject was asked to perform two sets of strengthening exercises each time, 2 times per week. A rest interval between sets was 2 to 3 minutes. Aerobic exercise program consisted of 5 min stretching of the exercise muscles, 5 min warm-up, 20–30 min aerobic exercise, and 5 min cooling-down. The intensity of aerobic exercise was set between 50% and 80% of $\dot{V}O_{2\,peak}$ (peak oxygen uptake). Each subject in the COMB group was requested to perform aerobic exercise 3 times per week. The COMB program was also continued for 3 months. Participants in each program were instructed to use daily exercise logs for self-monitoring of the duration, intensity, and frequency of exercise. During the study period, a physical therapist was assigned to monitor the progress of the exercise program by calling each subject every 2 weeks. Compliance with the exercise program was assessed by actual exercise frequency divided by the predicted frequency.

2.3. Assessment. Besides background information, spinal mobility (including Schober's test, finger-to-floor distance, occiput-to-wall distance, and range-of-motion of the cervical spine), chest expansion, exercise tolerance test, pulmonary function test, grip strength, Bath Ankylosing Spondylitis Global Score (BAS-G), Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Disease Activity Index (BASDI), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and hemoglobin (Hb) were measured at the baseline and immediately after 3-month exercise. Throughout the study, the evaluators did not know the assigned group of each subject. Peak oxygen consumption ($\dot{V}O_{2\,peak}$), finger-to-floor distance, chest expansion, and BASFI were chosen as primary outcome measures according to previous studies [16].

2.3.1. Background Information. Each subject was requested to fill out a self-report data form containing questions about age, gender, body weight, body height, symptom duration,

smoking history, marital status, exercise habit, occupational activities, recreational activities, medications, and health history.

2.3.2. Schober's Test. It was an increase in distance between 2 skin marks between the fifth spinal process and 10 cm above from erect standing to maximal forward bending [17].

2.3.3. Finger-to-Floor Distance. It was the shortest distance between fingers and floor on maximal forward flexion of the low back, with knees straight [18].

2.3.4. Occiput-to-Wall Distance. When the patient was standing with buttocks and heels against a wall and trying to touch the wall with the occiput while keeping a horizontal gaze, the distance between the occiput and the wall is measured [18].

2.3.5. Range-of-Motion of the Cervical Spine. Flexion, extension, bilateral rotation, and bilateral side bending of the cervical spine were measured with a special goniometer (CROM) [19].

2.3.6. Chest Expansion. It was measured with a tape at the level of the 4th intercostal space. The difference between maximal inspiration and maximal expiration was calculated [18].

2.3.7. Cardiopulmonary Exercise Test. Exercise tolerance of the subjects was measured by open-circuit spirometry. The test was performed on a bicycle ergometer with the participant in an upright position. It was started with an initial load of 0 watts, with an increment of 10–20 watts/min until exhaustion or appearance of symptoms. BP, ECG, HR, and oxygen saturation were monitored during the test. A physiatrist was present during all testing. Expired gas was analyzed by an automated system instrument (Vmax 29 Cardiopulmonary Exercise Testing Instrument, SensorMedics Corporation, Yorba Linda, California). Variables of exercise tolerance test included HR, BP, oxygen uptake ($\dot{V}O_2$), metabolic equivalent (MET), work, oxygen pulse, and respiratory exchange ratio (RER) at peak cardiovascular response and at ventilatory threshold (VT) [20, 21].

2.3.8. Pulmonary Function Test. Pulmonary function tests included measurement of the forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1/FVC, peak expiratory flow (PEF), total lung capacity (TLC), residual volume (RV), RV/TLC, and functional residual capacity (FRC).

2.3.9. Grip Strength. It was measured with a hand dynamometer in the dominant hand.

2.3.10. BAS-G. It is a single item question regarding a patient's sense of well-being over the last week and the past six months. The mean of the two scores gives a BAS-G score of 0 (the best) to 10 (the worst) [22].

2.3.11. BASFI. It contains 10 questions assessing activities of daily living and is scored on a 10 cm visual analogue scale (VAS). A final score is obtained by calculating the mean of the 10 items [23].

2.3.12. BASDAI. It consists of 6 questions relating to fatigue, back pain, pain and/or swelling of peripheral joints, localized tenderness, and duration and severity of morning stiffness in the previous week. Each question is answered with a 10 cm VAS and the total score (0 to 10; 0 = the best, 10 = the worst) is calculated according to the instructions [24].

2.3.13. Laboratory Tests. ESR, CRP, and Hb were measured for evaluation of disease activity.

2.4. Data Analysis. For demographic data, independent t-test or Mann-Whitney U test (if distribution was nonnormal) was used for continuous variables, and chi-square test or Fisher's exact test was performed for categorical variables. For between-group comparison, independent-sample t-tests were conducted to investigate if there were any differences in the baseline data as well as the changed score between the baseline and the postexercise data between the COMB and the ROM groups. When the assumption of normality or equality of variance was not met, Mann-Whitney U test was performed instead. For within-group comparison, we used paired-sample t-test or Wilcoxon signed-rank test if the assumption of normality was not met to evaluate whether postexercise data was significantly different from the baseline data in either the COMB or the ROM group. Based on two independent-sample groups (mean differences and their variances) with $\alpha = 0.05$, 2 tails, and sample size of each group being 9 and 10, respectively, powers were calculated. The power was sufficient for occiput-to-wall distance (98%), cervical rotation to the left (97%), Schober's test (92.9%), $\dot{V}O_2$ % of standard (98%), FEV1/FVC (98%), and BASFI (95%). Statistical analyses were performed using SPSS 15.0 for Windows. A value of $P < 0.05$ was used as an indicator of statistical significance.

3. Results

The mean compliance with the exercise program in the COMB group was 48%, while the ROM group had a mean compliance with exercise of 54%. There was no significant statistical difference in compliance between the two groups.

In comparison of spinal range-of-motion and chest expansion, no significant statistical differences between the COMB and ROM groups at baseline were observed except for cervical extension (Table 2), which was more limited in the ROM group ($P < 0.05$). Within-group comparison between baseline and postexercise showed significant improvement in finger-to-floor distance only in the COMB group ($P = 0.033$). However, there was no significant difference between the COMB and ROM groups with regard to changed score between the baseline data and the postexercise data in the spinal range-of-motion and chest expansion.

Table 2: Comparison of chest expansion and spinal range of motion at the baseline and after 3-month exercise between combined home exercise (COMB) group and range-of-motion home exercise (ROM) group.

Measures	COMB group ($n = 9$)			ROM group ($n = 10$)			Both groups
	Baseline mean (SD)	Postexercise mean (SD)	Within-group $^{*}P$ value	Baseline mean (SD)	Postexercise mean (SD)	Within-group $^{\dagger}P$ value	Between-groups $^{\ddagger}P$ value
Schober's test	3.3 ± 2.0	3.8 ± 2.3	0.260	1.4 ± 1.1	2.2 ± 2.0	0.092	0.567
Finger-to-floor distance, cm	19.9 ± 13.8	14.9 ± 12.7	0.033	28.2 ± 12.3	25.4 ± 12.8	1.000	0.141
Chest expansion, cm	2.4 ± 1.6	2.6 ± 0.8	0.553	1.7 ± 2.1	2.5 ± 1.9	0.059	0.391
Occiput-to-wall distance, cm	4.9 ± 5.1	2.7 ± 5.9	0.223	9.7 ± 9.2	9.8 ± 9.4	0.680	0.665
C-ext, degree	41.9 ± 17.7	44.9 ± 23.8	0.596	23.7 ± 17.7*	28.7 ± 25.9	0.497	0.967
C-flex, degree	37.4 ± 19.3	37.8 ± 18.7	1.000	23.7 ± 15.8	26.4 ± 17.4	0.400	0.615
C-LR, degree	48.1 ± 28.1	53.3 ± 25.0	0.236	32.5 ± 26.4	35.2 ± 21.9	0.482	0.836
C-RR, degree	45.9 ± 26.2	52.6 ± 24.6	0.202	30.0 ± 27.4	34.8 ± 24.9	0.778	0.681
C-LSB, degree	27.1 ± 17.2	31.9 ± 21.5	0.446	18.6 ± 17.9	17.6 ± 17.9	0.610	0.283
C-RSB, degree	33.8 ± 20.9	34.1 ± 22.2	0.779	16.2 ± 17.6	18.6 ± 19.0	0.113	0.362

C-ext: cervical extension; C-flex: cervical flexion; C-LR: left rotation of the cervical spine; C-RR: right rotation of the cervical spine; C-LSB: left side bending of the cervical spine; C-RSB: right side bending of the cervical spine. $^{*}P$ values for differences in the baseline data between the COMB and the ROM groups; $^{\dagger}P$ values for differences in the postexercise data between the COMB and the ROM groups; $^{\ddagger}P$ values for differences in the changed score between the baseline and the postexercise data between the COMB and the ROM groups.

The cardiopulmonary exercise variables at baseline and after 3-month exercise in both the COMB and the ROM groups are shown in Table 3. There was no significant difference in the baseline data of cardiopulmonary exercise test between the two groups. For within-group comparison of exercise tolerance test variables, significant improvement regarding $\dot{V}O_2$, $\dot{V}O_2$ of standard, metabolic equivalent (MET), and HR at peak cardiovascular response and $\dot{V}O_2$, MET, and HR at ventilatory threshold were found in the COMB group; however, significant reduction of $\dot{V}O_2$, $\dot{V}O_2$% of standard, and MET at the peak cardiovascular response and increase of resting HR were found in the ROM group. On comparison of the changed scores between the baseline data and after 3-month exercise data, the COMB group displayed significantly greater improvement in terms of $\dot{V}O_2$, $\dot{V}O_2$ of standard, and MET at peak cardiovascular response and $\dot{V}O_2$, MET at ventilatory threshold.

Table 4 displays comparison of pulmonary function test between the two groups. Either at baseline or after 3-month exercise, no significant difference was observed between the two groups in terms of variables of pulmonary function test. For within-group comparison of the exercise effect, no significant statistical difference was demonstrated in each group except for peak expiratory flow (PEF) in the COMB group. For between-group comparison regarding the changed score between the baseline data and the postexercise data, no significant statistical difference was observed in all of the pulmonary function test data.

On the follow-up of disease activity and functional ability, no significant statistical difference was found between the two groups in ESR, CRP, Hb, grip strength, BAS-G, BASFI, and BASDAI, either at baseline or after 3-month exercise (Table 5). Within-group comparison showed significant improvement ($P = 0.028$) in BASFI after 3-month exercise program (Table 5) only in the COMB group. Between-group comparison also demonstrated significant statistical difference ($P = 0.041$) in changed score of BASFI between the baseline data and postexercise data, in favor of the COMB group.

4. Discussion

Our study showed that Taiwanese patients with AS participating in combined home exercise (range-of-motion, strengthening, and aerobic exercise) could improve aerobic capacity as well as BASFI. In this study, the average improvement rate in $\dot{V}O_{2\,peak}$ was about 12%. On the contrary, patients with AS in the ROM group had some decrease in $\dot{V}O_{2\,peak}$. However, one subject in the ROM group had anemia (Hb = 9.0 gm/dL) for unknown reason, two reduced physical activities due to too much engagement in working, and none of the subjects in the ROM group participated in aerobic or strengthening exercise, which could partly explain the cause of aerobic capacity reduction.

For exercise prescription in patients with AS, previous studies emphasized range-of-motion exercise and posture instructions [13, 25]. The health-related components of physical fitness include aerobic fitness, muscle strength and endurance, flexibility, and body composition. For promoting physical fitness, exercise components usually consisted of aerobic, muscle strengthening, and range-of-motion or stretching exercise [26]. Also, increased cardiovascular morbidity and mortality in patients with AS have been reported [2]. For these reasons, we think that exercise for patients with AS should include aerobic component and muscle strengthening as well as range-of-motion exercise.

Table 3: Comparison of the exercise tolerance variables at the baseline and after 3-month exercise between combined home exercise (COMB) group and range-of-motion home exercise (ROM) group.

Variables	COMB group ($n = 9$)			ROM group ($n = 10$)			Both groups
	Baseline mean (SD)	Postexercise mean (SD)	Within-group $^{*}P$ value	Baseline mean (SD)	Postexercise mean (SD)	Within-group $^{\dagger}P$ value	Between-groups $^{\ddagger}P$ value
Resting state							
HR (beats/min)	77.3 ± 8.7	82.6 ± 11.0	0.173	75.0 ± 7.4	81.3 ± 10.5	0.036	0.870
SBP (mmHg)	120.0 ± 8.7	113.9 ± 6.6	0.155	120.2 ± 13.6	110.6 ± 12.6	0.139	0.595
DBP (mmHg)	73.4 ± 7.4	72.6 ± 4.1	0.905	72.8 ± 7.5	73.1 ± 10.1	0.959	0.775
SpO_2%	96.1 ± 0.8	96.0 ± 0.9	0.655	95.9 ± 1.3	95.9 ± 1.3	1.000	0.931
Peak response							
$\dot{V}O_2$ (mL/kg/min)	20.4 ± 4.2	22.9 ± 4.2	0.008	20.1 ± 5.5	17.9 ± 4.3	0.032	0.001
$\dot{V}O_2$% of standard	53.3 ± 8.0	60.1 ± 8.0	0.008	56.2 ± 10.7	50.7 ± 9.1	0.024	0.001
MET	5.8 ± 1.2	6.5 ± 1.2	0.008	5.7 ± 1.6	5.1 ± 1.2	0.032	0.001
Work (W)	124.3 ± 22.2	131.3 ± 31.8	0.138	118.4 ± 34.2	111.4 ± 30.0	0.139	0.055
O_2 pulse	8.5 ± 2.4	9.0 ± 2.3	0.075	8.3 ± 2.3	7.9 ± 2.2	0.221	0.027
HR (beats/min)	155.1 ± 14.2	162.6 ± 16.4	0.033	152.7 ± 17.2	147.7 ± 21.6	0.474	0.060
SBP (mmHg)	177.0 ± 26.2	178.1 ± 16.7	0.906	182.9 ± 21.7	179.4 ± 20.5	0.221	0.806
DBP (mmHg)	93.9 ± 6.3	97.3 ± 10.1	0.259	95.0 ± 9.6	98.1 ± 12.3	0.507	0.712
RER	1.09 ± 0.9	1.07 ± 0.11	0.261	1.08 ± 0.3	1.06 ± 0.05	0.358	0.967
Ventilatory threshold							
$\dot{V}O_2$ (mL/kg/min)	11.0 ± 2.5	12.3 ± 2.2	0.021	11.8 ± 2.8	11.2 ± 3.4	0.415	0.041
MET	3.2 ± 0.7	3.5 ± 0.6	0.021	3.4 ± 0.9	3.2 ± 1.0	0.414	0.049
Work (W)	68.2 ± 24.7	69.7 ± 22.8	0.285	64.4 ± 20.6	60.8 ± 26.0	0.359	0.252
HR (beats/min)	117.8 ± 10.5	123.8 ± 15.0	0.037	119.2 ± 13.6	117.0 ± 18.8	0.541	0.093

HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; SpO_2: peripheral oxygen saturation; $\dot{V}O_2$: oxygen uptake; $\dot{V}O_2$% of standard: ratio in percentage of oxygen consumption over a standard oxygen uptake; RER: respiratory exchange ratio; MET: metabolic equivalent. $^{*}P$ values for differences in the baseline data between the COMB and the ROM groups; $^{\dagger}P$ values for differences in the postexercise data between the COMB and the ROM groups; $^{\ddagger}P$ values for differences in the changed score between the baseline and the postexercise data between the COMB and the ROM groups.

Table 4: Comparison of pulmonary function test at the baseline and after 3-month exercise between combined home exercise (COMB) group and range-of-motion home exercise (ROM) group.

Variables	COMB group ($n = 9$)			ROM group ($n = 10$)			Both groups
	Baseline mean ± SD	Postexercise mean ± SD	Within-group $^{*}P$ value	Baseline mean (SD)	Postexercise mean (SD)	Within-group $^{\dagger}P$ value	Between-groups $^{\ddagger}P$ value
FVC (L)	3.2 ± 0.8	3.1 ± 1.0	0.594	3.3 ± 0.8	3.0 ± 1.0	0.333	0.369
FEV1 (L)	2.9 ± 0.7	2.5 ± 0.8	0.674	2.9 ± 0.7	2.5 ± 0.8	0.766	0.624
FEV1/FVC (%)	89.2 ± 6.2	83.1 ± 7.0	0.811	88.2 ± 6.0	84.7 ± 6.3	0.473	0.364
PEF (L/sec)	7.6 ± 2.3	7.2 ± 2.1	0.021	8.8 ± 1.6	8.0 ± 2.4	0.074	0.514
VC (L)	3.2 ± 0.8	3.1 ± 1.0	0.515	3.3 ± 0.9	3.1 ± 1.0	0.610	0.327
TLC (L)	5.0 ± 1.0	4.9 ± 0.9	0.767	5.1 ± 1.0	5.0 ± 1.0	0.878	0.744
RV (L)	1.7 ± 0.6	1.8 ± 0.4	0.859	1.7 ± 0.6	1.9 ± 0.5	0.506	0.653
RV/TLC (%)	35.0 ± 10.3	38.1 ± 10.8	0.812	34.4 ± 10.4	39.2 ± 10.2	0.540	0.486
FRC (L)	3.2 ± 0.7	3.1 ± 0.8	0.678	3.1 ± 0.7	3.2 ± 0.7	0.683	0.568

FVC: forced vital capacity; FEV1: forced expiratory volume at 1 second; PEF: peak expiratory flow; VC: vital capacity; TLC: total lung capacity; RV: residual volume; FRC: functional residual capacity. $^{*}P$ values for differences in the baseline data between the COMB and the ROM groups; $^{\dagger}P$ values for differences in the postexercise data between the COMB and the ROM groups; $^{\ddagger}P$ values for differences in the changed score between the baseline and the postexercise data between the COMB and the ROM groups.

Table 5: Comparison of grip strength, functional ability, and disease activity variables at the baseline and after 3-month exercise between combined home exercise (COMB) group and range-of-motion home exercise (ROM) group.

Variables	COMB group ($n = 9$)			ROM group ($n = 10$)			Both groups
	Baseline mean (SD)	Postexercise mean (SD)	Within-group $^{*}P$ value	Baseline mean (SD)	Postexercise mean (SD)	Within-group $^{\dagger}P$ value	Between-groups $^{\ddagger}P$ value
Grip strength (kg)	28.6 ± 11.0	30.5 ± 12.0	0.109	29.5 ± 10.7	31.1 ± 9.2	0.262	0.682
BAS-G (0–10)	5.6 ± 2.7	3.6 ± 2.0	0.085	5.0 ± 2.8	4.1 ± 3.5	0.285	0.567
BASFI (0–10)	3.7 ± 3.3	1.9 ± 2.3	0.028	3.5 ± 2.9	3.5 ± 3.1	0.859	0.041
BASDI (0–10)	4.2 ± 1.9	3.7 ± 1.8	0.441	4.5 ± 2.1	4.5 ± 3.0	0.953	0.414
ESR (mm/h)	36.8 ± 28.6	24.8 ± 12.0	0.343	24.7 ± 23.1	25.0 ± 28.3	0.673	0.743
CRP (mg/dL)	1.27 ± 1.10	0.79 ± 0.56	0.260	1.07 ± 1.24	0.9 ± 0.99	0.345	1.000
Hb (gm/dL)	14.1 ± 1.1	14.1 ± 0.9	0.812	14.2 ± 1.8	14.1 ± 2.3	0.683	0.653

BAS-G: Bath Ankylosing Spondylitis Global Score; BASFI: Bath Ankylosing Spondylitis Functional Index; BASDI: Bath Ankylosing Spondylitis Disease Activity; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; Hb: hemoglobin. $^{*}P$ values for differences in the baseline data between the COMB and the ROM groups; $^{\dagger}P$ values for differences in the postexercise data between the COMB and the ROM groups; $^{\ddagger}P$ values for differences in the changed score between the baseline and the postexercise data between the COMB and the ROM groups.

Measurement of spinal range-of-motion and chest expansion did not show significant improvement except for finger-to-floor distance in the COMB group (Table 2). Although previous studies demonstrated that participation of patients with AS in 3 to 4 weeks of intensive physiotherapy sessions could help to increase chest expansion, finger-to-floor distance, thoracolumbar rotation, and lateral trunk flexion [16, 27, 28], the studies were done on an inpatient, and usually the number of patients able to attend intensive 3- to 4-week inpatient training program was very limited. Uhrin et al. found that unsupervised recreational exercise with duration more than 200 minutes per week could reduce the severity and pain in patients who had AS for 15 years or less [11]. In Russell's study, a single exercise session induced a small but significant increase in lumbar extension for the vigorous exercise group but no significant change for moderate exercise or nonexercise group [27]. In AS patients with long duration, severe contracture or fusion of spinal and peripheral joints was frequently present, and gentle range-of-motion was not effective in improving the mobility of joints. In our study, both groups of patients with AS had average duration more than 11 years (especially in the ROM group), and fusion or severe contracture in the spine was present in some patients; besides, home exercise may be too gentle to induce change in the range-of-motion of the spine and peripheral joints.

Another cause of no improvement in spinal range-of-motion may be due to low compliance. As has been reported previously, the compliance for home exercise is between 30 and 90%, usually in the lower range [21]. Generally speaking, the compliance with inpatient exercise is highest, followed by supervised outpatient or organized exercise program, and home-based exercise is the lowest. However, in a study by Lim et al., an 8-week home-based exercise program increased joint mobility and functional capacity and decreased pain and depression in patients with AS [29]. In that study, the researchers monitored the patients by telephone every day. Because of lack of manpower, we monitored the patients by phone only once in 2 weeks. If we could have monitored the patients more frequently, the compliance would have been increased, and the effect of exercise might also have been improved. A home-based exercise program is cheaper, time-saving, and more easily accessible to patients. It might still be an effective intervention for patients with AS if the compliance with exercise could be improved.

Our study showed no significant difference between baseline and postexercise data for most of the pulmonary function tests except for PEF in the COMB group (Table 4). No improvement in VC, FVC, and other pulmonary function tests after exercise could partly be reflected by the nonsignificant change in chest expansion (Table 2). Viitanen et al. conducted a 3- to 4-week inpatient training and showed that average increase in VC was 200 mL in men and 270 mL in women [28]. However, at 15-month follow-up after the training, both chest expansion and vital capacity had significantly deteriorated from the baseline [30]. Tomlinson et al. also reported significant improvement in mobility, posture, and lung function from 3-week intensive inpatient physiotherapy [31]. Again, difference in the outcome could be explained by different exercise program (home exercise versus inpatient physiotherapy).

In our study, significant improvement in the functional ability after home exercise was observed only in the COMB group for BASFI (Table 5). Previous study has also shown that BASFI is sensitive to the functional change in patients with AS [22, 29]. van Tubergen et al. also found that combined spa therapy and exercise in addition to medications and physical therapy was associated with significant improvement in BASFI [9]. Another report from Sweeney et al. also demonstrated that a home-based exercise intervention showed a trend for improvement in BASFI [32]. Our study was consistent with those previous reports.

The strengths of this study are as follows. (1) It was prospective, randomized, and blinded to the evaluators. (2) Exercise intervention study in Taiwanese patients with AS has never been reported before. (3) Comparison of different home exercise programs has rarely been reported, even in the Western population. (4) The baseline and postexercise

evaluations were very extensive, including aerobic capacity, pulmonary function, range-of-motion of the spine, chest expansion, functional ability, and other disease-related measures. However, our study has some limitations, First, the sample size of 9 or 10 in each group was small, and the statistical power for chest expansion (21%), finger-to-floor distance (40.2%), MET at peak cardiovascular response (12%), and FVC (11.5%) was low. More cases are needed for evaluating change of spinal range- of- motion and pulmonary function test. Secondly, the study did not provide long-term follow-up (e.g., 6 months, 1 year), and thus we did not know whether the exercise effects would be maintained after a long exercise program. Thirdly, we monitored patients once in 2 weeks. A more frequent monitoring by phone or other means might increase the exercise effect. Fourthly, the average duration of disease in patients with AS was more than 11 years, and some patients had severe contracture or fusion of the spinal joints. If we could select patients with shorter duration or with a more flexible spine, the exercise effect might be improved.

5. Conclusions

This study demonstrated that a 3-month home-based combined (aerobic, strengthening, and range-of-motion) exercise program significantly improved aerobic capacity and functional ability (BASFI) in patients with AS and was superior to home-based range-of-motion exercise alone. Exercise prescription for patients with AS should include range-of-motion (or stretching), strengthening, and aerobic components and should be started in the early stage of the disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Dr. Chih-Cheng Chuang and Dr. James Cheng-Chung Wei contributed equally to this work.

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