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CLINICAL STUDY

Effect of Sanhuangyilong decoction plus methotrexate on tumor necrosis factor alpha and interferon gamma in serum and synovial fluid in rheumatoid arthritis patients with symptom pattern of damp heat obstruction

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Abstract

OBJECTIVE: To investigate the effect of Sanhuangyilong decoction plus methotrexate (MTX) on Interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) in the serum and synovial fluid of rheumatoid arthritis (RA) patients with damp-heat-obstruction symptom pattern, Sanhuangyilong decoction and the role of TNF-α and IFN-γ in the development of RA.

METHODS: RA inpatients with damp-heat-obstruction symptom pattern (partly with knee joint effusion) were selected as the research subjects. Before the treatment, healthy subjects and osteoarthritis (OA) patients with knee joint effusion were assigned to the serum control group and the synovial fluid control group, respectively; during the treatment, RA patients with damp-heat-obstruction symptom pattern were divided into two groups: one is combined group that was administered Sanhuangyilong decoction plus MTX; the other group was MTX group that received MTX only. The expression levels of TNF-α and IFN-γ in the serum and synovial fluid were measured with enzyme-linked immunosorbent assay (ELISA) before and after the treatment, and the peripheral blood levels of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and disease activity score in 28 joints (DAS28) were determined.

RESULTS: Before treatment, the serum levels of TNF-α and IFN-γ in the RA patients with damp-heat-obstruction symptom pattern were higher than those in healthy control group ($P < 0.05$).The expression levels of TNF-α and IFN-γ in the synovial fluid of the RA patients were higher than those in the serum of the RA patients ($P < 0.05$). The expression levels of TNF-α and IFN-γ in the synovial fluid of the RA patients were higher than those of the sy-

novial fluid of the osteoarthritis patients ($P < 0.05$). The expression of TNF-α and IFN-γ in the serum and synovial fluid of the RA patients had no correlation with the inflammatory activity index ESR, CRP, or DAS28 ($P > 0.05$). After 2 weeks of treatment, the expression level of TNF-α and IFN-γ in the combined group had increased, although the difference was not statistically significant ($P > 0.05$); in contrast, ESR, CRP, and DAS28 decreased, and the difference was statistically significant ($P < 0.01$). After 4 weeks of therapy, TNF-alpha and IFN-γ, ESR, CRP, and DAS28 in the combined group decreased compared with the before-treatment levels ($P < 0.01$). After 2 w of treatment, the differences in the TNF-α and IFN-γ expression levels in the combined group were not statistically significant ($P > 0.05$) compared with that in the MTX group, although there were statistically significant differences in the ESR, CRP, and DAS28 ($P < 0.05$). After 4 weeks of treatment, differences in TNF-α, IFN-γ, ESR, CRP, and DAS28 in the combined group compared with MTX group were statistically significant ($P < 0.01$).

CONCLUSION: TNF-α and IFN-γ might be involved in the development of RA. The RA patients with damp-heat-obstruction symptom pattern show better benefits from the treatment of Sanhuangyilong decoction plus MTX, and the treatment is superior to that of using MTX only.

Key words: Arthritis, rheumatoid; Dampness-heat; Cytokines; Blood sedimentation; C-reactive protein; Methotrexate; Sanhuangyilong decoction

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterised by chronic, erosive arthritis, which primarily involves symmetrical polyarticular swelling, pain, stiffness and multi-system involvement. The pathogenesis of RA is not clear. Research reports show that Th1 (T helper 1) and Th17 (T helper 17), the main pro-inflammatory Th subgroup, induce the pathogenesis of RA,[1] and many cytokines secreted by them play an important role in the pathogenesis of RA. Interferon gamma (IFN-γ) is the major cytokine secreted by Th1 cells; however, tumor necrosis factor alpha (TNF-α) is predominantly secreted by Th17 cells. Both cytokines play a specific role in the pathogenesis of RA. The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level are commonly used to reflect the disease activity of RA as clinical and laboratory indexes, and the disease activity score in 28 joints (DAS28)[2] is a comprehensive index for evaluating RA disease activity.

Based on the symptoms patterns minified in RA patients, in terms of theory of Traditional Chinese Medicine (TCM), RA is often diagnosed as the obstruction of *Qi* and blood in the meridians due to the invision of external pathogenic wind, cold, wet and heat, manifested as soreness, pain, numbness, a heavy sensation, swelling of the joints and limbs, limitation of movement and the formation of chronic inflammation. The Bi (symptom) pattern in Plain Questions[3] states that "if the wind, cold and dampness invade the body together, Bi (symptom) pattern might develop", demonstrating that Bi (symptom) pattern is associated with several pathogenic *Qi*. Shuo Wen Jie Zi[4] which was written in the Han Dynasty, includes the interpretation of "Bi, the pathogenic dampness", which indicates that pathogenic dampness is a major cause of rheumatism. The highest moisture levels in China occur in the Southwest. From 1993-1995, meteorologists investigated Beijing, Shanghai, Chengdu, and Guangzhou, which are in the north, southeast, southwest, and south of China, respectively, and the results show that the annual average relative humidity is 53% , 75% , 82% and 75%, respectively.[5] The view that "dampness and heat, arthralgia" is described as early as in the "synopsis of prescriptions of the Golden Chamber",[6] including the hypotheses that "patients suffering from pathogenic dampness have pain and fever" and "dampness being the disease, [which is] manifested as pain and fever of the body". Zhang *et al* [7] noted that the dampness-heat symptom pattern was closely related with the occurrence, development and prognosis of a variety of rheumatic diseases. In Traditional Chinese Medicine (TCM), RA is divided into 6 symptom patterns: damp-heat, phlegm and blood stasis, a deficiency of *Qi* and *Yin*, cold dampness, blood stasis obstruction and deficiency of spleen and kidney. Jiang *et al* [8] analysed the distribution of TCM symptom patterns in 475 in-patients with RA, finding that most of the occurrences were of damp-heat symptom pattern, which accounted for 41.7% of the cases. Le *et al* [9] retrospectively analysed the relationship between the TCM symptom pattern type and the immune index in 143 RA patients, showing that the anti-cyclic citrullinated peptide antibody and rheumatoid factor (RF) have the highest positive rate in damp-heat symptom pattern. Damp-heat symptom pattern predominates in the active stage of RA, and RA patients with damp-heat symptom pattern according to the standard were selected as the subjects of the study.

After years of study, our research team found that the Sanhuangyilong decoction displays a certain effectiveness in the treatment of RA with high activity by eliminating dampness.[10] This project aimed to investigate the effects of Sanhuangyilong decoction combined with MTX on TNF-α and IFN-γ, and the their role in the development of RA.

MATERIALS AND METHODS

Clinical data

From January 2011 to January 2013, 118 RA inpatients diagnosed with damp-heat symptom pattern were registered in the Department of Rheumatism Combined Traditional Chinese and Western Medicine (Rheumatic Disease Medical Centre, Chengdu Military Region), General Hospital of Chengdu Military Region. A total of 28 cases were excluded for various reasons, including failing to obtain a sample during treatment, detecting the emergence of complications and poor patient compliance; the remaining 90 cases were assigned to the RA patient group. Thirty outpatients or inpatients suffering from osteoarthritis (OA) with knee joint synovial fluid were assigned to the OA group with synovial fluid, and 30 healthy people served as the healthy control group.

The study was approved by Hospital ethics committee, and registered in the World Health Organization database at the General Hospital of Chengdu Military Area Command Chinese People's Liberation Army (ChiCTR-TRC-14004520). Informed consent was obtained from all participants.

Diagnostic criteria

We used two Western Medicine diagnostic criteria for rheumatoid arthritis: American College of Rheumatology (ACR) 1987[11] and/or ACR/The European League Against Rheumatism (EULAR)2009.[12] The diagnosis of osteoarthritis (OA) refers to the diagnostic criteria of ACR 1986.[13]

We used a Traditional Chinese Medicine (TCM) diagnostic criterion for identifying the subtypes of Bi symptom pattern, which were identified by the following 3 major symptoms, 2 secondary symptoms and tongue and pulse examinations. The main symptoms were as follows: joint redness, swelling, heat and pain; joint stiffness; joint swelling; joint tenderness and joint deformation. The secondary symptoms were as follows: joint heaviness; local joint inflammation; and thirst without a desire to drink. The appearance of the tongue and pulse readings were as follows: red tongue or dark red tongue; a whitish, greasy, furry or yellow tongue coating; and soft and rapid pulse or slippery and rapid pulse.

Inclusion and exclusion criteria

The following inclusion criteria were used in the study: (a) a diagnosis of RA and OA; and (b) a TCM diagnosis of damp-heat symptom pattern type of Bi symptom pattern. The researchers obtained the approval of the ethics committee of the General Hospital of the Chengdu Military Region of the People's Liberation Army of China. The subjects provided their signed informed consent.

The following exclusion criteria were used in the study: (a) pregnant or lactating women and subjects with allergies; (b) an association with other autoimmune diseases; (c) serious heart, brain, liver, kidney, blood, endocrine and gastrointestinal diseases; (d) disturbance of consciousness or mental abnormality; or (e) other reasons.

Grouping and treating

The RA group comprised 90 RA patients, in which 28 patients with knee joint synovial fluid were assigned to the RA group with synovial fluid. The RA patient group included 80 females and 10 males, aged 45-65 years old. The average age was (56 ± 7) years, the course of the disease was 0.25-40 years, and the average course of the disease was (13 ± 13) years. During the treatment, we followed the provisions of the ethics committee and distributed random numbers with random tables in accordance with a randomisation method; the RA patients were divided into the combined group of 45 patients and the MTX group of 45 patients. The combined group included 38 females and 7 males, with a mean age of (56 ± 8) years old; the MTX group included 37 females and 8 males, with a mean age of (55 ± 8) years old. There were no significant differences between the two groups in age and gender ($P > 0.05$).

The healthy control group included 30 age-and sex-matched healthy persons, including 26 females and 4 males, aged 46-64 years old with a mean age of (55 ± 7) years.

The OA group included 30 age-and sex-matched osteoarthritis patients with knee joint synovial fluid, including 27 females and 3 males, aged 48-66 years old, with a mean age of (57 ± 7) years.

There were no significant differences between the three groups in age and gender ($P > 0.05$) (Table 1).

Treatment protocols

Combined group: Sanhuangyilong decoction combined with methotrexate tablets (MTX) taken orally. The composition of the decoction was follows: Huangqin (*Radix Scutellariae Baicalensis*) 15 g, Huanglian (*Rhizoma Coptidis*) 15 g, Huangbai (*Cortex Phellodendri Amurensis*) 15 g, Qinjiao (*Radix Gentianae Macrophyllae*) 30 g, Weilingxian (*Radix et Rhizoma Clematidis Chinensis*) 30 g, Baishao (*Radix Paeoniae Alba*) 30 g, Xixin (*Herba Asari Mandshurici*) 6 g, Dilong (*Pheretima Aspergillum*) 10 g, and Fuling (*Poria*) 30 g, decocted in the TCM Dispensary of the General Hospital of Chengdu Military Region in China with an automatic decoction machine. The oral prescription was for 100 mL, three times a day. The methotrexate tablets were from Shanghai Xinyi Pharmaceutical Co., Ltd., No. H31020644; 2.5-mg tablets were administered in a 10-15 mg dose once per week. The MTX group was treated with oral methotrexate tablets. The two groups had 2 weeks as 1 course of treatment with continuous observation for 2 courses. During the treatment, the two groups were given diclofenac sodium sustained release tablets (75-mg Voltaren tablets, Beijing Novartis, No. H10980297;

Table 1 Comparison of the general data of the patients ($\bar{x} \pm s$)

Category	RA patients group		Healthy control group	OA control group
	Combined group	MTX group		
Cases (*n*)	45	45	30	30
Male/Female (*n*)	7/38[a]	8/37[b]	4/26	3/27
Age (years)	56±8[a]	55±8[b]	55±7	57±7

Notes: combined group was treated with Sanhuangyilong decoction (100 mL, three times a day) combined with methotrexate tablets (10 -15 mg , once per week). MTX group was treated with methotrexate tablets (10-15 mg, once per week). The two groups were treated 4 weeks. Healthy Control group: age-and sex-matched healthy persons. OA Synovial Fluid group: age-and sex-matched osteoarthritis patients with knee joint synovial fluid. RA: rheumatoid arthritis; MTX: methotrexate; OA: osteoarthritis. Combined group compared with the healthy control group and the OA control group, there are no significant differences in sex and age, [a]$P > 0.05$. MTX group compared with the healthy control group and the OA control group, there are no significant differences in sex and age, [b]$P > 0.05$.

dosage 75 mg orally, 2 times daily) for 4 weeks. When necessary, the treatment course was altered with 5-mg prednisone tablets (Henan Topfond Pharmaceutical Co., Ltd., No. H41020283; dosage: 2.5 mg orally, three times daily).

Observation index and method

The specimen collection was performed as follows: the specimens were collected before the treatment and after 2 weeks and 4 weeks of the treatment; 2-3 mL samples of peripheral blood were collected from the RA patients and healthy controls in the morning (with an empty stomach) before and after the treatment; the samples were centrifuged at 3000 r/min for 10 min; the upper serum was aspirated and the desired sample was stored at − 80 ℃. The synovial fluids from the RA and OA patients were obtained by a lateral patellar with a 5 mL syringe needle and stored at − 80 ℃ for centralised detection. TNF-α and IFN-gamma detection was performed with an enzyme-linked immunosorbent assay (ELISA) in a kit purchased from Sichuan RuiSheng Technology Co., Ltd. ESR levels were detected by the Westergren method and expressed as mm/h. CRP levels were assayed using an automatic rate nephelometic immunoassay and expressed as mg/L. The reference values were 0-15 mm/h for ESR and 0-3 mg/L for CRP. DAS28 consists of human 28 joint tenderness counts, swelling counts, the ESR or CRP level and the patient's comprehensive assessment project, and the disease activity is divided into the following four levels: remission (< 2.6), mild activity (2.6-3.2), moderate activity (3.2-5.1) and severe activity (> 5.1).

Statistical analysis

SPSS11.5 statistical analysis software (SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis, and the data are represented in`mean ± standard deviation ($\bar{x} \pm s$). The comparisons between the groups were tested by a paired-samples *T*-test, and the analysis of the correlation between the indicators was performed by Pearson's correlation analysis. A *P* value < 0.05 was considered statistically significant.

RESULTS

Study recruitment and follow-up

Of the initial 118 participants, 90 were considered eligible and randomized to a group (Figure 1). The main reasons for in ineligibility were failing to obtain a sample during treatment, detecting the emergence of complications and poor patient compliance. Patients were randomized to the combined group (n = 45), MTX group (n = 45). All participants were followed up at 4 weeks.

Comparison of the expression level of TNF-α in various groups

Compared with the healthy control group, the TNF-α expression level in the peripheral blood of RA patients was significantly higher (t = 8.758, P < 0.05); compared with the synovial fluids from the patients with OA, the expression of TNF-α was significantly higher synovial fluid of RA patients (t = 2.51, P < 0.05); compared with the peripheral blood of patients with RA, the expression of TNF-α was significantly higher in the synovial fluid of RA patients (t = 2.496, P < 0.05; Figure 2A, Table 2).

Comparison of the expression level of IFN-γ in all groups

Compared with the healthy control group, the IFN-γ expression level in the peripheral blood of RA patients significantly higher (t = 6.086, P < 0.05); compared with the synovial fluids from the patients with OA, the expression of IFN-γ was significantly higher in the synovial fluid of RA patients (t = 2.523, P < 0.05); compared with the peripheral blood of patients with RA, the expression of IFN-γ was significantly higher in the synovial fluid of RA patients (t = 2.708, P < 0.05; Figure2B, Table 2).

Correlation analysis between the expression level of TNF-α, IFN-γ in RA serum and ESR, CRP, and DAS28

Pearson's correlation analysis showed that there is no correlation between the expression level of TNF-α in RA serum and ESR levels, CRP levels, or DAS28 score (r = − 0.108, − 0.225, − 0.228; P > 0.05); there is no correlation between the expression level of serum IFN-γ and ESR levels, CRP levels, or DAS28 score (r = − 0.104, r = − 0.285, r = − 0.414, P > 0.05).

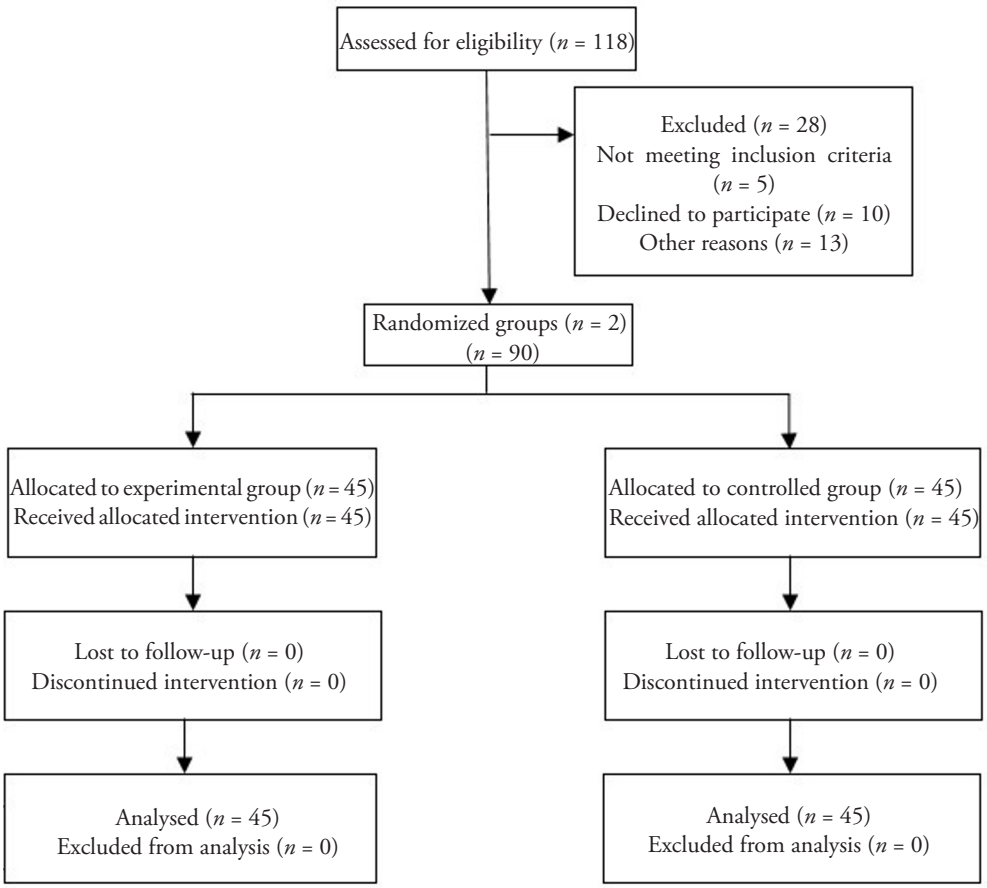


Figure 1 Study participant flow diagram

Correlation between the expression level of TNF-α, IFN-γ in RA synovial fluid and ESR, CRP, DAS28

Pearson's correlation analysis showed no correlation between the expression level of TNF-α in RA synovial fluid and ESR levels, CRP levels, or DAS28 score (r = − 0.256, 0.177; − 0.65, P > 0.05); there is no correlation between the expression level of synovial fluid IFN-γ and ESR levels, CRP levels, or DAS28 score (CRP, r = − 0.199, r = − 0.412, r = − 0.015, P > 0.05).

Comparison of the expression level of TNF-α and IFN-γ before and after treatment in RA patients

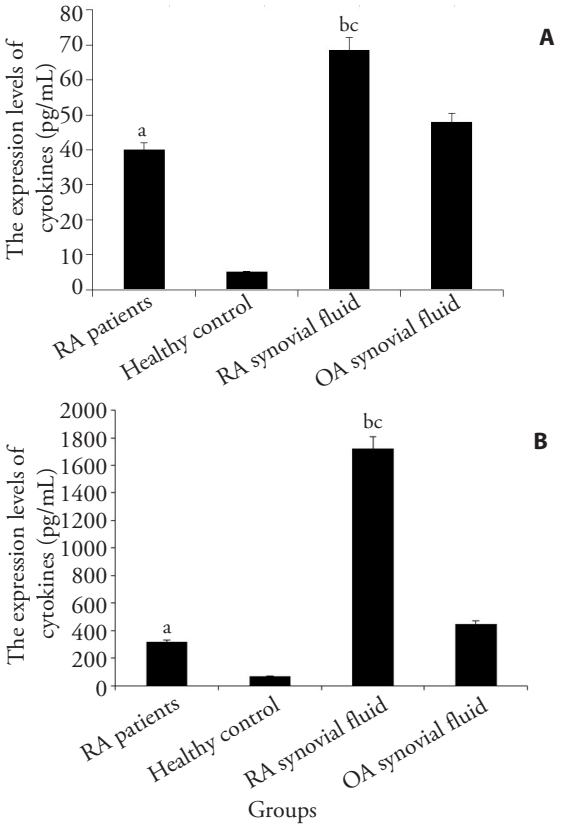
After 2 weeks, TNF-α and IFN-γ expression levels were increased in the combined group and the MTX group compared with the identical group before treatment; however, the differences were not statistically significant (P > 0.05); likewise, differences between the combined and MTX groups were not significant (P > 0.05). After 4 weeks, compared with those before treatment, TNF-α and IFN-γ expression levels significantly decreased in the combined group and MTX group (P < 0.01); in addition, there was a significant difference in the levels between the combined and MTX groups (P < 0.01) (Figure 3, Table 3).

Comparison of inflammatory changes before and after treatment in patients with RA

At 2 weeks and 4 weeks, compared with the levels before treatment, ESR levels, CRP levels, and DAS28 scores were significantly decreased in the combined group and the MTX group (P < 0.01 or P < 0.05). At 2 weeks and 4 weeks, the ESR levels, CRP levels, or DAS28 scores were significantly different between the combined and MTX groups (P < 0.05 or P < 0.01; Figure 4, Table 4).

DISCUSSION

The results of this study show that the serum TNF-α and IFN-γ levels in RA patients with damp-heat-obstruction symptom patternwere significantly higher than those in the healthy control group and that the synovial fluid TNF-α and IFN-γ levels were significantly higher than those in the peripheral blood and osteoarthritis synovial fluid. The expression of TNF-α and IFN in the serum of the peripheral blood and synovial fluid has no obvious correlation with ESR levels, CRP levels, or DAS28 score. Other studies have reported that the TNF-α levels in the peripheral blood and synovial fluid in RA patients were significantly higher than those in the healthy control group.[22] The TNF-α levels in the synovial fluid of knee joints in RA children have also been shown to be significantly higher than those in healthy children, and there is no correlation between TNF-α levels and peripheral blood WBC, ESR, CRP or immunoglobulin levels.[23] The serum IFN-γ levels of RA patients in the active phase were significantly



Figue 2 Expression levels of TNF-α, IFN-γ in the serum, synovial fluid in all groups

A: TNF-α; B: IFN-γ. RA patients group: comprised 90 RA patients. Healthy control group: included 30 age-and sex-matched healthy persons. RA synovial fluid group: comprised 28 RA patients with knee joint synovial fluid. OA synovial fluid group: included 30 age-and sex-matched osteoarthritis patients with knee joint synovial fluid. RA: rheumatoid arthritis; OA: osteoarthritis; TNF-α: tumor necrosis factor-alpha; IFN-γ: interferon-gamma. Compared with the healthy control group, $^{a}P < 0.05$; compared with OA Synovial Fluid, $^{b}P < 0.05$; compared with RA patients, $^{c}P < 0.05$.

higher than those of RA patients in the stable phase,[24] and they were significantly increased compared to healthy controls.[25,26] The results of this study are consistent with those reported in the literature.

The results of this study suggest that for RA patients with damp heat obstruction symptom pattern, after the initial 2 weeks of treatment with Sanhuangyilong decoction combined with the antirheumatic drug methotrexate, in tablet form, the expression levels of serum TNF-α and IFN-γ were increased. ESR levels, CRP levels, and DAS28 score, all of which are inflammatory markers, were significantly reduced, and the symptoms and signs of the patients improved significantly. After 4 weeks of treatment, the levels of TNF-α, IFN-γ, ESR and CRP as well as the DAS28 scores were significantly decreased in the combined treatment group. Studies in other countries reported that in RA patients treated with etanercept, the IL-6, IL-1 and CRP levels decreased rapidly; however, the TNF-α and sTNFR-Ⅱ increased markedly within 24 h, plateauing after 2 weeks.[27] This result might be because the synovial fluid of patients in the active

Table 2 Comparison of the expression level of TNF-α, IFN-γ in all groups (pg/mL, $\bar{x} \pm s$)

Group	*n*	TNF-α	IFN-γ
RA Patients	90	40.0±17.0[a,c]	317.6±163.5[a,c]
Healthy Control	30	5.1±1.6	69.6±32.2
RA Synovial Fluid	28	68.7±31.6[b]	1723.1±1421.3[b]
OA Synovial Fluid	30	48.0±16.7	450.2±126.5

Notes: RA patients group: comprised 90 RA patients. Healthy Control group: included 30 age-and sex-matched healthy persons. RA Synovial Fluid group: comprised 28 RA patients with knee joint synovial fluid. OA Synovial Fluid group: included 30 age-and sex-matched osteoarthritis patients with knee joint synovial fluid. TNF-α: tumor necrosis factor-alpha; IFN-γ: interferon-gamma; RA: rheumatoid arthritis; OA: osteoarthritis. Compared with the healthy control group, $^{a}P < 0.05$; compared with OA Synovial Fluid, $^{b}P < 0.05$; compared with RA Synovial Fluid, $^{c}P < 0.05$.

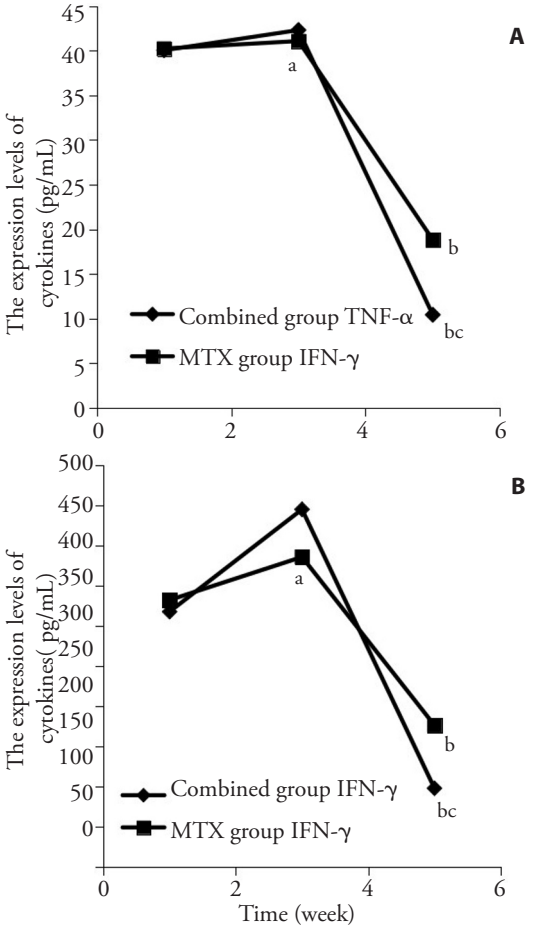


Figure 3 Expression levels of TNF-α, IFN-γ in the serum before and after treatment between the two groups

A: TNF-α; B: IFN-γ. Combined group was treated with Sanhuangyilong decoction (100 mL, three times a day) combined with methotrexate tablets (10-15 mg, once per week) and diclofenac sodium sustained release tablets (75 mg, 2 times daily) or prednisone tablets (2.5 mg, 3 times daily). MTX group was treated with methotrexate tablets (10-15 mg , once per week) and diclofenac sodium sustained release tablets (75 mg, 2 times daily) or prednisone tablets (2.5 mg, 3 times daily). MTX: methotrexate; TNF-α: tumor necrosis factor-alpha; IFN-γ: interferon-gamma. After 2 weeks, compared with before treatment, $^{a}P > 0.05$; After 4 weeks, compared with before treatment, $^{b}P < 0.01$; compared with MTX group, $^{c}P < 0.01$.

Table 3 Comparison of the expression level of TNF-α, IFN-γ before and after treatment in RA patients (pg/mL, $\bar{x} \pm s$)

Group	Phase	*n*	TNF-α	IFN-γ
Combined	Before treatment	45	40±17	318±163
	2 weeks of treatment	45	42±13[a]	445±335[a]
	4 weeks of treatment	45	10±5[bc]	97±33[bc]
MTX	Before treatment	45	40±17	332±166
	2 weeks of treatment	45	41±10[a]	386±317[a]
	4 weeks of treatment	45	19±9[b]	176±146[b]

Notes: combined group was treated with Sanhuangyilong decoction (100 mL, three times a day) combined with methotrexate tablets (10-15 mg, once per week) and diclofenac sodium sustained release tablets (75 mg, 2 times daily) or prednisone tablets (2.5 mg, 3 times daily). MTX group was treated with methotrexate tablets (10-15 mg , once per week) and diclofenac sodium sustained release tablets (75 mg, 2 times daily) or prednisone tablets (2.5 mg, 3 times daily). MTX: methotrexate; TNF-α: Tumor Necrosis Factor-alpha; IFN-γ: Interferon-gamma. After 2 weeks, compared with before treatment,[a]$P > 0.05$; After 4 weeks, compared with before treatment, [b]$P < 0.01$; compared with MTX group, [c]$P < 0.01$.

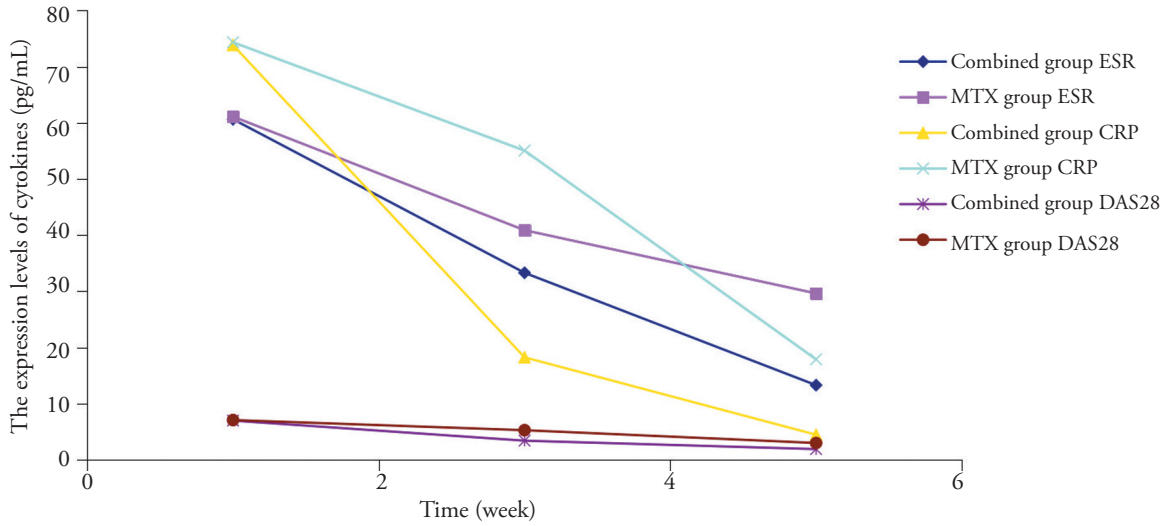


Figure 4 Comparison of the ESR levels, CRP levels, and DAS28 score before and after treatment in the patients with RA
Combined group was treated with Sanhuangyilong decoction (100 mL, three times a day) combined with methotrexate tablets (10-15 mg, once per week) and diclofenac sodium sustained release tablets (75 mg, 2 times daily) or prednisone tablets (2.5 mg, 3 times daily). MTX group was treated with methotrexate tablets (10-15 mg, once per week) and diclofenac sodium sustained release tablets (75 mg, 2 times daily) or prednisone tablets (2.5 mg, 3 times daily). MTX: methotrexate; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: disease activity score in 28 joints.

Table 4 Comparison of the ESR levels, CRP levels, and DAS28 score before and after treatment in the patients with RA ($\bar{x} \pm s$)

Group	Phase	*n*	ESR (mm/h)	CRP (mg/L)	DAS28
Combined	Before treatment	45	60.6±34.4	73.9±60.2	7.0±0.8
	2 weeks of treatment	45	33.3±29.2[ac]	18.2±19.1[ac]	3.4±1.2[ac]
	4 weeks of treatment	45	13.3±10.6[ad]	4.4±3.3[ad]	1.9±0.4[ad]
MTX	Before treatment	45	61.1±33.8	74.4±59.7	7.1±0.9
	2 weeks of treatment	45	40.9±30.6[b]	55.1±34.2[b]	5.3±2.1[b]
	4 weeks of treatment	45	29.6±28.6[a]	17.9±15.2[a]	3.0±1.0[a]

Notes: combined group was treated with Sanhuangyilong decoction (100 mL, three times a day) combined with methotrexate tablets (10-15 mg , once per week)and diclofenac sodium sustained release tablets (75 mg, 2 times daily) or prednisone tablets (2.5 mg, 3 times daily). MTX group was treated with methotrexate tablets (10-15 mg, once per week) and diclofenac sodium sustained release tablets (75 mg, 2 times daily) or prednisone tablets (2.5 mg, 3 times daily). MTX: methotrexate; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: disease activity score in 28 joints. At 2 weeks and 4 weeks, compared with the levels before treatment, ESR levels, CRP levels and DAS28 were significantly decreased in the combined group and MTX group, [a]$P < 0.01$, or [b]$P < 0.05$. At the corresponding time, ESR levels, CRP levels, or DAS28 scores in the combined group were significantly decreased in MTX group, [c]$P < 0.05$, or [d]$P < 0.01$.

disease stage stores a large number of cytokines that quickly migrate to serum after sudden drug stimulation and exhibit a growing trend for cytokines in serum after only a short time. RA patients with damp-heat-obstruction symptom pattern have a yang deficiency of the spleen and kidney, and pathogenic damp heat invades the body, brews cementation and is difficult to eliminate quickly, resulting in joint inflammation. The TNF-α and IFN-γ cytokines in serum and synovial fluid, with internal and external factors, might non-specifically activate T and B lymphocytes and mediate autoimmune injury. After treatment with the San Jiao bitter cold dampness prescription, which clears heat and eliminates dampness, activates collaterals, and relieves pain, immunosuppressive therapy combined with MTX could rapidly stimulate the accumulation of cytokines in serum; however, the accumulation could be quickly reduced by the continuous anti-inflammatory action of the drugs. After the inflammatory activities are controlled, a satisfactory effect could be quickly achieved.

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