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Review Article

Methotrexate combined with leflunomide reduces the serum levels of CRP and IL-18 in patients with ankylosing spondylitis

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Abstract: Objective: To investigate the effects of methotrexate combined with leflunomide on the CRP and IL-18 levels in patients with ankylosing spondylitis (AS). Methods: A total of 87 patients with AS admitted to Panyu Central Hospital from January 2017 to January 2019 were enrolled as the study cohort. Among them, 45 of the patients received methotrexate combined with leflunomide treatment and were designated as the experimental group, and 42 patients treated just with methotrexate were designated as the control group. We compared the two groups in terms of the following: clinical efficacy, changes in the clinical symptoms (morning stiffness time of lumbago, chest-expanding degree, finger-floor distance, occipital wall gap), the Bath AS disease activity index (BASDAI), the Bath AS functional index (BASFI), the spinal pain score, the incidence of adverse reactions, the inflammation markers (ESR, CRP, IL-18), and other indicators. Results: The effective rates were 91.11% in the experimental group and 80.95% in the control group ($P < 0.05$). After the treatment, the two groups' indicators were significantly improved compared with the indicators before the treatment, including morning stiffness time of lumbago, the chest-expanding degree, the finger-floor distance, the occipital wall gap, the VAS scores, BASDAI, and BASFI ($P < 0.05$). Compared with the control group, the morning stiffness time of lumbago, chest-expanding degree, the finger-floor distance, the occipital wall gap, the VAS scores, BASDAI, and BASFI were significantly improved in the experimental group ($P < 0.05$). After the treatment, the ESR, CRP, and IL-18 levels in the experimental group were significantly lower than the corresponding levels in the control group ($P < 0.05$), and the incidence of adverse reactions in the experimental group was significantly lower than it was in the control group ($P < 0.05$). Conclusion: The use of methotrexate combined with leflunomide in the treatment of AS can achieve a good clinical effect and effectively improve patients' clinical symptoms and inflammatory indicators. It is safe and worthy of promotion.

Keywords: Methotrexate, combination, leflunomide, ankylosing spondylitis, CRP, IL-18

Introduction

According to published data, the number of patients with ankylosing spondylitis (AS) is gradually increasing, and the inconvenience and pain caused by the disease are affecting more and more people [1]. AS is a kind of progressive inflammatory disease involving the axial bone, which also affects the spine, sacroiliac, and limb joints [2, 3]. However, patients with AS do not show any clinical symptoms at the early stages; there are only non-specific symptoms such as fatigue, an intermittent low fever, and anorexia, leading to a prolonged time to diagnosis and missing the optimal time for effective treatment [4]. Moreover, middle-aged and elderly people are more likely to suffer from AS, and the pain they experience in the early stages is mostly unilateral and intermittent, but it can develop into persistent bilateral pain after several months of poor disease control. Some patients may have severe hip pain or peripheral radiative pain at the later stages of the disease [5, 6]. Current research on AS has not specifically illustrated its etiology or pathogenesis. Its drug treatment mainly starts with anti-rheumatics, non-steroidal anti-inflammatories, and hormonal treatment. The treatment can reduce the patients' pain when administered in the clinic. However, it is not effective in delaying the development of the disease, its toxic and side effects also appear during long-

term use, and there are defects such as high drug compliance and easy relapse [7, 8]. Therefore, the long-term application of the existing clinical drug treatment program is worthy of scientific discussion. It is vital to find drugs with low side effects and high efficiency for the treatment of AS patients.

Methotrexate can disrupt the coenzyme tetrahydrofolate synthesis of purine nucleotides and pyrimidine deoxynucleotide in vivo by inhibiting dihydrofolate reductase, thereby limiting the transfer of a carbon group between the nucleotides of purines and pyrimidines during DNA biosynthesis [9]. Leflunomide is an immunosuppressive agent that inhibits the activity of dihydroorate dehydrogenase and the synthesis of pyrimidine, and it has the effect of inhibiting the proliferation of B and T lymphocytes [10]. Some studies have pointed out that ankylosing spondylitis infection may be involved in the progression of autoimmune diseases by regulating the serum CRP and IL-18 levels [11, 12]. Both of the above two drugs affect the immune system and patients' inflammatory responses by affecting coenzyme and pyrimidine, etc. By monitoring the changes in the indicators of conventional inflammatory factors in patients with AS, the visual and immediate therapeutic effects of patients receiving the drug treatment in this study can be analyzed. Therefore, this experiment investigated the serum levels of CRP and IL-18 and other inflammatory factors in patients with ankylosis.

Data and methods

General data

A total of 87 patients with AS admitted to Panyu Central Hospital from January 2017 to January 2019 were enrolled as the study cohort. Among them, 45 cases of patients received methotrexate combined with leflunomide treatment and were designated as the experimental group, and 42 patients treated just with methotrexate were designated as the control group. There were 51 males and 36 females in the study ranging in age from 19-45 years old, with an average of (31.99±5.16) years old and an average disease course of (0.82±0.31) years. The inclusion criteria were as follows: (1) Patients whose symptoms of AS were in line with the *Guidelines for the Diagnosis and Treatment of Ankylosing Spondylitis* [13, 14]. (2) Patients whose thoracic activity was less than that of normal subjects of the same age and gender and whose disease course lasted more than 3 months. (3) Patients without previous contraindications. (4) Patients with AS whose treatment drugs had been discontinued before January. The exclusion criteria were as follows: (1) Patients with severe joint deformities or disability. (2) Patients with severe abnormal diseases of the internal organs or blood. (3) Female patients who were lactating or pregnant. This study was approved by the medical ethics committee of Panyu Central Hospital. All the patients signed the informed consent.

Drug therapy

Control group: (1) Methotrexate (produced by Shanghai Xinyi Pharmaceutical Co., Ltd., SFDA approval number: H31020644, specification: 2.5 mg/tablet) was added based on the routine treatment of non-steroidal antibiotics, liver protection and ca-supplement. Usage: 5 mg/time in week 1, 10 mg/time in week 2, 15 mg/time in week 3. When ESR < 20 mm/h, Bath AS disease activity index (BASDAI) < 2, adjusting it to 15 mg/time, once every two weeks. (2) Leflunomide (Suzhou Changzheng-Xinkai Pharmaceutical Co., Ltd, SFDA approval number: H20000550, specifications: 10 mg × 10 s × 1 plate) was added to the experimental group's treatment in addition to the methotrexate also being taken by the control group. Usage: for patients whose weight > 50 kg, the initial dose was 50 mg/time, 3 times/day. After maintenance for 3 days, it was adjusted to 50 mg per week. For patients whose weight < 50 kg, the initial dose was 40 mg/time, and 3 times/day. After maintenance for 3 days, it was adjusted to 40 mg per week. When ESR < 20 mm/h, BASDAI < 2, 50 mg or 40 mg orally every 10 days. ALT and GGT increased < 20%, continued, ALT and GGT increased > 20%, peripheral blood leukocytes < 3.0 × 10^9/L, platelets < 90 × 10^9/L, discontinued.

Serological testing methods

An ESR special anticoagulation tube was used to measure the ESR level. It was placed in an automatic ESR analyzer (Shanghai Huanxi Medical Devices Co., Ltd.) after being shaken uniformly. Venous blood (3 ml) was extracted from the patients before and after the treatment, letting it stand for 20 min and then cen-

trifuging it at room temperature for 1500 × g for 20 min. The supernatant was taken for testing. The CRP was determined using a fully automatic immune perspective turbidimetry assay (reagent provider: Beckman, USA). The serum IL-18 was quantified using ELISA, and the procedures were in strict accordance with the instructions of the kit (kit provider: Beijing Lab Scientific Co., Ltd.).

Observational indicators

(1) The treatment effects on the AS patients: the main observation indicators are the clinical symptoms such as joint swelling and pain, lower back pain, etc. The efficacy was judged and classified into full control, markedly effective, effective, and ineffective according to the observation indicators of basically disappeared, significantly improved, slightly improved, and no improvement or aggravation. Total effective rate of treatment = full control + markedly effective + effective. (2) Clinical symptoms: the records included the following items. Chest-expansion degree: the patients were standing upright and fully exhaling and inhaling. The chest circumference difference at the 4th intercostal space level was measured with a graduated tape. Occipital wall gap: the distance from the wall to the vertical occipital protuberance. Finger-floor distance: the average distance between the middle fingers of your hands and the ground with your feet close together and your lower limbs upright and bent. (3) The Bath index was collected by specially-assigned person for a blinded evaluation [15, 16]: BASDAI: including fatigue, pain in the central axis and peripheral joints, tendon end pain, morning stiffness degree and time, for a total of 6 issues. The higher the score was, the more serious the discomfort was. Bath AS functional index (BASFI): including the patients' 8 functional activities and 2 life ability to solve the problem, and the higher the score, the worse the activity. (4) VAS score [17]: a 10-scale Vernier caliper marked with 0 and 10 points at the ends was used. The higher the score of the pain perception scale was, the stronger the pain sensation was. (5) Any adverse reactions and changes in the inflammatory indicators occurred during the treatment in each group were recorded and counted.

Statistical methods

SPSS 16.0 statistical software was used in this study. The measurement data were expressed as the mean ± standard deviation. t tests were used to analyze the measurement data. The enumeration data were expressed as rates (%) and analyzed using χ^2 tests. When $P < 0.05$, it was considered that there was statistical significance.

Results

General data

There were no significant differences between the two groups in terms of gender, age, or whether there were joint lesions, inflammatory pain, heart lesions, and other indicators ($P > 0.05$). More details are shown in **Table 1**.

Comparison of the clinical efficacy of the different treatment methods

The clinical effective rate (91.11%) of the experimental group was remarkably higher than the control group's rate (80.95%) ($P < 0.05$). More details are shown in **Table 2**.

Changes in the clinical symptoms in the two groups

There was no significant difference in the clinical symptoms of the two groups before the treatment ($P > 0.05$). After the treatment, morning stiffness time of lumbago, finger-floor distance, and the occipital wall gap in the two groups were significantly reduced, the chest-expanding degree in the two groups was significantly increased, and the changes in the experimental group were more significant ($P < 0.05$), as shown in the **Figure 1**.

Changes in the two groups' BASDAI and BASFI scores before and after the treatment

Before the treatment, the BASDAI and BASFI scores in the two groups were not significantly different ($P > 0.05$). After the treatment, the two scores in both groups decreased significantly, with a more remarkable decrease in the experimental group ($P < 0.05$), as shown in the **Figure 2**.

Spinal pain score analysis before and after the treatment of AS patients in the two groups

The VAS spinal pain scores were basically the same in the two groups before the treatment ($P > 0.05$). After the treatment, the scores were decreased in both groups, and the VAS score in the experimental group was lower than the

Table 1. Comparison of the general data of patients with AS

Item	Experimental group n=45	Control group n=42	t/X^2	P
Gender (case)			0.185	0.667
Male	27 (60.00)	24 (57.14)		
Female	18 (40.00)	18 (42.86)		
Age (years old)	31.96±5.23	32.14±5.11	0.162	0.872
Average disease course	0.81±0.31	0.83±0.30	0.305	0.761
Joint lesions (case)			0.035	0.852
with	37 (82.22)	35 (83.33)		
without	8 (17.78)	7 (16.67)		
Inflammatory pain (case)			1.229	0.268
with	34 (75.56)	29 (69.05)		
without	11 (24.44)	13 (30.95)		
Heart lesions (case)			0.180	0.671
with	23 (51.11)	20 (47.62)		
without	22 (48.89)	22 (52.38)		
Neurologic disease (case)			0.321	0.987
with	12 (26.67)	9 (21.43)		
without	33 (73.33)	33 (78.57)		
Lung lesion (case)			0.199	0.655
with	16 (35.56)	14 (33.33)		
without	29 (64.44)	28 (66.67)		
Low fever (case)			0.020	0.887
with	25 (55.56)	24 (57.14)		
without	20 (44.44)	18 (42.86)		
Thinness and anorexia (case)			0.561	0.454
with	31 (68.89)	27 (64.29)		
without	14 (31.11)	15 (35.71)		
Mild anemia (case)			0.185	0.667
with	27 (60.00)	24 (57.14)		
without	18 (40.00)	18 (42.86)		

Table 2. Comparison of the clinical efficacy of the different treatment methods

Groups	Experimental group n=45	Control group n=42	X^2	P
Full control	20 (44.44)	8 (19.05)	-	-
Markedly effect	12 (26.67)	12 (28.57)	-	-
Effective	9 (20.00)	14 (33.33)	-	-
Ineffective	4 (8.89)	8 (19.05)	-	-
Effective rate(%)	41 (91.11)	34 (80.95)	4.153	0.042

score in the control group (P < 0.05), as shown in **Table 3**.

Comparison of the adverse reactions in the AS patients with the different treatment methods

The incidence of adverse reactions was lower in the experimental group than it was in the control group (P < 0.05). As shown in **Table 4**.

Comparison of the inflammatory indicators in the two groups

ESR, CRP, and IL-18 were significantly lower in the experimental group than they were in the control group (P < 0.05), as shown in the **Figure 3**.

Discussion

AS is an inflammatory arthritis that mainly affects the spine and also affects the sacroiliac joint and the adjacent joints to varying degrees. The pathological changes are mainly manifested as inflammatory changes in the chronic tendon, ligament, and joint capsule attachment points, as well as the fibrosis changes [18]. At present, the specific pathogenesis of AS remains unclear. Due to the long-term chronic aseptic inflammation in its pathological process, the inflammatory response may be an important mechanism for the disease progression of AS [19]. Studies have revealed [20, 21] that inflammatory cytokine IL-18 produced by mononuclear macrophages and acute-phase protein CRP synthesized by hepatocytes are important pro-inflammatory cytokines. It is speculated that the changes in the inflammatory factors in AS patients may directly reflect an improvement of their conditions. The treatment program for AS is single and blinded. There are fewer antirheumatic drugs for treating AS and improving the condition, and the severity of the patients' illnesses will lead to different responses to the treatment drugs. In clinical practice, however, only drugs with poor efficacy or with serious adverse drug

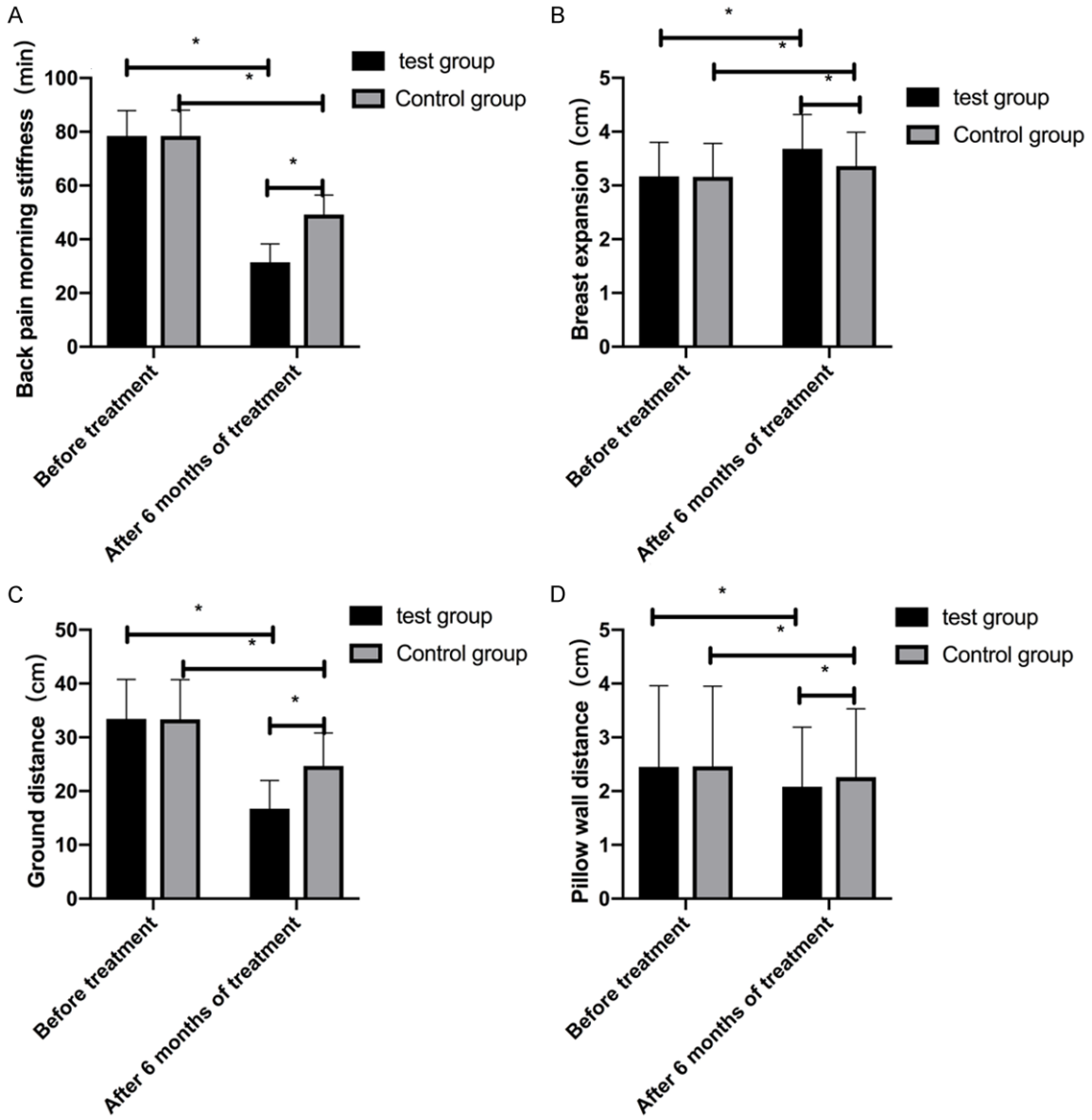

Figure 1. Changes in the two groups' clinical symptoms before and at after 6 months of treatment. A: There was no significant difference in the morning stiffness times of lumbago in the two groups before the treatment, but the morning stiffness time of lumbago in the two groups decreased significantly after 6 months of treatment, and the decrease was more significant in the experimental group. B: There was no significant difference in the chest-expanding degrees in the two groups before the treatment, but the chest-expanding degrees increased significantly in the two groups after 6 months of treatment, and the increase was more significant in the experimental group. C: There was no significant difference in the finger-floor distance in the two groups before treatment, but the finger-floor distance in the two groups decreased significantly after 6 months of treatment, and the decrease was more significant in the experimental group. D: There was no significant difference in the occipital wall gap in the two groups before treatment, and the occipital wall gap in the two groups decreased significantly after 6 months of treatment, and the decrease was more significant in the experimental group. Note: *represents $P < 0.05$.

reactions can be changed and severely limited their scope of use. Patients tend to transition from occasional pain, joint injuries and acute spinal fusion to eventual disability [22, 23]. Therefore, the treatment scheme of methotrexate combined with leflunomide was designed to treat AS patients enrolled in this experiment, and the changes in the patients' clinical symptoms and adverse reactions were recorded.

In this study, it was found that the treatment effect on the patients in the experimental group

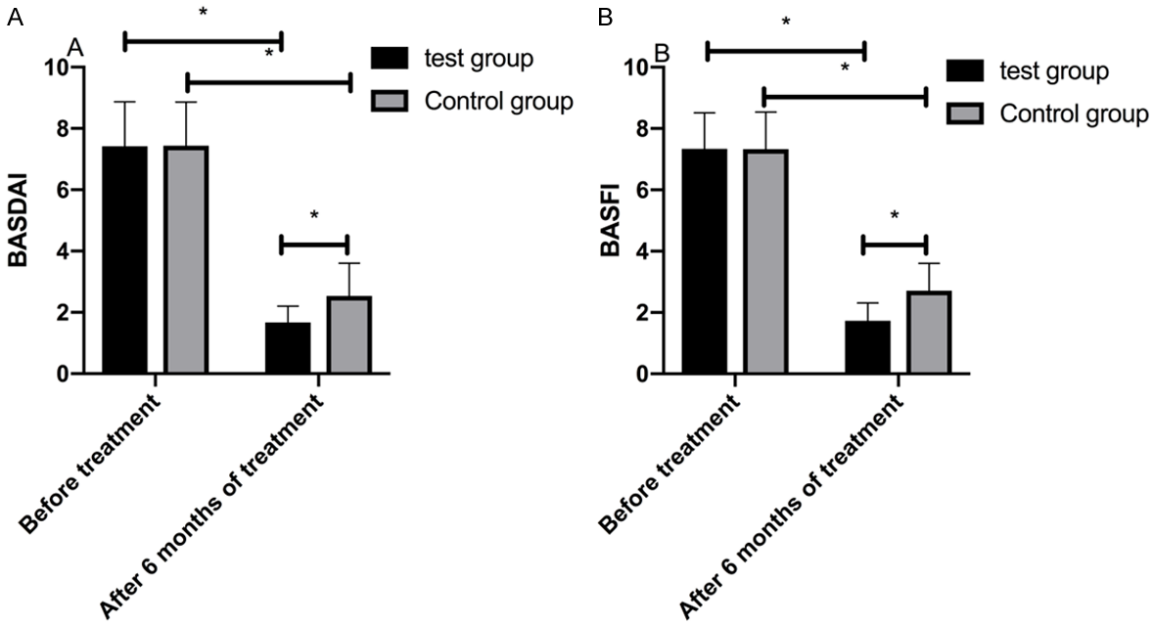

Figure 2. Changes in the two groups' BASDAI and BASFI scores before and after the treatment. A: There was no significant difference in the BASDAI scores in the two groups before the treatment, but after 6 months of treatment, the BASDAI scores in the two groups significantly decreased, and the decrease was more significant in the experimental group. B: There was no significant difference in the BASFI score in the two groups before the treatment, but after 6 months of treatment, the BASFI scores in the two groups significantly decreased, and the decrease was more significant in the experimental group ($P < 0.05$). Note: *represents $P < 0.05$.

Table 3. Spinal pain score analysis before and after the treatment of AS patients in two groups

Groups	Experimental group n=45	Control group n=42	t	P
Before treatment	5.78±0.98	5.79±0.94	0.049	0.961
After 6 months of treatment	3.62±0.51	4.89±0.72	9.544	< 0.001
t	12.630	4.926		
P	< 0.001	< 0.001		

Table 4. Comparison of the adverse reactions in the AS patients with the different treatment methods

Groups	Experimental group n=45	Control group n=42	X^2	P
Hepatic injury	2 (4.44)	4 (4.76)	-	-
Allergic eruption	1 (2.22)	2 (2.38)	-	-
Leukocytopenia	1 (2.22)	2 (2.38)	-	-
Diarrhea	2 (4.44)	4 (4.76)	-	-
Transaminase elevation	1 (2.22)	2 (2.38)	-	-
Total adverse reaction rate (%)	7 (15.56)	14 (33.33)	7.812	0.005

was better than the treatment effect on the patients administered methotrexate alone. In order to explore the improvement of the specific curative effect, the morning stiffness of lumbago, and the chest and spinal activity of the patients were recorded and analyzed, and it was found that the recovery of the patients in the experimental group was better than the recovery in the control group. These results suggest that the combination of the two drugs can improve the symptoms of activity limitation more significantly. However, the gold standard for AS patient activity has not been defined. The subjective judgment analysis of the patients' BASDAI and BASFI scores showed that the two groups' scores were decreased after the treatment, and the scores of the experimental group decreased more significantly. Studies have shown [24] that both BASDAI and BASFI are based on patients' subjective responses to questionnaires. The index was highly subjective. It was positively related to the symptoms of lower back pain in

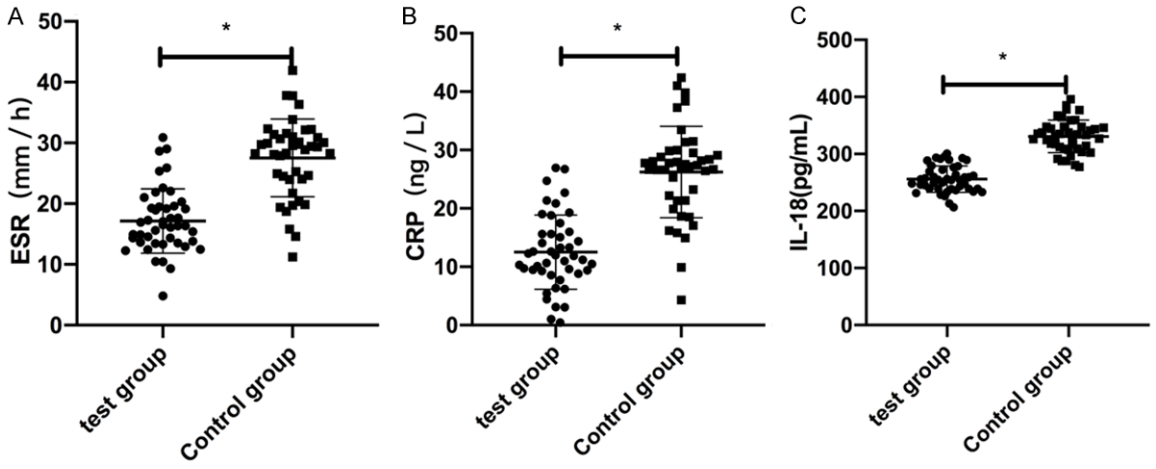

Figure 3. Comparison of the inflammatory indicators in the two groups. A: The ESR level in the experimental group was significantly lower than it was in the control group. B: The CRP level in the experimental group was significantly lower than it was in the control group. C: The Il-18 level in the experimental group was significantly lower than it was in the control group. Note: *represents $P < 0.05$.

the AS patients, but not related to the peripheral joint swelling and pain. Since they have not been combined with auxiliary and clinical physical examinations, the reliability of the results is low, and then can only be used as reference data to reflect some of the conditions of AS. Combined with the analysis of the patients' symptoms, methotrexate and leflunomide may have certain effects on the improvement of patient activity, and the combined effect of the two is more obvious. In order to further understand the changes in the AS patients, we also included the patients' pain perceptions in the results and found that the VAS scores of spinal pain in both groups decreased after the treatment, and the patients in the experimental group performed better at pain control. Previous studies [25, 26] have shown that methotrexate significantly improves joint symptoms in AS patients at certain doses. However, in the course of treatment, patients often suffer from the discomfort of nausea and vomiting caused by the use of methotrexate, and the body's drug resistance leads to limited medical promotion and application. In order to avoid the adverse reactions of methotrexate, it is often used in combination with leflunomide clinically, thus suppressing the immune response and alleviating the joint pain symptoms by inhibiting the release of the inflammatory factors and the release and reproduction of lymphocytes. Combined with the adverse reaction results recorded in this paper, the adverse reactions caused by the combined medication are relatively small, which is consistent with the conclusions in the literature, indicating that the combined medication has stronger pain relief and medical safety than methotrexate alone. As well as with the inflammatory changes of AS after treatment, we found that the ESR, CRP, and IL-18 levels in the experimental group were significantly lower than they were in the control group. It has been reported in studies [27-29] that the long-term onset of AS can induce joint damage, which is related to long-term chronic inflammation, leading to high coagulation status, abnormal metabolism of blood lipids, vascular endothelial function defects and bone fibrosis. Previous published research also pointed out [30, 31] that the elevation of the ESR, CRP, and IL-18 levels may be correlated with the degree of metabolic activity at the lesion site of AS and the fibrosis and inflammation of bone, indicating that methotrexate and leflunomide have an inflammation inhibitory effect on patients with AS, and a combination of the two has a more notable anti-inflammatory effect.

In conclusion, the combination of methotrexate and leflunomide in the treatment of AS can achieve a good clinical effect and effectively alleviate the clinical symptoms and patients' functional inflammatory changes, with a high degree of safety. However, in this study, there are still some deficiencies in the exploration of the inflammatory changes of AS in the direction of inflammatory factors. For example, only the

changes in the expressions of CRP and IL-18 were studied. Therefore, more inflammatory factors will be analyzed in order to achieve high-efficiency anti-inflammatories and to provide better treatment for AS patients.

Disclosure of conflict of interest

None.

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