

Original Article

DMARDs combined with acupuncture therapy to treat RA: study of effects on dsDNA/NETs level and mechanism analysis

Xiaojun Wang^{1,2}, Huaiyu Wang¹, Xiaohong Lv², Xiaoling Wang³

Departments of ¹Hematology, ²Rheumatology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi, China; ³Department of Hematology and Rheumatology, Ankang Hospital of Traditional Chinese Medicine, Ankang 725000, Shaanxi, China

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Abstract: Objective: This study aimed to analyze the efficacy of DMARDs combined with acupuncture therapy in rheumatoid arthritis (RA) patients. Methods: A total of 200 RA patients were equally divided into Group A (GA, n=100, DMARDs combined with acupuncture therapy) and Group B (GB, n=100, DMARDs) by a Random Number Table. Joint swelling and pain, DAS28 score and dsDNA/NETs levels were compared between the two groups. Results: At the 4th and the 8th weeks of treatment, GA yielded better indices for symptoms and signs, lower ESR, RF, CRP and dsDNA/NETs levels in comparison with the GB (P<0.05). At the 8th week, the two groups were statistically different in the number of patients with joint function at Levels I, II and IV (P<0.05) but not in the incidence of adverse reactions which was 7.00% for GA and 9.00% for GB (P>0.05). As compared with GB at the 8th week of treatment, the proportion of GA patients with disease activity (DA) in remission or low activity stages was higher, and fewer patients were in the moderate or high activity stages (P<0.05). Conclusion: RA patients greatly benefited from DMARDs and acupuncture therapy, including significantly mitigated clinical symptoms, improved DAS 28 scores and better controlled dsDNA/NETs levels.

Keywords: DMARDs, acupuncture, RA, dsDNA/NETs, mechanism

Introduction

RA (Rheumatoid arthritis) is a chronic and inflammatory autoimmune disease involving most of the joints of the body. The persistence of synovitis, cartilage damage and bone erosion are reported [1], and RA's association with other factors (RF), including cardiovascular diseases, or increasing risks of other system complications are demonstrated in the clinic [2].

So far, the pathogenic mechanisms of this disease are not clearly defined but considered to be related to immune system disorders, environment and heredity, according to analysis [3]. The immune system's immune cells and antibodies can accurately recognize and effectively eliminate invaders, especially viruses and pathogens. The blood of patients with autoimmune diseases has a lot of antibodies for their own tissues, which can further result in

inflammatory-related diseases. Whenever this defense system malfunctions, RA may happen [4]. In western countries, RA is extensively treated by disease modifying antirheumatic drugs (DMARDs) which can mitigate symptoms to a certain degree, but may lead to clear adverse reactions if taken in a sustained manner, and thus can affect a patients' medication adherence.

In traditional Chinese medicine, RA is classified as arthromyodynia, and its causes include aging, macronosia, wind-cold-dampness, injury from falling down, as well as improper arrangement of labor and rest. According to TCM, arthritis develops as a result of wind-cold-dampness and meridian obstruction, and is accompanied with pain [5]. TCM therapies for RA contain oral administration of decoctions, external application of traditional Chinese medicine, massage, cupping, medicated diets and acupuncture;

among which, acupuncture has been used in TCM for more than a thousand years. Ancient books record how acupuncture can dissipate the heat in patients with heat syndrome, clear fire and nourish vitality in patients with deficiency syndromes, reheat the qi in patients with cold syndrome, and disperse the fire in patients with excess syndromes. The clinical application of acupuncture has gone through several stages. Initially, it was used to treat curable diseases, and then it was mainly used for the treatment of diseases with acupuncture and moxibustion, which had a good therapeutic effect on the main symptoms and signs. Later, it was used to treat diseases with no definite substance to the nature of the disease and help relieve some of the derived symptoms. Currently, progress has been made in the treatment of diseases for which cures are not exactly known, or for which there are already clearly efficient means of treatment, and which are rarely treated with acupuncture. In any case, the clinical use of acupuncture is gradually increasing. As for the application advantages of acupuncture, animal experiments have shown that acupuncture treatment can help to restore the activity of spleen lymphocytes, enhance the immune function of the body, and play a positive role in regulating the immune system. Pathological studies have found that acupuncture can reduce the congestion and edema of articular synovium cells, reduce synovium cell proliferation and inflammatory cell infiltration, and has a good mechanism of improving immunity and anti-inflammation. Modern medical studies have found that acupuncture therapy can speed up blood circulation, reduce swelling, relieve pain and inflammation, improve blood flow in a lesion, eliminate some of the pathogenic inflammatory factors to relieve or even eliminate clinical symptoms [6]. Although many studies have been conducted on treating RA with acupuncture, there are few comparing the advantages and disadvantages of acupuncture and DMARDs. In this study, the application value of acupuncture was discussed by comparing the effects of the two therapies in 200 patients.

Materials and methods

Materials

A total of 200 RA patients admitted to our hospital from May 2018 to May 2019 were divided into GA (n=100) and GB (n=100) by

a Random Number Table. Inclusion criteria: patients who were preliminarily identified with disease according to the diagnostic criteria of RA in Chinese [7] and western [8] medicines, but had not been treated prior to the study were included and provided a fixed domicile for return visit; they were informed of the study content and agreed to be involved. This study was approved by our hospital. Exclusion criteria: patients for whom no definitive diagnosis result was obtained, or patients who were complicated with severe diseases in the stomach and intestinal tract, or patients who were recently treated by probiotics or antibiotics, or patients who were pregnant or lactating or who were allergic to the drugs studied were excluded.

Methods

Patients in GB were treated with DMARDs, including diclofenac sodium (specification: 2 ml, 75 mg of sodium diclofenac and 20 mg of lidocaine hydrochloride, Approval by H2009-0085, manufactured by Anhui Wanbei Pharmaceutical Co. Ltd.) intramuscularly injected at the dose of 75 mg q.d., leflunomide (specification: 10 mg*10 s*1 plate, Approval by H200-00550, manufactured by Suzhou Changzheng-Cinkate Pharmaceutical Co., Ltd.) and methotrexate (specification: 2.5 mg*100 s, Approval by H31020644, Shanghai Shangyao Xinyi Pharmaceutical Co., Ltd.) taken orally with a dose of 20 mg q.d. and 10 mg q.d. The treatment continued for 8 weeks.

For patients in GA, in addition to the western medicines given to GB, they were also provided acupuncture at Qihai, Guanyuan, Zusanli, Tianshu, Xuehai, Jiushenque, Sanyinjiao, and Zhongwan, or Ashi as the moxibustion if necessary. Before acupuncture, the points were disinfected with iodophor. Huanqiu Acupuncture Needles for Single Use (0.25 mm × 40 mm) were inserted into the points to bring about the desired sensation and retained in place for half an hour, during which, the even reinforcing-reducing method was applied; moxibustion was performed on Shenque and Ashi for half an hour q.d. A course of treatment lasted for 8 weeks.

Observation indices

Indices of symptoms and signs: before treatment, at the 4th and the 8th weeks of treatment,

the patients' morning stiffness duration, gripping power, walking time (time required to finish a walk of 20 m in the morning), joint swelling and pain were documented. Joint swelling [9] was categorized as Level 0 (no swelling), Level 1 (joint swelling but not rising above the proximal bone protrusion), Level 2 (obvious joint swelling flush with the bone protrusion while nearby soft tissues depressed and disappeared), and Level 3 (severe swelling rising above the proximal bone protrusion). Joint pain [10] was evaluated according to joint tenderness scored between 0 and 3 and joint pain in activities varying from 0 to 6 scores. Four grades were used to express joint tenderness, 0 for no tenderness, 1 for mild tenderness, 2 for moderate tenderness with rebounding when pressed forcefully, and 3 for severe tenderness with rebounding when pressed gently. Joint pain in activity was indicated by numbers from 0 to 3, 0 for no pain, 1 for mild pain, 2 for moderate pain and 3 for sharp pain that caused patients to rejected participating in activities.

Joint function: grades I to IV were used to describe the severity of joint dysfunction [11]. Patients in grade I are able to live a normal life without obstructions; patients in grade II may be challenged in the case of tasks with more activities, except for ordinary daily life; patients in grade III moderate malfunction with abilities limited to simple daily life tasks, and patients in grade IV severe malfunction that even simple daily life can't be handled properly. The evaluation was performed before and at the 8th week of treatment.

Lab indices: erythrocyte sedimentation rate (ESR), rheumatoid factors (RF) and C-reaction protein (CRP) were measured before treatment, at the 4th and the 8th weeks of treatment by Westergren method, electrochemical luminescence method and ELISA method, respectively.

DA: The DAS 28 scale [12] was used to evaluate DA upon hospitalization, before treatment, and at the 8th week of treatment. The scale involves 28 joints including shoulders, elbows, wrists, metacarpophalangeal joints, proximal interphalangeal joints, and knee joints. DAS 28 score is calculated according to the following expression: $0.56 \times \text{number of joints with tenderness} + 0.28 \times \text{number of joints swelling} + 0.7 \times \text{ESR} \times 1.08 + 0.16$. The disease is in the

remission stage if the score is at or under 26, in the low activity stage if the score is between 27 and 32, in the moderate activity stage if the score varies from 33 to 51, and in the high activity stage if the score reaches or exceeds 52.

dsDNA/NETs level: PicoGreen dsDNA Quantitation Kit (American Invitrogen) was used for testing; 5 ml of blood was drawn from the veins of patients and collected into a general tube. After centrifugation, the serum was stored under -20°C. PicoGreen was diluted by TE buffer (1:200) to prepare a dye working solution which was then kept in a dark environment, incubated and evenly mixed with the serum to be tested at the ratio of 1:1. The mixture was stored under room temperature without light for 5 min and then used to determine the fluorescence value for dsDNA/NETs level calculation.

Adverse reactions: the incidences of gastrointestinal discomfort, rash, hidrosis, aleukocytosis, and liver dysfunction after treatment were recorded and compared between the two groups.

Statistical analysis

Statistical analysis was performed with SPSS 22.0. In case of numerical data expressed as Mean \pm Standard Deviation, comparison studies were carried out through independent-samples *t* test. In case of nominal data expressed as [n (%)], comparison studies were carried out through chi-squared test for intergroup comparison. Intragroup and intergroup comparisons of multiple time points were performed through ANOVA analysis and *F* test. For all statistical comparisons, significance was defined as $P < 0.05$.

Results

General materials

No statistical difference was found between the two groups in average age, average course of disease, average BMI, proportions of male and female patients, proportions of patients with/without complications, and proportions of patients at different DA levels upon admission ($P > 0.05$) (Table 1).

Efficacy of DMARDs combined with acupuncture therapy in RA patients

Table 1. Intergroup comparison of general materials ($\bar{x} \pm s$)/[n (%)]

Materials		GA (n=100)	GB (n=100)	t/X ²	P
Gender	Male	43 (43.00)	46 (46.00)	0.182	0.669
	Female	57 (57.00)	54 (54.00)		
Age (y)		59.86±15.38	62.37±17.81	1.067	0.287
Course of disease (month)		18.46±3.61	17.99±3.28	0.964	0.336
BMI (kg/m ²)		23.15±2.46	22.94±2.61	0.586	0.559
Complication	Yes	28 (28.00)	31 (31.00)	0.216	0.642
	No	72 (72.00)	69 (69.00)		
DA upon admission	Remission stage	0 (0.00)	0 (0.00)	1.638	0.827
	Low activity stage	16 (16.00)	14 (14.00)		
	Moderate activity stage	58 (58.00)	59 (59.00)		
	High activity stage	26 (26.00)	27 (27.00)		

Table 2. Intergroup comparison of improvements in symptoms and signs before and after treatment ($\bar{x} \pm s$)

Group	Time	Morning stiffness period (min)	Gripping power (kpa)	walking time (s)	Joint swelling (score)	Joint pain (score)
GA (n=100)	Before treatment	30.15±12.28	8.15±2.31	38.45±5.96	2.11±0.36	4.62±0.75
	At the 4 th week of treatment	22.24±8.94*	11.94±3.55*	30.22±4.18*	1.75±0.29*	3.12±0.53*
	At the 8 th week of treatment	13.23±5.67*	14.38±2.43*	11.25±3.66*	1.00±0.23*	1.64±0.28*
GB (n=100)	Before treatment	31.59±13.25	7.89±2.19	37.91±6.23	2.16±0.39	4.57±0.72
	At the 4 th week of treatment	27.86±9.63*	9.11±2.58*	34.61±5.42*	1.86±0.31*	3.86±0.60*
	At the 8 th week of treatment	18.79±6.38*	11.34±2.33*	18.99±5.33*	1.38±0.28*	2.23±0.37*
t1		4.277	6.449	10.250	2.591	9.244
P1		0.000	0.000	0.000	0.010	0.000
t2		6.514	9.030	11.971	10.487	12.715
P2		0.000	0.000	0.000	0.000	0.000

Note: t1 and p1 are the comparative statistical values at the 4th week, while t2 and p2 are for the 8th week. *P<0.05 vs conditions before treatment.

DMARDs combined with acupuncture therapy improve the indices of symptoms and signs

In terms of morning stiffness duration, gripping power, walking time, joint swelling score, and joint pain score, the two groups were not statistically different from each other before treatment (P>0.05). However, both achieved improvements, which were more significant in GA, at the 4th and the 8th weeks of treatment (P<0.05) (Table 2).

DMARDs combined with acupuncture therapy improve joint function

Before treatment, the joint function grading results of GA were not significantly different from GB's (P>0.05). At the 8th week of treatment, statistical difference was observed in GA for the proportions of patients with joint function at levels I, II, III and IV (P<0.05), while in GB, the number of patients at level II was significantly

higher, and the numbers of patients at other levels were not statistically different as compared with the figures before treatment (P>0.05). At the 8th week of treatment, a statistical difference was observed between the two groups in the proportions of patients at levels I, II and IV (P<0.05) but not in the proportion of patients at level III (P>0.05) (Table 3).

DMARDs combined with acupuncture therapy ameliorate lab indices

The ESR, RF and CRP of GA and GB were (79.28±10.34) mn/hr, (196.83±30.53) lu/ml and (59.86±8.13) mg/L, (78.93±11.15) mn/hr, (195.78±31.48) lu/ml and (58.72±9.34) mg/L respectively, before treatment (P>0.05). They were (42.31±10.56) mn/hr, (105.28±16.39) lu/ml and (47.51±9.22) mg/L, (48.62±11.32) mn/hr, (124.32±18.46) lu/ml and (55.62±10.29) mg/L respectively, at the 4th week of treatment (P<0.05). They were respectively

Table 3. Intergroup comparison of joint function grading before and after treatment [n (%)]

Group	Time	Level I	Level II	Level III	Level IV
GA (n=100)	Before treatment	0 (0.00)	16 (16.00)	59 (59.00)	25 (25.00)
	At the 8 th week of treatment	12 (12.00)*	43 (43.00)*	37 (37.00)*	8 (8.00)*
GB (n=100)	Before treatment	0 (0.00)	14 (14.00)	60 (60.00)	26 (26.00)
	At the 8 th week of treatment	4 (4.00)	29 (29.00)*	49 (49.00)	18 (18.00)
χ^2		4.348	4.254	2.938	4.421
P		0.037	0.039	0.087	0.036

Note: χ^2 , and p are the comparative statistical values at the 8th week. * $P<0.05$ vs conditions before treatment.

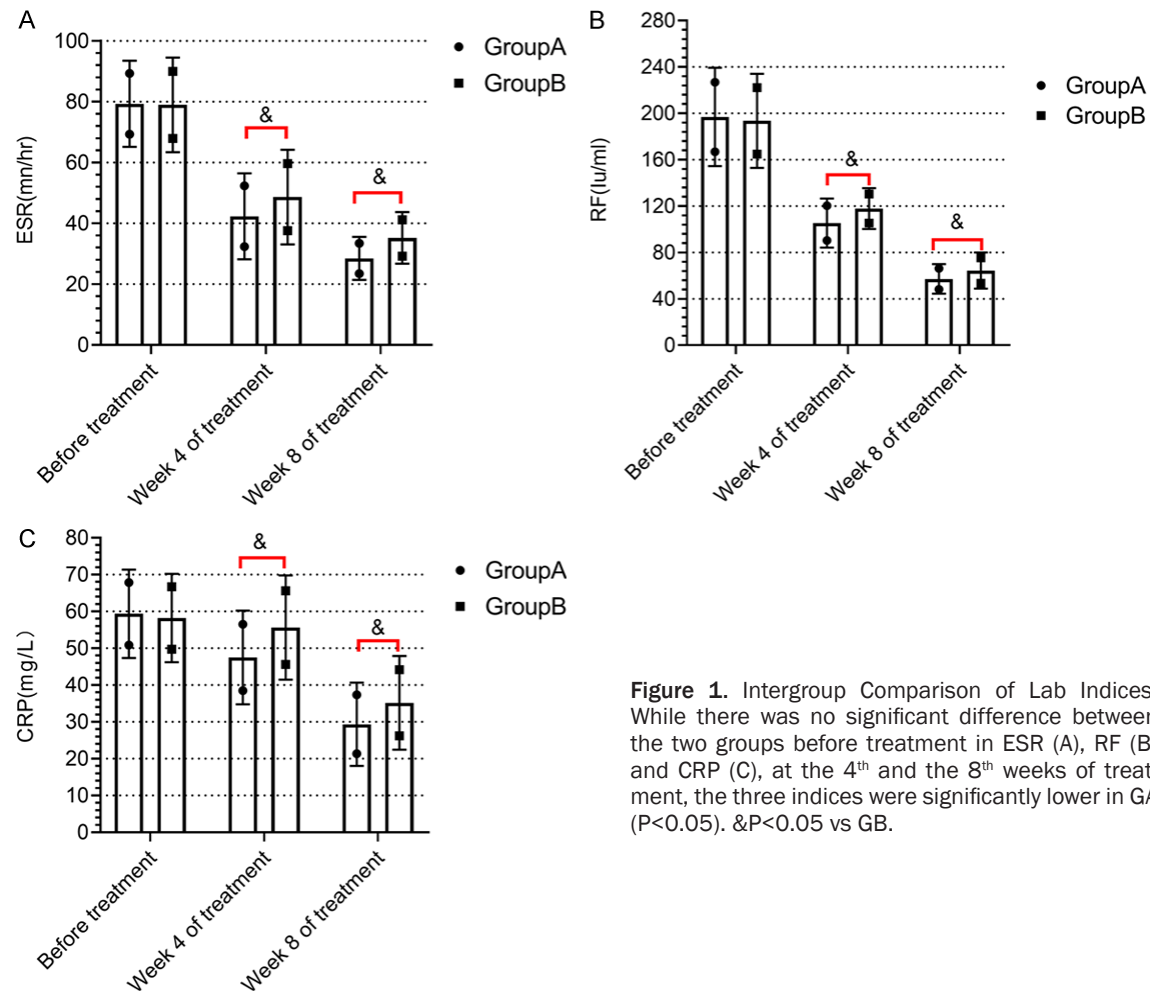


Figure 1. Intergroup Comparison of Lab Indices. While there was no significant difference between the two groups before treatment in ESR (A), RF (B) and CRP (C), at the 4th and the 8th weeks of treatment, the three indices were significantly lower in GA ($P<0.05$). & $P<0.05$ vs GB.

(28.45±5.64) mn/hr, (57.15±9.83) lu/ml and (29.34±8.45) mg/L, (35.18±6.92) mn/hr, (64.38±11.12) lu/ml and (35.19±9.82) mg/L at the 8th week of treatment ($P<0.05$) (**Figure 1**).

DMARDs combined with acupuncture therapy improves DA

The DAS 28 scores at the 8th week of treatment indicated that in GA, the proportions of patients

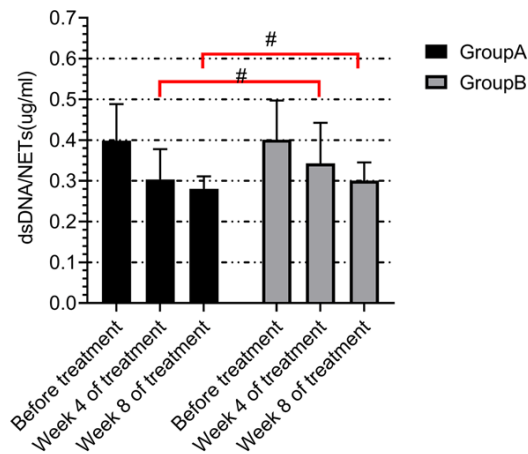
with DA in remission/low activity stage were significantly higher and the proportions of patients with DA in moderate/high activity stage were significantly lower than in GB ($P<0.05$) (**Table 4**).

DMARDs combined with acupuncture therapy reduce dsDNA/NETs level

The dsDNA/NETs levels of GA and GB were (0.435±0.108) ug/ml and (0.433±0.105) ug/

Table 4. Intergroup comparison of ad at the 8th week of treatment [n (%)]

Group	Remission stage	Low activity stage	Moderate activity stage	High activity stage
GA (n=100)	20 (20.00)	45 (45.00)	25 (25.00)	10 (10.00)
GB (n=100)	10 (10.00)	30 (30.00)	40 (40.00)	20 (20.00)
χ^2	3.922	4.800	5.128	3.922
<i>P</i>	0.048	0.028	0.024	0.048

**Figure 2.** Intergroup Comparison of dsDNA/NETs Level. The dsDNA/NETs level in GA was not significantly different from the GB's before treatment but was clearly lower at the 4th and the 8th week of treatment ($P<0.05$). # $P<0.05$ vs GB.

ml before treatment ($P>0.05$); then they were (0.319 ± 0.087) ug/ml and (0.356 ± 0.093) ug/ml at the 4th week of treatment ($P<0.05$); and finally they were (0.281 ± 0.026) ug/m and (0.302 ± 0.034) ug/ml at the 8th week of treatment ($P<0.05$) (**Figure 2**).

DMARDs combined with acupuncture therapy do not increase adverse reactions

Seven cases of adverse reactions were reported in GA, including 2 cases of rash, 2 cases of hidrosis and 3 cases of gastrointestinal discomfort, leading to an incidence of 7.00%. While in GB, 9 patients claimed adverse reactions such as aleukocytosis (1), liver dysfunction (1), hidrosis (1), rash (3) and gastrointestinal discomfort (3), resulting in the incidence of adverse reactions of 9.00% ($P>0.05$) (**Table 5**).

Discussion

In China, RA has an incidence between 0.32% and 0.36%, and is expected in all age groups

[13] but mostly in people around 40 years old. A total of 75% of the RA patients were women and 25% were men. For RA, the condition progresses slowly, and morning stiffness is a common symptom which may last half an hour or even one hour in the early stages. Joints involved include the fingers, wrists, ankles, elbows, knees, etc. In addition, there will be

joint swelling and pain, gradually limiting patients' movement, severely affecting and compromising their quality of life [14]. Traditional Chinese medicine brings RA into the category of arthralgia syndrome, which means blocking and obstruction. Arthralgia is a result of wind, coldness and dampness [15]. Traditional Chinese medicine believes that arthralgia syndrome is essentially caused by deficiency of liver, spleen and kidney, promoted by wind, coldness, dampness, heat and blood stasis. Its main pathologies are qi and blood stasis, as well as meridian obstruction. Accordingly, therapies aim to remove dampness, stasis and heat, unblock collaterals, dispel wind and coldness, and be supported by regimes to reinforce the body resistance [16].

In this study, acupuncture therapy was performed at points including Sanyinjiao to replenish the vital essence and remove heat, nourish the blood and promote blood circulation, and Zusanli to strengthen the spleen and stomach, remove dampness through diuresis, stop pain and reinforce the body resistance [17]. Xuehai is a point of spleen meridian of foot-Taiyin, and where the blood from spleen meridian gathers. Stimulating this point can generate and promote blood circulation, and remove blood stasis. Subject to the Ren channel, Qihai is stimulated to cultivate and replenish the primordial Qi, while Guanyuan is related to constitution consolidation, primordial Qi cultivation, and tonifying Xiajiao; Tianshu takes charge of regulating qi-flowing for strengthening of the spleen, clearing and activating the channels and collaterals; Moxibustion of Shenque unblocks channels for Qi passage, while Zhongwan helps harmonize stomach, tonify spleen, calm the adverse-rising energy, and alleviate water retention [18, 19]. By acupuncture those points, collaterals were unblocked, coldness was dispelled, body constitution was consolidated and reinforced, blood stasis, damp-

Table 5. Intergroup comparison of adverse reactions (Case, %)

Group	Rash	Sweating	Gastrointestinal upset	Leukopenia	Abnormal liver function	Total incidence
Group A (n=100)	2	2	3	0	0	7.00
Group B (n=100)	3	1	3	1	1	9.00
χ^2						0.751
<i>P</i>						0.163

ness and wind were removed, and blood circulation was invigorated. In this study, western medicines were combined with acupuncture to treat patients in GA. The better indices of symptoms and signs in GA at the 4th and the 8th weeks of treatment, including morning stiffness duration, gripping power, walking time, joint swelling scores, and joint pain score ($P<0.05$), indicated that the combination of acupuncture improved the mitigation speed and effects of RA symptoms and signs. At the 8th week of treatment, most of the patients in GA were at levels I, II and IV for joint function, and at the remission/low activity stage for DA according to DAS 28 ($P<0.05$), indicating that treatment combination with acupuncture can significantly improve the joint functions of RA patients and reduce the DA. RF is a type of serum immune globulin and an antibody produced in our body against denatured immunoglobulin. Verheul MK et al. [20] found in their study that RF was detected in the serum of about 80% of the RA patients, and can be taken as an effective index of RA diagnosis. ESR and CRP, are both important inflammatory indices, that can reflect the DA of RA positively without impact from patients' age, lipids and red blood cell status, and can be depended on to judge inflammatory infection [21]. In this study, patients in the GA had lower ESR, RF and CRP levels at the 4th and the 8th weeks of treatment ($P<0.05$), indicating that acupuncture therapy can effectively improve patients' inflammatory level and immunity.

Besides, the two groups were not statistically different in the incidence of adverse reactions (7.00% in GA vs 9.00% in GB) ($P>0.05$), indicating that patients' safety is not threatened by acupuncture therapy, and better efficacy is expected under the premises of safety. NETs are a type of 3D net structure consisting of histone, DNA and some antibacterial proteins and formed by neutrophil granulocytes through new cell death pathways, which can kill pathogens. Studies believe that measuring the

dsDNA in the serum provides an indirect way to learn about the level of NETs [22, 23], and NETs play a promoting role in the development and progression of autoimmune diseases such as gouty arthropathy, polyangitis, and systemic lupus erythematosus. Therefore, the level of dsDNA/NETs can be used to judge if the RA conditions are controlled [24]. In this study, lower dsDNA/NETs levels in the GA at the 4th and the 8th weeks of treatment was a sign of significant condition alleviation after acupuncture therapy, and an embodiment of the therapy's values. The specific mechanism is that acupuncture therapy manages to reduce the synovitis exudation and accelerates liquid absorption, cartilage and sclerotin repair to prevent joints from more serious injuries. Treating RA by acupuncture therapy emphasizes reinforcing the body resistance and removing blood stasis, in order to maximally motivate the body's disease resistance for faster recovery. In the early stages, points promoting the health of spleen and stomach can be acupunctured and assisted with local puncturing. When it progresses to the middle and advanced stages, recommended points change to those replenishing liver and kidneys in order to slow down or avoid the disease from being more serious. In the process of treatment, Qi in the body shall always be maintained [25]. Brodin N et al. [26] also revealed in their study that acupuncture can adjust the immunity of our body and affect endocrine, signaling pathways of synovium and synovial cells for the purposes of relieving conditions, resisting infection, slowing down joint damage and improving joint function.

In conclusion, the combination of DMARDs and acupuncture therapy to treat RA patients is not only highly safe, but also significant in improving their conditions and joint function, as well as controlling the dsDNA/NETs level. However, the number of samples is limited in this study, and the results are not comprehensively analyzed, leading to possibly biased conclusions. Future studies shall be based on a larger sam-

ple sizes and more analysis aspects to obtain more scientific and representative conclusions as references of RA patients' treatment.

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Disclosure of conflict of interest

None.

Address correspondence to: Xiaohong Lv, Department of Rheumatology, The First Affiliated Hospital of Xi'an Jiaotong University, No.277, West Yanta Road, Yanta District, Xi'an 710061, Shaanxi, China. Tel: +86-029-85323976; E-mail: lxh000a@163.com

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