ORIGINAL RESEARCH

Relationship Between Serum MMP-2, Siglec-1, Th1/Th2 Cell Ratio, and Disease Activity in Rheumatoid Arthritis

Zhaoyu Chen, PhD; Zijian Ma, PhD; Jiaxiang Gao, PhD; Yan Gao, PhD; Fengyao Mei, MBBS

ABSTRACT

Objective • To investigate the association of serum MMP-2, Siglec-1, and Th1/Th2 cell ratio with disease activity in rheumatoid arthritis (RA).

Methods • Between August and November 2020, Peking University People's Hospital recruited 40 patients with RA and 40 healthy individuals. Various methods such as ELISA, flow cytometry, and RT-PCR were used to assess the levels of sCR1, MMP-2, MMP-9, and Siglec-1 in the participants. Correlation analysis was conducted between Siglec-1 expression and DAS28 and hs-CRP. T lymphocyte subsets; cytokines IFN-γ and IL-4, were assessed using flow cytometry and ELISA in both patient groups.

Results • Rheumatoid arthritis was linked to lower levels of serum sCR1 and higher levels of MMP-2 and MMP-9 compared to healthy individuals ($P < .05$). The percentage of Siglec-1-positive cells in PBMCs was significantly higher in patients with RA than in the healthy group ($P < .05$), with monocytes being the predominant cells expressing Siglec-1. Patients with RA exhibited a significantly higher expression of Siglec-1 mRNA compared to those in a healthy condition ($P < .05$), and the expression of Siglec-1 in these patients was positively correlated with DAS28 and hs-CRP ($P < .05$). Study patients demonstrated a notably lower level of peripheral blood CD8+ cells and a higher CD4+/CD8+ ratio when compared to healthy individuals ($P < .05$). There was no statistically significant difference in CD3+CD4+ levels between the 2 groups ($P > .05$). Rheumatoid arthritis was associated with a higher level of peripheral blood IFN-γ and a lower IL-4 level than healthy individuals ($P < .05$).

Conclusion • There was a strong link between sCRl, MMP-2, and MMP-9 and the progression of rheumatoid arthritis. These markers can effectively monitor disease activity in patients with rheumatoid arthritis. Siglec-1 is highly expressed in peripheral blood and can be used to track disease activity and inflammation in these patients. Regulating Th1/Th2-mediated homeostasis may help alleviate symptoms in individuals with rheumatoid arthritis. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic and progressive erosive destruction of joints. The underlying mechanism of this disease involves the interaction between various types of cells, of which fibroblast-like synovial cells play a key role. These cells exhibit an aggressive phenotype, releasing inflammatory mediators that lead to synovial inflammation and subsequent damage to bone. Studies indicate that the prevalence of rheumatoid arthritis in China is between 0.23 and 0.36%, while in Europe and the United States, the prevalence is approximately 1%. The disease is a major contributor to disability and labor loss in China, imposing significant economic and psychological burdens.

The pathogenesis of rheumatoid arthritis is complex, and the cause of the disease is unknown. Apart from affecting the joints, it also leads to damage in multiple systems and organs. Some patients experience protracted and challenging recovery, with symptoms prone to recurrence. Due to the current limitations of scientific and technological developments and research conditions, understanding RA and its pathological aspects remains unclear. It is believed that the pathogenesis of RA is influenced by various factors such as genetics, immunity, and the environment.[1]

The clinical manifestations of rheumatoid arthritis include pain, swelling, and decreased function of the affected joints. The pathological changes of rheumatoid arthritis primarily involve the proliferation of synovial tissue, inflammatory cell infiltration, and progressive irreversible destruction of cartilage.[2] The uncertainty of the pathogenesis of clinical rheumatoid arthritis has resulted in difficulties in disease management.

The current clinical treatment option consists of comprehensive or augmentation therapy initiated after diagnosis, depending on disease activity to alleviate the clinical symptoms. Anti-arthritis drugs are used for the management of arthritis, and their prolonged use is associated with serious adverse effects. Alternative medical approaches are required to meet the medical needs of patients with RA. The unfavorable effects and drug toxicity of Western drugs result in unsatisfactory treatment.

Traditional Chinese medicine (TCM) believes that rheumatoid arthritis belongs to the category of "arthritis." This condition is believed to result from a combination of diseases caused by the synergy of internal and external factors. The invasion of exogenous pathogens such as rheumatism, cold, and heat manifests as disease symptoms. At the same time, imbalances of qi and blood of ying and wei and the deficiency of viscera are the root of the disease. The combination of invasion of exogenous pathogens and internal deficiency leads to the occurrence of rheumatoid arthritis. The key pathological changes of the disease are phlegm, dampness, and blood stasis, leading to prolonged joint pain. Furthermore, phlegm turbidity and blood stasis indicate a poor prognosis. Treatment for RA can be tailored based on the specific pathogenesis of each case.

There have always been differences in traditional Chinese medicine syndrome types of rheumatoid arthritis. This can be attributed to the etiology and pathogenesis of the condition. Different doctors hold diverse perspectives on the fundamental core pathogenesis and syndrome differentiation of rheumatoid arthritis. In TCM, Radix Et Rhizoma Tripterygii wilfordii, Paeoniae Radix Alba, and Whiteback Thundergodvine are common herbal medicines used for RA with favorable effects.

Serum-soluble complement receptor type 1 (sCR1) inhibits and regulates complement activation. It is the only complement regulatory protein that suppresses the c3/c5 convertase of the 3 complement activation pathways: classical, bypass, and MBL.[3] Additionally, it assists factor I in C3b cleavage. Matrix metalloproteinases (MMP) are important proteases that degrade or remodel the extracellular matrix (ECM) and function in cell migration, tumor infiltration, and tumor metastasis.[4] In healthy individuals, sialic acid-binding Ig-like lectins (Siglecs)-1 are consistently expressed on specific tissue macrophages but are inactive on peripheral blood cells. Inflammatory activation of the mononuclear macrophages causes a rapid increase of Siglec-l in peripheral blood monocytes. The pathophysiology of rheumatoid arthritis, as a chronic systemic autoimmune illness, remains unknown.

In recent years, there has been a suggestion that rheumatoid arthritis is caused by both natural and acquired immunity. It has also been proposed that the development of rheumatoid arthritis is linked to the humoral immune response triggered by degenerative IgG. Rheumatoid arthritis synovial tissue has an abundant expression of Siglec-1, which is a crucial marker of mononuclear macrophage activation. Extensive clinical studies on T lymphocyte subsets and helper T cells (Th1/Th2) have revealed that an imbalance in the levels of Th1/Th2 cells and their secreted cytokines is closely related to the development of rheumatoid arthritis.[5] Accordingly, 40 patients with rheumatoid arthritis treated in Peking University People's Hospital between August 2019 and November 2020 were recruited to investigate the association of serum MMP-2, Siglec-1, and Th1/Th2 cell ratio with disease activity in rheumatoid arthritis and provide a clinical reference.

MATERIALS AND METHODS

Baseline Data

Between August 2019 and November 2020, 40 patients with rheumatoid arthritis treated in our institution (Department of Rheumatology and Immunology, Peking University People's Hospital) were recruited in the disease group, and 40 healthy individuals were recruited in the healthy group following physical examination.

The disease group consisted of 17 males and 23 females, ranging in age from 24 to 78 years, with a mean age of 51.72±10.67 years. The disease duration in this group varied from 1 to 19 years, with a mean duration of 8.26±4.54 years. The characteristics of the healthy group were 20 males and 20 females, aged between 23 and 70 years, and a mean age of 49.48±11.20 years. Statistical analysis revealed no significant differences between the 2 groups ($P > .05$). (Table 1). The original sample size calculation estimated that 40 patients in each group would be needed to detect a 3-point difference between groups in a 2-sided significance test with a power of 0.8 and an alpha error level of 0.05.

The trial was carried out in accordance with the Good Clinical Practice guidelines developed by the International Council for Harmonisation and in compliance with the trial protocol. The protocol received approval from the institutional review boards or independent ethics committees at each study site. All patients provided written informed consent per the Declaration of Helsinki principles. An independent data monitoring committee monitored safety and efficacy data, ethics number: MK-PO20190706.

Inclusion and Exclusion Criteria

To be included in the study, prospective participants were required to have met (1) the diagnostic criteria of the American College of Rheumatology (ACR) diagnostic

Table 1. Comparison of Baseline [n(%)]

	Disease group (n=40)	Healthy group (n=40)	t or χ^2	P value
Groups			0.453	.501
Male	17	20		
Female	23	20		
Mean age (year)	51.72±10.67	49.48±11.20	0.916	.362
Mean duration of disease (year)	8.26±4.54	-	-	-

criteria and (2) having not previously been provided with medication of slow-acting rheumatic drugs.

Prospective subjects were excluded explicitly if they met any of the following criteria: (1) a combination of 2 or more autoimmune diseases, such as systemic lupus erythematosus, Sjögren's syndrome, ankylosing spondylitis, etc.; (2) concomitant use of other slow-acting anti-rheumatic drugs; (3) women of childbearing age who have childbearing requirements; (4) those with a clear history of allergy to the drugs used in the study; (5) patients with severe primary cardiovascular and cerebrovascular, liver and kidney, and hematopoietic system diseases; (6) patients with or suspected of having tumors; (7) patients with or suspected of having hepatitis B, tuberculosis, or other infectious diseases; (8) patients with or suspected mental illness; (9) individuals who are currently participating or planning to participate in other clinical trials; (10) patients with severe deformity diseases; and (11) individuals with a history of alcohol or drug abuse.

Methods

In both groups, the researchers collected 5ml of morning fasting venous blood. The blood was left to rest at room temperature for 2 hours and then centrifuged at 3000 r/min for 10 minutes to isolate the serum. The serum was stored in a refrigerator at -20°C. The researchers then used the enzyme-linked immunosorbent assay (ELISA) method to determine sCRl, MMP-2, and MMP-9 levels.

To isolate PBMCs from peripheral blood and extract total cellular RNA, the researchers obtained 3ml of ethylenediaminetetraacetic acid dipotassium salt (EDTA-K2) anticoagulated whole blood with an equal volume of 0.9% sodium chloride injection for dilution. They placed it on top of a 5ml lymphocyte separation solution. PBMCs were separated by density gradient centrifugation at 2500 r/min for 30 min with a centrifugation radius of 12cm. The PBMC layer was collected, and the cells were rinsed twice with a phosphate buffer solution (PBS). Then, cell precipitate was collected, and total cellular RNA was extracted using an RNA extraction kit. All procedures were strictly followed in accordance with the instructions. The quality and concentration of the total RNA obtained were determined using a 1.2% agarose gel electrophoresis and UV spectrophotometer.

The researchers employed the RT-PCR method to determine the expression of Siglec-1 mRNA. As instructed, the cDNA synthesis was carried out using the Invitrogen reverse transcription kit, and the resulting cDNA was frozen at -20°C. The PCR primers for amplification were as follows: Siglec-1- forward primer 5' -GGCTGTTACGATGGTTT-ATGATGT-3', reverse primer 5' -AATCAAAGGCATCATT-TTAGGGATA-3', resulting in an amplicon of 82 base pairs; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) - forward primer 5' -CCATCAATGACCCCTT-CATTG-3' and reverse primer 5' -CATGGGTGGAATCATATT-GGAAC-3', resulting in an amplicon of 66 base pairs.

The PCR amplification was performed using the SYBR Green method with the following cycling conditions: 50°C for 2 minutes, 95°C for 1 minute, and 95°C for 15 seconds, followed by 60°C for 1 minute for a total of 40 cycles. Post-amplification, the temperature was increased to 60°C or the melting curve analysis to confirm the amplification product's specificity and determine the threshold cycle (Ct) value.

In measuring Siglec-1 protein expression using flow cytometry, the researchers first treated the isolated peripheral blood mononuclear cells (PMBCs) with erythrocyte lysate to remove any remaining red blood cells. Then, 2×10^5 cells were incubated with mouse anti-human Siglec-1 monoclonal antibody at a concentration of 10μg/ml in flow washing solution called PBA (PBS+0.5% bovine serum albumin+0.05% sodium azide) for 60 minutes. Next, 5μg/ml of goat anti-mouse secondary antibody was added and incubated for 30 minutes, followed by 20μl of FITC-labeled mouse anti-human CDl4 monoclonal antibody for 30 minutes. After each incubation, the cells were rinsed with PBA solution. An isotype control consisting of an IgG antibody of the same species and type as the primary antibody was also included. The cell sample was resuspended with 500μl of PBS and analyzed on a flow cytometry with a total count of 2×104 cells. The results were then analyzed using Coulter's CXP analysis software.

To measure the disease activity score of 28 joints (DAS28) and ultrasensitive C-reactive protein (hs-CRP) required using a Dade Behring instrument and supporting reagents. The calculation for DAS28 involved the following formula: $DAS28=0.56\times\sqrt{}$(number of pressure joints) + $0.28\times\sqrt{}$(number of swollen joints) + $0.70\times\ln$(erythrocyte sedimentation rate) + $0.014\times\sqrt{}$(patient health status score).

Venous blood was collected from 2 groups of patients according to different experimental requirements. The blood was separated from 3ml of blood and stored at -70°C. To analyze the serum, the levels of 2 cytokines, interferon-γ (IFN-γ) and interleukin (IL)-4, were measured using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). Additionally, another portion of the blood, 2ml in volume, was treated with heparin as an anticoagulant. This portion was used to determine the levels of T lymphocyte subsets CD3+, CD4+, and CD8+ through flow cytometry analysis.

Statistical Analysis

The mean difference between the 2 groups underwent examination via the student's t-test for variables that followed a normal distribution. At the same time, the Mann-Whitney U test was employed for variables that did not adhere to normality. Data analyses were conducted using. SPSS 20.0 and GraphPad Prism 8 was utilized for graphical presentation. Measurement data were expressed as ($\bar{x} \pm s$) and subjected to analysis through the independent sample t test. The count data were expressed as the number of cases (rate) and analyzed using the chi-square test. Statistical significance was considered at a $P < .05$.

RESULTS

sCR1, MMP-2, and MMP-9

In individuals with rheumatoid arthritis, the levels of serum sCR1 were found to be significantly lower compared

Table 2. Comparison of sCR1, MMP-2, and MMP-9 Levels ($\bar{x} \pm s$)

Groups	n	sCR1 (ng/ml)	MMP-2 (ng/ml)	MMP-9 (ng/ml)
Disease group	40	86.36±7.52	1331.51±121.36	2234.47±246.39
Healthy group	40	42.38±7.21	2761.58±124.61	3579.41±216.73
t	-	26.699	-51.997	-25.922
P value	-	<.001	<.001	<.001

Figure 1. Flow Cytometry Results

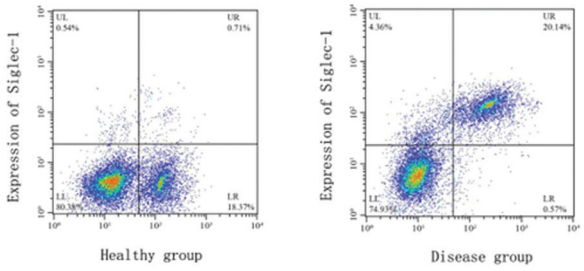
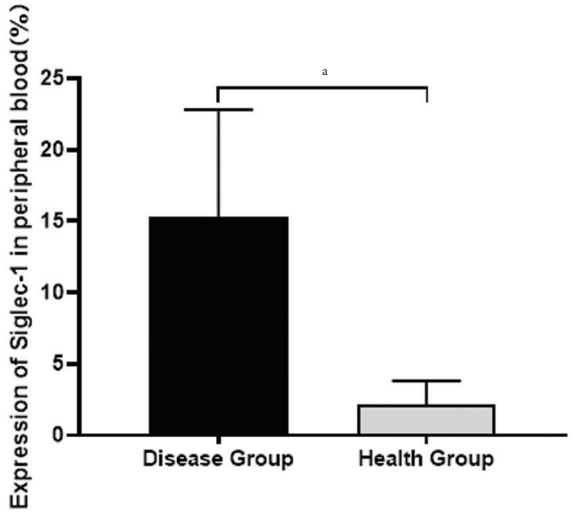
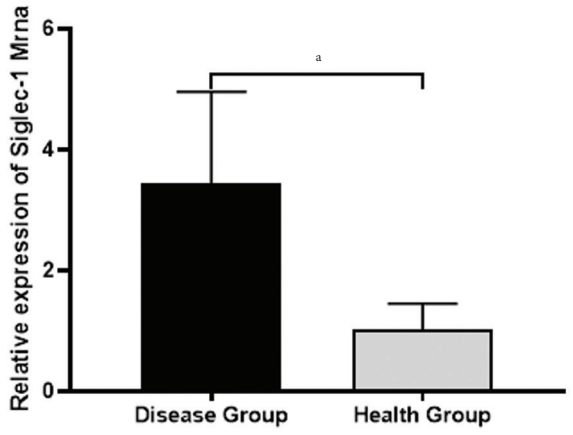


Figure 2. Comparison of Siglec-1 Expression in Peripheral Blood ($\bar{x} \pm s$)



[a]indicates $P < .05$.

Figure 3. Comparison of mRNA Expression of Siglec-1 ($\bar{x} \pm s$)



[a]indicates $P < .05$

Table 3. Comparison of T-lymphocyte Subsets ($\bar{x} \pm s$)

Groups	n	CD3+	CD4+	CD8+	CD4+/CD8+
Disease group	40	0.63±0.17	0.47±0.30	0.14±0.03	3.19±2.12
Healthy group	40	0.65±0.18	0.41±0.23	0.27±0.11	1.45±0.27
t	-	-0.511	1.004	-7.211	5.149
P value	-	.611	.318	<.001	<.001

Table 4. Comparison of Th1/Th2 Cytokines ($\bar{x} \pm s$)

Groups	n	IFN-γ (ng/L)	IL-4 (ng/L)
Disease group	40	97.62±21.54	7.58±2.19
Healthy group	40	34.32±13.18	16.34±5.68
t	-	15.854	-9.101
P value	-	<.001	<.001

to those of healthy subjects ($P < .05$), while the levels of MMP-2 and MMP-9 were higher ($P < .05$). (Table 2)

Expression of Siglec-1 in Peripheral Blood

Through a flow cytometric assay, it was discovered that the expression of Siglec-1 in PMBCs was significantly higher in patients (15.28±7.54%) compared to healthy subjects (2.17±1.64%) ($P < .05$). Using forward scatter (FS) and side scatter (SS) gating allowed the separation of PBMCs into lymphocyte and monocyte populations. The proportion of Siglec-1 positive cells in PBMCs was notably higher in patients than in healthy individuals, and monocytes were the predominant cell type expressing Siglec-1 ($P < .05$). (Figures 1 & 2)

Expression of Siglec-1 mRNA

Rheumatoid arthritis resulted in a significant increase in the expression of Siglec-1 mRNA (3.44±1.52) compared to a healthy state (1.02±0.43) ($P < .05$). Furthermore, the expression of Siglec-1 in patients with rheumatoid arthritis showed positive correlations with DAS28 (r=0.89) and hs-CRP (r=0.48) ($P < .05$). (Figure 3)

T-lymphocyte Subsets

The patients who met the criteria displayed a noticeable decrease in the level of CD8+ cells in their peripheral blood and an elevated CD4+/CD8+ ratio compared to individuals who were in good health ($P<0.05$). There was no significant difference in the levels of CD3+CD4+ cells between the 2 groups ($P > .05$). (Table 3)

Th1/Th2 Cytokines

Rheumatoid arthritis was associated with a higher peripheral blood IFN-γ level and a lower IL-4 level versus a healthy status ($P<0.05$). (Table 4)

DISCUSSION

Rheumatoid arthritis is a chronic, progressive, autoimmune disease that is progressively damaging and can cause severe disabling-related conditions. The etiology is related to genetics, infection, hormones, and the environment. The significant pathological alterations involve abnormal synovial and surrounding connective tissue growth and the gradual loss of articular cartilage. Rheumatoid arthritis is the most common autoimmune disease seen in rheumatology

departments. A common manifestation of RA is a mixed syndrome of cold and heat. Numerous clinical studies have confirmed the efficacy and safety of Guishaozhimu Decoction and Tripterygium wilfordii extract in treating rheumatoid arthritis. Currently, methotrexate is the primary treatment option for RA; however, a significant number of patients do not respond well to it.

Soluble complement receptor type I, or sCRI, is the extracellular component of complement receptor type I. It functions as a unit of CRI and plays a role in suppressing complement activation. This complement regulatory protein is highly expressed in serum, limiting complement activation. Previous research has shown that small compounds derived from the amino acid sequence and structural composition of sCRl can inhibit complement activation. This finding is significant regarding therapeutic applications for certain autoimmune illnesses and the suppression of inflammatory responses.[6]

MMP-2 and MMP-9 are protein hydrolases involved in the degradation and remodeling of the ECM. It has been noted that these enzymes are closely associated with tumor infiltration and tumor metastasis[7] in cancer patients. On the other hand, patients with rheumatoid arthritis show a connection between MMP-2 and MMP-9 expression, inflammatory infiltration, and tissue damage.[7]

The results of the present study showed that rheumatoid arthritis was associated with significantly lower levels of serum sCR1 and higher levels of MMP-2 and MMP-9 compared to healthy subjects (P<0.05). This suggests that serum sCRl, MMP-2, and MMP-9 could potentially be used as diagnostic tools for rheumatoid arthritis. Additionally, these markers may serve as potential therapeutic targets for treating rheumatoid arthritis.[8]

Neovascularization plays a crucial role in nourishing pannus growth and sustaining inflammation in the synovial tissue of patients with RA. Critical studies have shown that RA patients with increased joint blood flow exhibit higher disease activity. Animal studies have also demonstrated a positive correlation between synovial neovascularization and disease activity in patients with arthritis. This suggests that forming new blood vessels is crucial in mediating disease progression.

Gelatinase, an enzyme, facilitates the migration of endothelial cells through the breakdown of the vascular basement membrane of new blood vessels, thus contributing to the formation of neovascularization. The activation of MMP-2 increases vascular endothelial cell migration by inducing chemotaxis through activation of laminin-5. MMP-2 also regulates the activity of vascular endothelial growth factors, which create new blood vessels and promote the growth and invasion of tumors in humans.[9-11] Siglec-1 consists of 17 extracellular Ig-like regions and transmembrane and intracellular regions. It has a short intracellular segment that lacks signaling pathways but primarily functions by recognizing structures containing sialic acid and facilitating interactions between hematopoietic and immune cells.[12]

Examining Siglec-1 and autoimmune disorders has recently become a prominent field of study.[13] There has been an increased expression of Siglec-1 in patients with rheumatoid arthritis and atherosclerosis.[14] Furthermore, HIV-infected patients with high viral load have shown a 10-fold higher expression of the Siglec-1 gene in their peripheral blood mononuclear cells compared to healthy individuals. Additionally, Siglec-1 expression is positively associated with proteinuria levels in proliferative glomerulonephritis. These clinical investigations provide evidence for the activation and high expression of Siglec-1 in inflammatory and autonomous disorders, indicating its direct involvement in disease pathogenesis.

Currently, it is widely accepted in the medical field that patients with a genetic predisposition to rheumatoid arthritis experience a process where pathogens or other stimuli attach themselves to pattern recognition receptors found on the surface of dendritic cells and macrophages in the bloodstream. This binding event triggers a swift immune system response, involving the activation of cytokines, inflammatory mediators, complement, natural killer cells, and neutrophils.[15] These dendritic cells and macrophages then migrate to lymph nodes and transmit antigens to the T and B cells, resulting in the production of autoimmune T and B cells that release inflammatory cytokines and antigen-specific IgG, thereby activating the acquired immune system. In the bursa, activated mononuclear macrophages and T cells that have been activated multiply and move, leading to a complex immune-inflammatory cascade response involving multiple cells and molecules. Therefore, the normal immunological responses facilitated by macrophages accelerate the development of rheumatoid arthritis.[16] Accordingly, the high expression of Siglec-1 in peripheral blood mononuclear cells and its role in the pathogenesis of rheumatoid arthritis constitute the critical concern of the present study.

Moreover, the proportion of cells in PMBCs that tested positive for Siglec-1 was significantly higher in patients than in healthy individuals ($P < .05$). Among those cells, monocytes were the dominant type expressing Siglec-1. Patients with RA exhibited a significantly higher level of Siglec-1 mRNA expression compared to those in a healthy condition ($P <$ .05). Furthermore, the expression of Siglec-1 in patients with rheumatoid arthritis was positively correlated with DAS28 and hs-CRP ($P < .05$), suggesting the great potential of Siglec-1 as a marker of disease activity and inflammation severity. Siglec-1 offers a convenient marker, as DAS28 is considered less objective and not conducive to the timely calculation and monitoring of patients' disease conditions.

Clinical studies have shown cellular immunity in the pathogenesis of rheumatoid arthritis. This involvement is likely linked to the activation of T cells, and the balance between Th1 and Th2 is considered crucial in regulating immune response.[17] In the current study, patients with RA had significantly lower levels of CD8+ cells in their peripheral blood and a higher CD4+/CD8+ ratio than healthy individuals. There was no statistically significant difference in CD3+CD4+ levels between the 2 groups. These findings suggest a decrease in suppressor T cells (Ts) and an increase in Th cells, leading to

Th cell hyperfunction[18] and a relative deficiency in Ts cell suppressor function. The exact cause of RA pathogenesis remains undetermined, but it is clear that the disease involves the abnormal activation of cellular immunity.

CD4+ T cells play a role in the secretion of cytokines in rheumatoid arthritis. When stimulated by antigen-presenting cells, primary CD4+ T cells express specific transcriptional regulators and differentiate into different T cell subsets. The predominant subsets were characterized by the secretion of Th1-, and Th2-related Th1 cells form TNF-α, IFN-γ and IL-2, IL-12 cytokines, and Th2 cells form IL-4, IL-5 and IL-6, IL-10, IL-13 cytokines. Inflammatory factors contribute to osteoclast formation, which worsens RA's effects.[19-21] Additionally, the overactivity of B-cells contributes to the differentiation of immature B-cells and their proliferation into antibody-forming cells. This results in a significant increase in B-cells in peripheral blood and uncontrolled expression of immunoglobulins, leading to autoimmune diseases.[22]

An elevated CD4+/CD8+ ratio is closely associated with the development of rheumatoid arthritis. Changes in disease progression are closely associated with alterations in peripheral blood T-cell subsets in patients with rheumatoid arthritis. Monitoring the disease involves normalizing CD8+ cell counts and CD4+/CD8+ ratios as symptoms are controlled and go into remission.[23]

The representative cytokine produced by Th1 cells is IFN-γ plays a crucial role in the T cell-mediated immune response.[24] Conversely, Th2 cells produce IL-4, primarily promoting the B cell-mediated humoral immune response.[25] These 2 cytokines mutually regulate each other to maintain a dynamic balance and assure normal cellular and humoral immune function in the body.[26] According to Zhang et al.,[27] there is a strong correlation between the imbalance of Th1/Th2 cell ratio and cytokines in the pathogenesis of rheumatoid arthritis. In individuals with RA, there is an elevated level of IFN-γ in peripheral blood and a lower level of IL-4 compared to healthy individuals. This suggests that the imbalance between Th1 and Th2 cells is involved in the progression of rheumatoid arthritis. By shifting the secretion pattern of cytokines towards Th2 (IL-4), it may be possible to convert Th1 cells into Th2 cells and improve the condition of the disease. Restoring the balance between Th1 and Th2 cells could be a novel therapeutic approach to control RA.

This study has several limitations. Firstly, the sample size of this trial is small and can only represent the correlation in this region or the surrounding areas. It does not provide a comprehensive evaluation of serum MMP-2, Siglec-1, Th1/Th2 cell ratio, or disease activity of rheumatoid arthritis from a large sample perspective. Secondly, the follow-up period was short, which prevented a thorough observation of long-term treatment effects. Thirdly, no quantitative correlation analysis was conducted between MMP-2, Siglec-1, Th1/Th2 levels, and various coagulation indicators. Therefore, further analysis and validation of the results are necessary. Fourthly, due to the limited observation time, imaging methods were not utilized to evaluate patients' conditions. To assess the long-term efficacy of treatment, ultrasound medicine, and medical imaging, methods could be introduced for the extended follow-up to the minute disease progression. Lastly, only direct medical expenses were considered in the minimum cost analysis, while the patients' direct non-medical, indirect, and hidden costs were not systematically considered. For patient comorbidity, only the overall number of drugs was counted without further investigation, leading to inaccuracies.

In addition to studying limitations, the current investigation was unsuccessful in documenting and conducting a thorough assessment of the number of individuals who specifically utilized non-steroidal anti-inflammatory drugs or glucocorticoids, as well as the associated circumstances of drug reduction and withdrawal. Subsequent studies that involve extended periods of observation and reporting are necessary to examine the effects of reducing or discontinuing non-steroidal anti-inflammatory drugs and hormones.

The study's findings have several clinical implications that can potentially influence current treatment strategies for rheumatoid arthritis. Regarding disease activity monitoring, the study identified a significant correlation between serum levels of soluble complement receptor type 1 (sCR1), matrix metalloproteinases (MMP-2 and MMP-9), and the progression of RA. These biomarkers can serve as indicators of disease activity in RA patients. Regular testing to monitor these markers can provide valuable information about the disease status and help guide treatment decisions.

The study demonstrated that Siglec-1, a marker of mononuclear macrophage activation, is highly expressed in RA patients' peripheral blood mononuclear cells (PBMCs). The expression of Siglec-1 was positively correlated with disease activity scores and inflammatory markers. Siglec-1 could be a non-invasive marker to monitor disease activity and inflammatory response in RA patients.

The study highlighted the imbalance between Th1/Th2 cells and their secreted cytokines in RA patients. RA patients showed elevated levels of Th1 cells and interferon-gamma (IFN-γ), while the levels of Th2 cells and interleukin-4 (IL-4) were decreased compared to healthy individuals. This imbalance is known to contribute to the development and progression of RA. Targeting Th1/Th2-mediated homeostasis may potentially alleviate symptoms in RA patients.

Identifying specific biomarkers and cellular imbalances associated with RA can contribute to a more personalized approach to treatment. By monitoring these markers and understanding individual disease activity profiles, healthcare providers can tailor treatment strategies to optimize outcomes for each patient. This may involve adjusting medication dosages, selecting appropriate therapies, or implementing combination therapies based on the patient's disease characteristics.

The study acknowledges the limitations and adverse effects of current Western medications used for RA. The identification of favorable effects of traditional Chinese medicine herbal medicines, such as Radix Et Rhizoma

Tripterygii wilfordii, Paeoniae Radix Alba, and Whiteback Thundergodvine, suggests that alternative medical approaches, including TCM, may have a role in managing RA and meeting the needs of patients. Integrating complementary and alternative therapies with conventional treatments may provide supplementary advantages and improve overall patient care.

In conclusion, a significant correlation exists between sCRl, MMP-2, and MMP-9 and the progression of rheumatoid arthritis. These 3 indices can potentially monitor disease activity in patients with rheumatoid arthritis. Siglec-1 is activated and expressed at high levels in peripheral blood PBMCs, making it a promising non-invasive marker for monitoring disease activity and inflammatory response in RA patients. By regulating Th1/Th2-mediated homeostasis, it may be possible to alleviate symptoms in patients with rheumatoid arthritis.

DATA AVAILABILITY STATEMENT

The datasets used during the present study are available from the corresponding author upon reasonable request.

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CONFLICT OF INTEREST

All authors declared that they have no financial conflict of interest.

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