



Emerging Role of Interleukin-38 (IL-38) in the Development of Rheumatoid Arthritis

Shengxiang Liang · Liting Chen · Ruilan Liang · Jiayi Ling ·
Minghui Hou · Song Gao · Minglin Ou · Min Yang

Received: October 31, 2023 / Accepted: January 12, 2024 / Published online: February 5, 2024
© The Author(s) 2024

ABSTRACT

Introduction: Rheumatoid arthritis (RA) is an incurable autoimmune disease. The role of interleukin-38 (IL-38), an anti-inflammatory cytokine, in RA is not fully understood, and its clinical relevance in RA remains unclear. This study aims to investigate the correlation of IL-38 with disease activity and the clinical manifestation of RA.

Methods: In this cross-sectional study, patients with treatment-naïve RA ($n = 63$) and healthy

controls (HC) ($n = 60$) were consecutively enrolled over a 15-month period. Patients with RA were categorized into three subgroups—low disease activity (LDA), moderate disease activity (MDA) and high disease activity (HDA)—using the Disease Activity Score in 28 joints based on C-reactive protein (DAS28-CRP). Circulating levels of IL-38, tumour necrosis factor (TNF), IL-6, IL-17, IL-1 β , and 25(OH)D were assessed using enzyme-linked immunosorbent assay (ELISA). Clinical data, including duration, tender joints count (TJC), swollen joints count (SJC), patient global assessment (PGA), evaluator global assessment (EGA), bone mineral density (BMD), clinical disease activity index (CDAI), simplified disease activity index (SDAI), DAS28-CRP, joint musculoskeletal ultrasound (MSUS), and serological indicators were recor-

Shengxiang Liang and Liting Chen contributed equally to this work.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40744-024-00640-x>.

S. Liang
Health Management Center, Guangxi Key Laboratory of Metabolic Reprogramming and Intelligent Medical Engineering for Chronic Diseases, The Second Affiliated Hospital of Guilin Medical University, Guilin 541100, China

L. Chen
Department of Rheumatology and Immunology, The First People's Hospital of Qinzhou, Qinzhou 535000, China

R. Liang · J. Ling · M. Hou · M. Yang (✉)
Department of Rheumatology and Immunology, The Affiliated Hospital of Guilin Medical University, Guilin 541001, China
e-mail: liangyangmin@163.com

S. Gao
Department of Ultrasound, The Affiliated Hospital of Guilin Medical University, Guilin 541001, China

M. Ou
Central Laboratory, Guangxi Health Commission Key Laboratory of Glucose and Lipid Metabolism Disorders, The Second Affiliated Hospital of Guilin Medical University, Guilin 541100, China

ded. We determined the correlation between IL-38 and disease activity, as well as clinical manifestation in RA.

Results: At the macroscopic level, musculoskeletal ultrasonography of joints in different stages of disease activity in RA suggests that, as the disease progresses, arthritis in the hand becomes more severe, accompanied by synovial thickening and pronounced blood flow signals in the joint area. The expression of IL-38, TNF, IL-6, IL-17 and IL-1 β significantly increased in patients with RA compared to HC. Noteworthy differences were observed in the blood flow signal score, synovial signal score, IL-38, TNF, IL-6, IL-17 and IL-1 β among the three subgroups (LDA, MDA and HDA). As disease activity increased in patients with RA, the blood flow signal score, synovial signal score and expression of TNF, IL-6, IL-17 and IL-1 β exhibited a gradual increase, while the expression of IL-38 showed the opposite pattern. Inverse correlations were identified between IL-38 and pro-inflammatory cytokines (IL-6, IL-17), as well as key clinical parameters, including disease duration, SJC, TJC and DAS28-CRP score.

Conclusion: IL-38, intricately linked to the pathogenesis of RA, emerges as a promising therapeutic target for the management of this debilitating disease.

Keywords: Correlation; Interleukin-38; Rheumatoid arthritis

Key Summary Points

Why carry out this study?

Previous studies have indicated that the imbalance between pro- and anti-inflammatory factors likely plays a pivotal role in the development of rheumatoid arthritis (RA).

Interleukin-38 (IL 38), an anti-inflammatory cytokine, has shown the ability to inhibit inflammation in both patients with RA and animal models. However, limited research exists on the correlation of IL-38 with disease activity and the clinical manifestation of RA.

What was learned from the study?

IL-38 is implicated in the autoimmune response associated with the disease activity of RA, suggesting its potential as a therapeutic target in the future.

Serum IL-38 may serve as a predictive biomarker for clinicians assessing disease activity in RA. Additionally IL-38 may serve as a therapeutic target for the management of this chronic condition of RA.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterized by symmetric multi-joint swelling, synovial cell hyperplasia, vascular opacification and destruction of articular cartilage and bone, ultimately resulting in joint deformity and dysfunction [1]. The prevalence rate of RA is 1.0% globally [2] and 0.42% in China [3], with notable increase each year. The disability caused RA is the highest among joint lesions in China [4]; hence, early diagnosis and intervention of RA are crucial for managing this devastating disease. The aetiology and pathogenesis of RA are complex, and it is widely believed that the imbalance of pro-inflammatory and anti-inflammatory factors may play a key role in its development [5, 6]. Therefore, it is imperative to explore the underlying mechanisms of RA as a fundamental step toward advancing novel therapeutic medications.

RA is a chronic autoimmune disease characterized by inflammatory reactions in joints and bone damage. Previous studies have demonstrated an increase in the levels of pro-inflammatory cytokines such as interleukin (IL)-1 β , IL-6, IL-17, and tumour necrosis factor (TNF) in both patients with RA and animal models. This suggests that these cytokines play a critical role in the development of RA [7]. Pro-inflammatory cytokines are known to be crucial regulators of osteoclast formation, differentiation and activation, ultimately leading to synovial hyperplasia and bone destruction [8]. The persistent

inflammation in the joints of patients with RA is believed to be the primary cause of accelerated bone destruction [9]. Therefore, inhibiting pro-inflammatory cytokines is considered a potential therapeutic approach for managing RA.

IL-38, a cytokine with characteristics similar to those of IL-1Ra and IL-36R, is located on human chromosome 2q13-14.1. IL-38 shares 41% and 43% homology with IL-1Ra and IL-36Ra, respectively [10]. It binds to IL-36R, inhibiting the pro-inflammatory function of the agonist ligands IL-36 α , IL-36 β and IL-36 γ [11]. IL-38 also inhibits the secretion of IL-6 and IL-8, regulates the differentiation and proliferation of Th17 and reduces the Th17/Treg ratio [12]. Furthermore, IL-38 reduces the expression of inflammatory factors, inhibits inflammation in collagen-induced arthritis (CIA) rats, promotes osteoblast production and thus reduces joint damage in rats [13].

Previous studies have demonstrated that elevated expression levels of IL-38 in the synovium of patients with RA and in the joints of mice with arthritis [14–16]. Xu et al. demonstrated an upregulation of circulating IL-38 in RA compared to healthy controls and a correlation between IL-38 and disease activities at 3 or 6 months post-treatment [17]. This may be attribute to the low inflammation in the microenvironment following anti-inflammatory management.

Although studies have confirmed the involvement of IL-38 in the development of RA, there are limited studies investigating the correlation of IL-38 with disease activity and the clinical manifestation of RA. Therefore, this study aims to evaluate the expression of IL-38 in the serum of patients with RA and analyse its correlation with disease activity, clinical manifestation as well as laboratory and ultrasound characteristics of RA, further contributing to this field.

METHODS

Patient Enrollment and Study Design

Consecutive patients with RA were enrolled from September 2020 to December 2021. The study included 63 patients (14 male) with primary RA who were hospitalized in The Department of Rheumatology and Immunology, The Affiliated Hospital of Guilin Medical University. These patients with RA were treatment-naïve prior to recruitment for the current study to avoid the effects of prior intervention(s). Subsequently these patients with RA were divided into different subgroups according to different criteria for comparative analysis. Healthy controls (HC) ($n = 60$, 15 male) were recruited during their routine annual health check-up from The Health Centre, The Affiliated Hospital, Guilin Medical University during the same period. This cross-sectional study utilised baseline data of these patients in the cohort.

The study was approved by the Ethics Committee of the Affiliated Hospital of Guilin Medical University (approval number YJSL202151) and was conducted in accordance with the Helsinki Declaration of 1964 and its later amendments. Each participant signed an informed consent form. No identifying information of participants was included in the manuscript.

The inclusion criteria applied for the patients with RA were as follows: older than 18 years and met the 1987 American College of Rheumatology (ACR) revised diagnostic criteria for RA classification [18] or satisfied the latest ACR/European League Against Rheumatism (EULAR) RA classification criteria published in 2010 [1].

The exclusion criteria applied for patients with RA were as follows: (1) comorbidity of other rheumatic diseases; (2) use of hormones or vitamin D analogues, and/or using antirheumatic drugs in the last 12 weeks; (3) comorbidity of other diseases that may affect vitamin D, e.g. diabetes, hypertension, tumours, abnormal parathyroid function, coronary heart disease and other chronic diseases; (4) elevated liver transaminases, blood creatinine, urea nitrogen at least 1.5 times the standard high value; (5)

combination of severe acute or chronic infectious diseases; (6) pregnant and lactating women.

Healthy controls were individuals who underwent routine health check-ups at the Health Screening Centre, The Affiliated Hospital, Guilin Medical College during the same time period. HC were excluded if they were (1) pregnant and lactating women; (2) individuals with incomplete information.

Sample Collection and Testing

Serum was obtained from each individual via venous fasting blood in the early morning of the following day. The serum was stored at -80°C till use and avoided repeated freeze-thawing. The levels of 25(OH)D (Human 25(OH) D Reagent Kit, Shanghai Enzyme Link Biotechnology Co.), TNF (Human TNF kit), IL-1 β (Human IL-1 β kit), IL-6 (Human IL-6 kit) and IL-17 (Human IL-17 kit) were determined using a kit from Wuhan Elite Bio-Technology Co.; IL-38 (Human IL-38 kit) from Wuhan Huamei Biological Engineering Co. The expression levels of the aforementioned cytokines were determined through ELISA, following the instructions from the manufactures.

Clinical Data Collection

Measurement of Clinical Indicators

The following data was collected: duration of disease, tenderness joints count (TJC), swollen joints count (SJC), patient global assessment (PGA), evaluator global assessment (EGA), anti-cyclic citrullinated peptide antibody (anti-CCP), rheumatoid factor (RF), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), bone mineral density (BMD) and bone mineral density (BMD) in patients with RA. Additionally, CCP, rheumatoid factor (RF), CRP, Z/T value, joint musculoskeletal ultrasound (MSUS) results and other relevant data were also obtained.

Composite disease activity scores, including Clinical Disease Activity Index (CDAI), Simplified Disease Activity Index (SDAI) and Disease

Activity Score in 28-joints based on C-reactive protein (DAS28-CRP) for RA, were calculated for RA. Disease activity was assessed using DAS28-CRP according to the 2012 update of the ACR recommendations [19]. DAS28-CRP > 5.1 indicates high active, $3.2 \leq \text{DAS28-CRP} \leq 5.1$ is moderate activity, $2.6 \leq \text{DAS28-CRP} < 3.2$ is low activity, DAS28-CRP < 2.6 is in clinical remission. Patients with RA were categorised into three different subgroups according to DAS28.

Articular Musculoskeletal Ultrasound

Using a Siemens (Germany) ACUSON X700 ultrasound machine equipped with a linear array probe operating at 14–18 MHz, we conducted the procedure following the 2017 European Rheumatology Consortium ultrasonography guidelines [20]. For each included patient, we scanned the metacarpophalangeal joints of both hands, encompassing 28 joints (MCP1–5, PIP1–5 and DIP2–5 in both hands) in both longitudinal and transverse vertical views during musculoskeletal ultrasonography.

All participants assumed a seated position, facing the operator, with their hands placed flat on the examination bed. The procedure was performed by the same senior attending sonographer or higher, with the operator unaware of the patient's joint pain. We observed manifestations of synovitis, including synovial thickness, blood flow signal, tendon/tendinitis and bone erosion. The site of the joint lesion was recorded.

Semi-quantitative scoring was carried out following the proposed scoring method [21]. The synovial condition was graded, assigning a score of 0–3 on the basis of the degree of synovial hyperplasia and blood flow signal. The total score for each patient was the sum of these individual scores. The grading method employed the Szkudlarek scoring method [21].

Synovial hyperplasia grading:

- Level 0 No synovial hyperplasia.
- Level 1 Mild thickening of the synovial membrane, not exceeding the line of the highest point of the bony surface.

- Level 2 Synovial thickening beyond the line of the highest point of the bone surface but not beyond the bone stem.
- Level 3 Synovial thickening beyond the line of the highest point of the bony surface and extending beyond at least one side of the diaphysis.

Blood flow signal grading:

- Level 0 No coloured blood flow signal in the joint.
- Level 1 Up to 3 coloured blood flow signals detected in the joints.
- Level 2 More coloured blood flow signal than grade 1, but blood flow filling area < 50% intra-articular area.
- Level 3 More than $\geq 50\%$ of the intra-articular area is filled with coloured blood flow signals.

Statistical Analysis

All data were statistically analysed using the SPSS 26.0 software. For data demonstrating normal distribution and homogeneous variance, values were expressed as mean \pm standard deviation (SD). Comparisons between two groups were conducted using an independent samples *t* test; comparisons among three groups utilised one-way analysis of variance (ANOVA). Pearson correlation analysis was employed for these datasets.

In the case of non-normally distributed data or data with dependent variances, values were expressed as median (interquartile range). Comparisons between two groups used the Mann–Whitney *U* test; the Kruskal–Wallis test was employed for comparisons among three groups. Correlation analysis for these datasets utilised Spearman correlation.

Count data were expressed as number of cases or percentages, with the chi-square test employed for comparison between two groups. Bonferroni-corrected probability (*P*) values were used for multiple comparisons, and statistical significance was set at $P < 0.05$. Statistical results were graphically represented using the GraphPad Prism 9 software package.

RESULTS

Baseline Information in RA and HC Groups

Between September 2020 and December 2021, 63 patients who fulfilled the 1987 ACR revised criteria for RA received care at the Department of Rheumatology and Immunology, The Affiliated Hospital of Guilin Medical University. Sixty-three patients with RA (14 male) and 60 healthy controls (15 male) were enrolled.

Patients with RA had a median (interquartile range) age of 57 (47, 65) years, with 77% being female. The median duration of the disease was 12 months (Table 1). Data on simplified and clinical disease activity indices (SDAI/CDAI) were collected, and a comparative analysis was performed using these indices. The results of the analysis conducted with SDAI and CDAI are presented in Supplementary Material Tables S1–S4, offering a comprehensive overview of the findings.

As shown in Supplementary Material Tables S1–S2, patients with low disease activity (LDA) based on CDAI or SDAI disease activity exhibited higher levels of IL-38 than patients with high disease activity (HDA), whereas the concentrations of TNF, IL-6 and IL-17 in patients with HDA were higher than those in LDA. With the increase in disease activity, the serum level of IL-1 β increased, and it was statistically significant in the SDAI disease activity group but not statistically significant in the CDAI disease activity group.

As demonstrated in Supplementary Material Tables S3–S4, in the CDAI disease activity group, patients with HDA showed higher scores of synovial signal and blood flow signal. However, the levels of vitamin D, as well as the values of BMD and Z/T in patients with HDA, were lower than in LDA, and all differences were statistically significant. Similar results were observed in the SDAI disease activity groups, but the differences in the values of BMD and Z/T were not statistically significant.

Table 1 Characteristics of patients with RA and healthy controls (at baseline)

Characteristics	Healthy controls (<i>n</i> = 60)	Patients with RA (<i>n</i> = 63)	<i>P</i> value
Age (year)	54.00 (50.00, 57.75)	57.00 (47.00, 65.00)	0.145
Sex (male/female)	15/45	14/49	0.717
BMI (kg/m ²)	21.91 (20.43, 23.62)	21.30 (19.40, 24.90)	0.619
Disease duration (months)	–	12.00 (28.60, 60.50)	–
Anti-CCP (U/mL)	7 (7, 7)	281.2 (9.62, 484.65)	< 0.001
RF (IU/mL)	6.40 (5.82, 6.90)	125.75 (23.92, 238.77)	< 0.001
ESR (mm/h)	–	71 (47, 109)	–
CRP (mg/L)	–	19.52 (7.84, 65.63)	–
Anti-CCP (<i>n</i> , %)	1 (1.66)	47 (74.60)	< 0.001
RF (<i>n</i> , %)	2 (3.33)	51 (80.95)	< 0.001
TJC	–	10.00 (5.00, 14.00)	–
SJC	–	2.00 (0, 10.00)	–
PGA	–	50 (31, 78)	–
EGA	–	50 (31, 76)	–
DAS28-CRP	–	4.84 (3.68, 6.08)	–
CDAI	–	20.30 (12.20, 34.50)	–
SDAI	–	25.04 (15.19, 37.16)	–

RA rheumatoid arthritis, BMI body mass index, Anti-CCP anti-cyclic citrullinated peptide, RF rheumatoid factor, ESR erythrocyte sedimentation rate, CRP C-reactive protein, TJC tenderness joints count, SJC swollen joints count, PGA patient global assessment, EGA evaluator global assessment, DAS28-CRP Disease Activity Score in 28-joints based on C-reactive protein, CDAI clinical disease activity index, SDAI simplified disease activity index

Comparison of Images Between RA and HC Groups

Hand images were collected from healthy individuals and patients with RA exhibiting different degrees of disease activity, categorised as mild, moderate and severe (Fig. 1, upper). Musculoskeletal ultrasonography of the joints revealed variations in synovial thickening and blood flow signals. As the disease activity progressed, arthritis in the hand became more severe, and the synovial thickening and filling of blood flow signal in the joint area became more extensive and pronounced (Fig. 1, middle and Lower).

Comparison of Cytokines in Different RA Disease Activity Groups

A total of 63 patients with RA were categorised into three subgroups based on DAS28-CRP scores: patients (*n* = 11) achieving LDA with DAS28-CRP scores of $2.6 \leq \text{DAS28-CRP} < 3.2$; patients (*n* = 24) achieving MDA with DAS28-CRP scores of $3.2 \leq \text{DAS28-CRP} \leq 5.1$ and patients (*n* = 28) achieving HDA with DAS28-CRP scores > 5.1 .

The average serum concentrations of IL-38 in patients with RA were significantly higher compared to those HC (Fig. 2a). TNF expression levels in patients with RA were significantly elevated in comparison with HC (Fig. 2b).

Similarly, IL-6 concentrations in patients were markedly higher than those in HC (Fig. 2c). Patients with RA exhibited significantly higher IL-17 concentrations compared to HC (Fig. 2d), and IL-1 β concentrations were also notably elevated in patients with RA compared to the HC (Fig. 2e).

Figure 2 further illustrates that the expression of IL-38 gradually decreased with increasing disease activity. Patients with LDA showed higher levels of IL-38 than those with HDA (Fig. 2f). In contrast, the expression of TNF, IL-6 and IL-17 gradually increased with increasing disease activity. The concentrations of TNF (Fig. 2g), IL-6 (Fig. 2h) and IL-17 (Fig. 2i) in

patients with HDA were higher than those with LDA. Additionally, with the increase in disease activity, the serum level of IL-1 β increased, although this was not statistically significant (Fig. 2j).

Correlation Analysis of IL-38 Levels and Clinical Indicators in RA Group

IL-38 in the RA group exhibited an inverse correlation with disease duration (Fig. 3a), SJC (Fig. 3b), TJC (Fig. 3c) and DAS28 (Fig. 3d). However, it did not show a significant correlation with time to morning stiffness, anti-CCP,

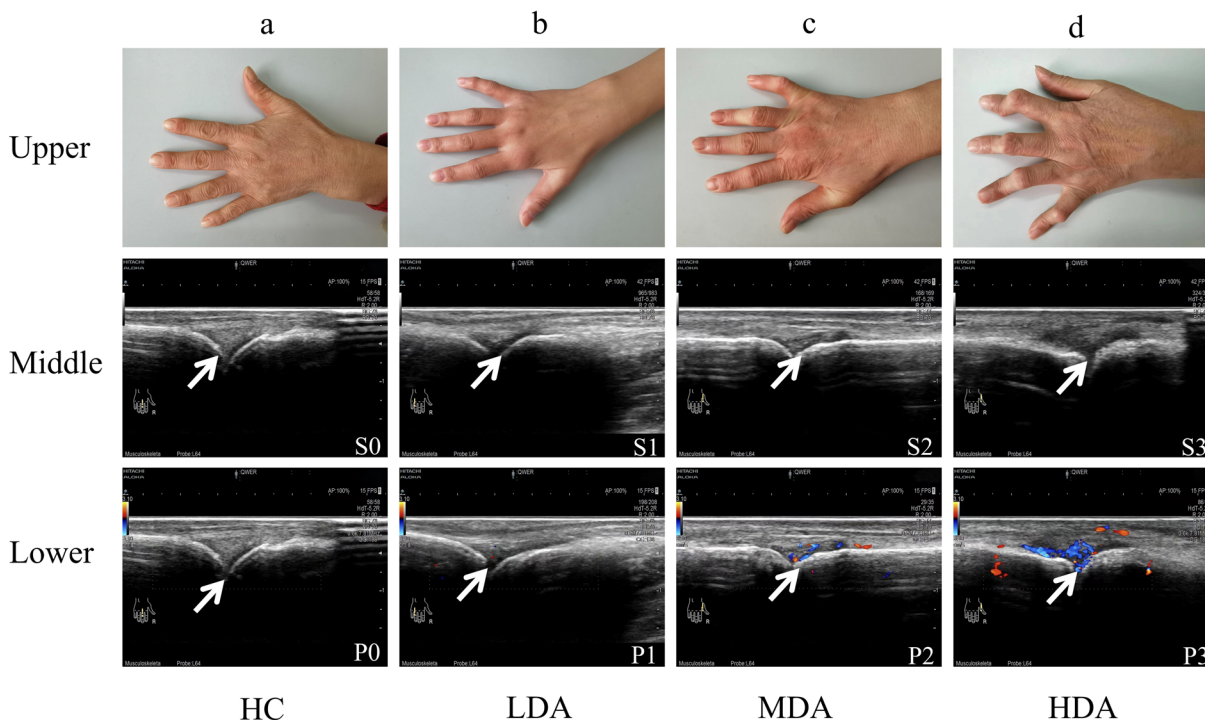


Fig. 1 Representative images of patients with RA and HC (upper, appearance; middle, US synovial signal; lower, US blood flow signal). **a** There is no abnormal appearance, ultrasound performance and blood flow in HC. **b** Mild swelling, up to 3 coloured blood flow signals are detected in the joints (indicated by white arrows) and mild thickening of the synovial membrane, not exceeding the line of the highest point of the bony surface in patients achieving LDA. **c** Moderate swelling, more coloured blood flow signal than grade 1, but blood flow filling area < 50% intra-articular area (indicated by white arrows) and synovial thickening beyond the line of the highest point

of the bone surface, but not beyond the bone stem in patients achieving MDA. **d** Severe swelling, more than $\geq 50\%$ of the intra-articular area is filled with coloured blood flow signals (indicated by white arrows) and synovial thickening beyond the line of the highest point of the bony surface and extending beyond at least one side of the diaphysis in patients achieving HDA. *RA* rheumatoid arthritis, *HC* healthy controls, *US* ultrasound, *LDA* low disease activity, *MDA* moderate disease activity, *HDA* high disease activity

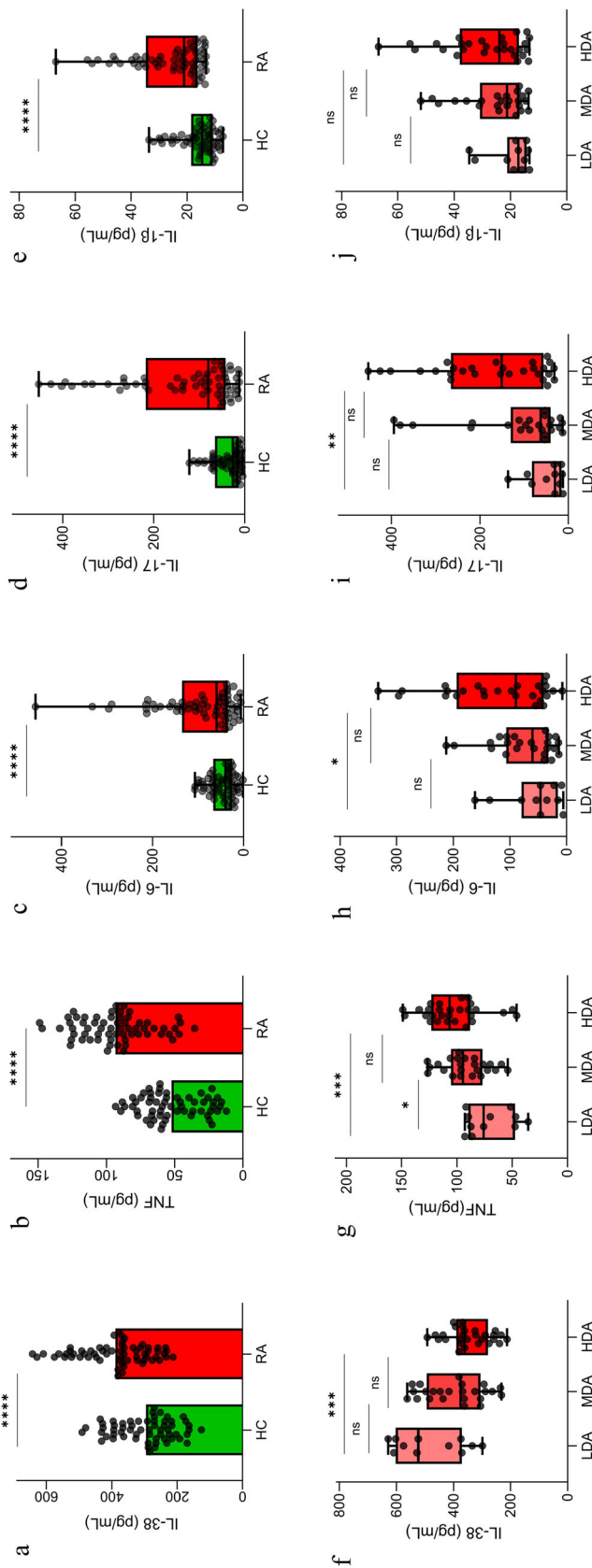


Fig. 2 Measurement of cytokine production between RA ($n = 63$) and HC ($n = 60$) (a–e IL-1β, IL-6, IL-17, IL-38 and TNF) by ELISA, as well as the difference between different activity (LDA [$n = 11$] versus MDA [$n = 24$] versus HDA [$n = 28$]), (f–j IL-1β, IL-6, IL-17, IL-38 and TNF). Data with a normal distribution are presented as mean \pm SD, while data with a non-normal distribution are expressed as median (interquartile range). Significance levels are denoted as follows: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, indicating significant differences as indicated. RA rheumatoid arthritis, HC healthy controls, IL-1β interleukin-1β, TNF tumour necrosis factor, ELISA enzyme-linked immunosorbent assay, LDA low disease activity, MDA moderate disease activity, HDA high disease activity, SD standard deviation

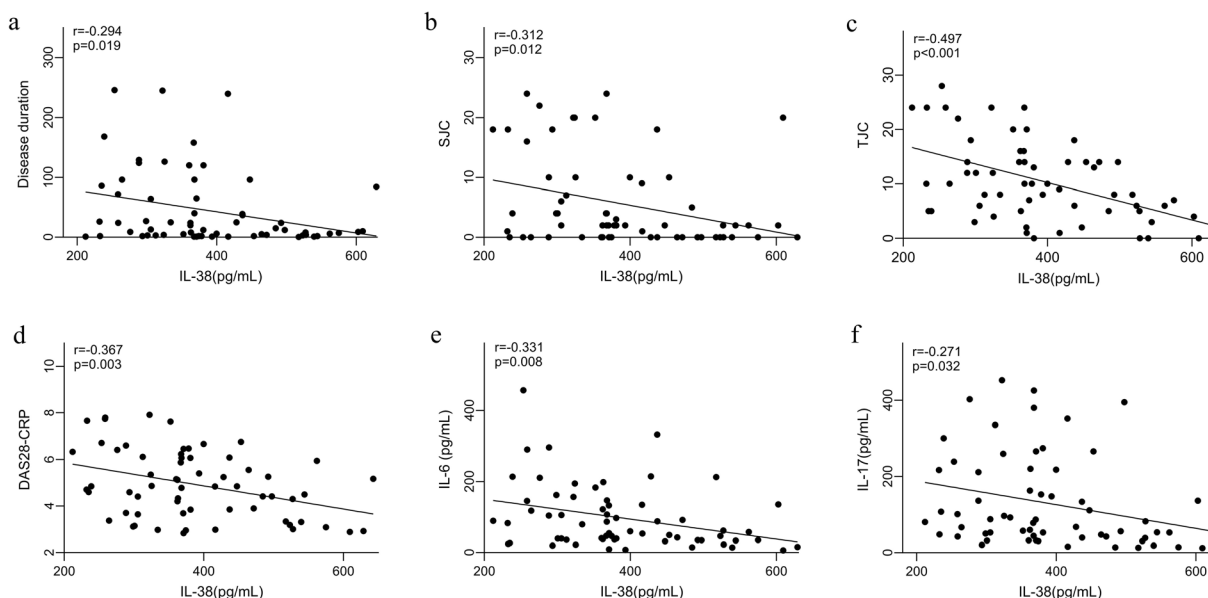


Fig. 3 Correlations between IL-38 levels and disease duration (a), SJC (b), TJC (c), DAS28-CRP (d), IL-6 (e), IL-17 (f) in patients with RA ($n = 63$). Significance levels are denoted as follows: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, indicating statistically significant differences

as indicated. *IL-38* interleukin-38, *SJC* swollen joint count, *TJC* tenderness joint count, *DAS28-CRP* Disease Activity Score in 28-joints based on C-reactive protein, *CRP* C-reactive protein, *RA* rheumatoid arthritis

RF, ESR, CRP, BMD, Z/T value, blood flow signal score and synovial signal score ($P > 0.05$, Table 2).

IL-38 was inversely correlated with IL-6 (Fig. 3e) and IL-17 (Fig. 3f) in the RA group ($P < 0.05$); there was no significant correlation with IL-1 β and TNF ($P > 0.05$, Table 3).

Comparison of Imaging Indexes in Different RA Disease Activity Groups

Among the three subgroups, there was a gradual increase in synovial signal score (Fig. 4a) and blood flow signal score (Fig. 4b) with increasing disease activity while the vitamin D (Fig. 4c), BMD (Fig. 4d) and Z/T values (Fig. 4e) gradually decreased. In the highly active group (DAS28 > 5.1), patients with RA had significantly higher synovial signal scores and blood flow signal scores, as well as lower BMD Z/T and vitamin D values compared to the low active group (2.6 points \leq DAS28 < 3.2 points). The low active group exhibited synovial signal scores, blood flow signal scores, BMD values,

Z/T values and vitamin D values, with all differences being statistically significant ($P < 0.05$) (Table 4, Fig. 4).

DISCUSSION

In our study, we observed that the expression level of IL-38 in the RA group was significantly higher than that in HC. Additionally, it showed a negative correlation with disease activity scores and pro-inflammatory cytokines (IL-6, IL-17). These findings suggest that IL-38 may play an anti-inflammatory role in RA and thereby influencing the disease activity of RA.

In our present investigation, we demonstrated an upregulation of circulating IL-38 in RA in comparison to the HC. This finding suggests that IL-38, an anti-inflammatory cytokine, exhibits elevated levels in response to highly pro-inflammatory mediators, both in the local microenvironment and circulation, compared to that of HC. This elevation suggests an attempt to suppress inflammation, which is supported by the role IL-38 plays in the

Table 2 Correlation analysis of IL-38 with clinical parameters in patients with RA

Parameter	R value	P value
Disease duration	− 0.294	0.019
SJC	− 0.312	0.012
TJC	− 0.497	< 0.001
DAS28-CRP	− 0.367	0.003
Time to morning stiffness	− 0.046	0.724
Anti-CCP	0.064	0.623
RF	− 0.012	0.928
ESR	− 0.039	0.782
CRP	− 0.055	0.678
BMD	0.096	0.482
Z/T value	0.091	0.509
Blood flow signal score	− 0.101	0.454
Synovial signal score	− 0.113	0.387

RA rheumatoid arthritis, SJC swollen joints count, TJC tenderness joints count, DAS28-CRP Disease Activity Score in 28-joints based on C-reactive protein, Anti-CCP anti-cyclic citrullinated peptide antibody, RF rheumatoid factor, ESR erythrocyte sedimentation rate, CRP C-reactive protein, BMD bone mineral density

Table 3 Correlation analysis of IL-38 levels and cytokines in RA group

Parameter	R value	P value
IL-1 β (pg/mL)	0.114	0.373
IL-6 (pg/mL)	− 0.331	0.008
IL-17 (pg/mL)	− 0.271	0.032
IL-38 (pg/mL)	−	−
TNF (pg/mL)	0.183	0.150

RA rheumatoid arthritis, IL-1 β interleukin-1 β , IL-6 interleukin-6, IL-17 interleukin-17, IL-38 interleukin-38, TNF tumour necrosis factor

gastrointestinal system [22]. However, it is plausible that the pro-inflammatory effect was too potent for the host to inhibit, leading to

persistent inflammation in the joints and subsequent joint destruction. Our results align with the study conducted by Xu et al., wherein elevated levels of circulating IL-38 were observed in patients with RA compared to non-RA cohorts [17]. This upregulation was confirmed at both the protein and mRNA levels.

Notably, we observed an unexpected inverse correlation between DAS28-CRP and circulating IL-38 in patients with RA. In contrast, Xu et al. demonstrated a positive correlation between the DAS28-CRP and IL-38 in RA. This discrepancy may be attributed to whether or not the patients with RA had prior treatment, because our patients with RA were treatment naïve. We speculate that the initial upregulation of IL-38 in patients with RA diminishes over time because of continuous joint destruction, leading to scar formation and obstructing the interaction between circulation and the local microenvironment. This dynamic process may explain the higher IL-38 levels in RA compared to HC, despite the inverse correlation with DAS28-CRP. The precise mechanism is currently being investigated for both diagnosis and/or target precision medicine. Note that our patients were treatment naïve, but the treatment status of the patients with RA from Xu et al.'s study is not specified [17]. These results emphasize the potential significance of IL-38 as a key player in the pathophysiology of RA and underscore its modulation as a therapeutic target, in line with emerging research in this field.

Additionally, Pei et al.'s study demonstrates that the expression of IL-38 is significantly reduced in RA synovium following 3 months of treatment [13]. Xu et al. conducted an in-depth evaluation of plasma IL-38 levels in RA and explored the potential of IL-38 as a biomarker for RA [17]. Their study reported elevated plasma levels of IL-38 in patients with RA compared to healthy controls in the training cohort, a finding consistent with our current investigation. In the validation cohort, IL-38 expression was notably higher in patients with RA compared to non-RA individuals, and treatment led to a significant reduction in IL-38 expression. Notably, in our study, IL-38 expression exhibited a gradual decrease with increasing disease activity, establishing a

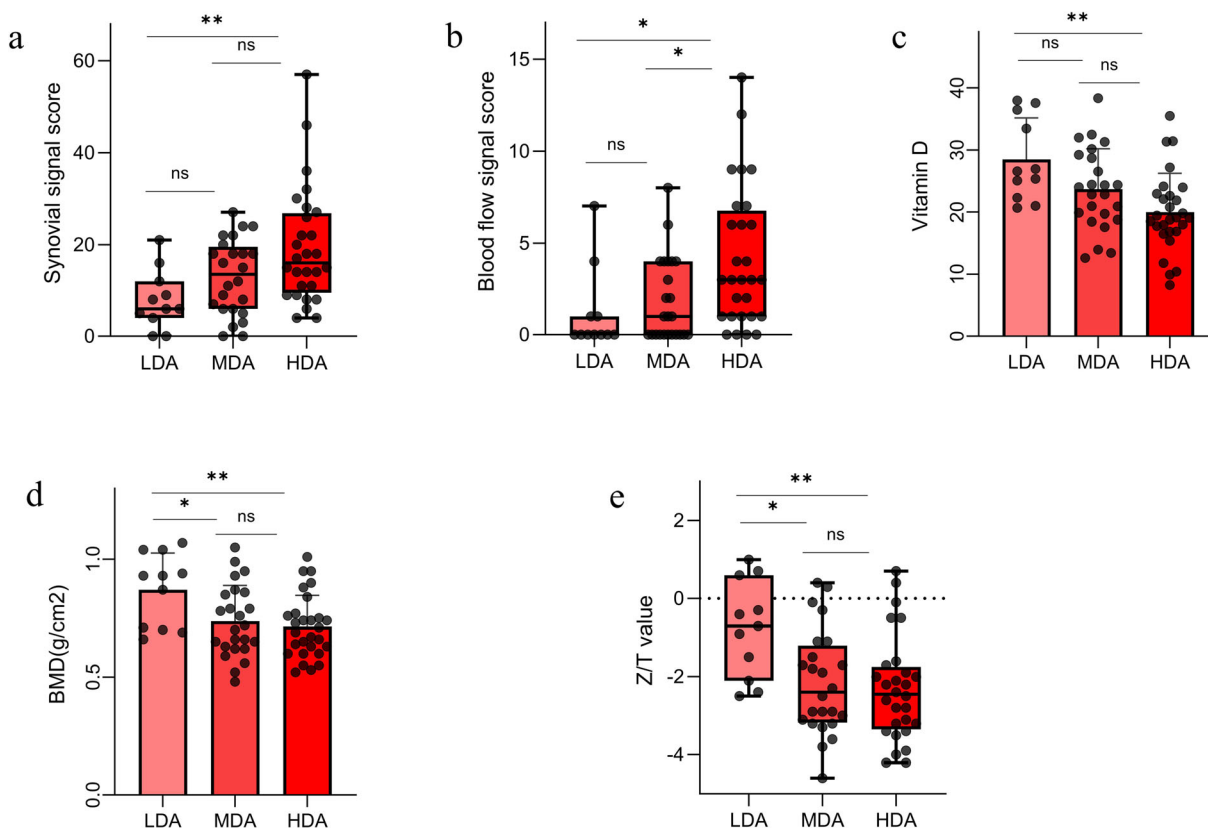


Fig. 4 Measurement of imaging parameters between different activity (LDA [$n = 11$] versus MDA [$n = 24$] versus HDA [$n = 28$]), (a–e synovial signal score, blood flow signal score, vitamin D levels, BMD and Z/T values). Data with a normal distribution are presented as mean \pm SD, while data with a non-normal distribution are

expressed as median (interquartile range). Significance levels are denoted as follows: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, indicating significant differences as indicated. LDA low disease activity, MDA moderate disease activity, HDA high disease activity, BMD bone mineral density, SD standard deviation

dynamic association with inflammation parameters both at baseline and during follow-up studies. At the identified optimal cut-off value of 341.90 pg/mL, the sensitivity, specificity, and area under the receiver operating characteristic curve were determined to be 72.3%, 90.6% and 0.84, respectively, in the training cohort, with similar outcomes observed in the validation cohort. These findings collectively underscore that serum IL-38 levels are distinctly elevated in patients with RA. The observed increase in IL-38 levels correlates with disease severity and is responsive to treatment, indicating its promising potential as a diagnostic and prognostic biomarker for monitoring disease progression in RA. These studies suggest

its potential as a diagnostic and prognostic biomarker to monitor disease progression in RA.

We further substantiated that heightened levels of IL-38 correlate with clinical manifestations, such as macroscopic indicators like swelling, pain and disease progression. Our investigation revealed associations between IL-38 and ultrasound findings, specifically increased blood flow (neovascularization). Additionally, a noteworthy correlation exists between circulating IL-38, an anti-inflammatory cytokine, and the pro-inflammatory cytokines IL-6/IL-17. These findings suggest that elevated IL-38 may be attempting to counterbalance heightened pro-inflammatory cytokines within the microenvironment, albeit unsuccessfully. The potential causes, including

Table 4 Comparison of imaging indexes in different disease activity groups

Parameter	LDA (<i>n</i> = 11)	MDA (<i>n</i> = 24)	HDA (<i>n</i> = 28)	<i>P</i> value
Synovial signal scores	6.00 (4.00, 12.00)	13.50 (6.00, 19.50)	16.00 (9.50, 26.75)	< 0.01 ^b
Blood flow signal scores	0 (0, 1.00)	1.00 (0, 4.00)	3.00 (1.00, 6.75)	< 0.05 ^{bc}
Vitamin D	28.47 ± 6.67	23.64 ± 6.53	19.93 ± 6.31	< 0.01 ^b
BMD values	0.87 ± 0.15	0.73 ± 0.15	0.71 ± 0.13	< 0.05 ^{ab}
Z/T values	− 0.70 (− 2.10, 0.60)	− 2.40 (− 3.17, − 1.20)	− 2.45 (− 3.35, − 1.75)	< 0.05 ^{ab}

LDA low disease activity, MDA moderate disease activity, HDA high disease activity, BMD bone mineral density

^aLDA versus MDA

^bLDA versus HDA

^cMDA versus HDA

insufficient receptors and/or damage to the downstream pathway, will be subject to further exploration. Furthermore, Xu et al. have demonstrated a correlation between IL-38 and disease activities at 3 or 6 months post-treatment [17]. Unfortunately, we currently lack corresponding post-treatment data. Moreover, Takenaka et al. have reported that synovial tissues from patients with RA strongly expressed IL-38 protein [23]. This prompts speculation about a potential correlation between circulating IL-38 and the RA disease score post-treatment. We are in the process of seeking ethical approval to delve into the immunological changes within the microenvironment of patients with RA, aiming to contribute further insights to this intriguing aspect of our research.

Considering IL-38 as a potential therapeutic target, there arises a concern that intervening with its levels might disrupt the delicate balance of host immunity, potentially leading to heightened inflammation in other regions or organs. The prospect of such potential adverse reactions warrants careful consideration in the exploration of IL-38 as a therapeutic avenue for RA.

Interestingly, we observed no correlation between IL-38 and anti-CCP, RF, BMD and Z/T values, despite reports of their involvement in inflammation. This finding may be attributed to the relatively small sample size and absence of longitudinal data. Addressing this limitation could involve increasing the sample size and

collecting samples longitudinally in future studies.

Additionally, the underlying mechanism of the observed inverse correlation between IL-38 and disease activity/IL-6/IL-17 was not fully explored and requires further investigation in future studies. Finally, a cross-sectional study would provide invaluable insights to elucidate the potential role of IL-38 in the development of RA. Regrettably, as a result of time constraints, we were unable to conduct such an in-depth investigation in the current study. Nonetheless, the pursuit of this crucial aspect is earmarked for future research endeavours, and we anticipate that it will contribute significantly to a comprehensive understanding of the intricate involvement of IL-38 in the pathogenesis of RA.

CONCLUSIONS

IL-38 may play a critical role in regulating the immune response and alleviating inflammation in RA, potentially influencing the disease activity of RA. Therefore, IL-38 could be considered a promising therapeutic target for managing RA.

ACKNOWLEDGEMENTS

The authors would like to thank all the staff and patients who participated in this study,

especially the patients for contributing data to this study.

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Author Contributions. Min Yang conceived and designed this study. Liting Chen, Song Gao, Ruilan Liang, Jiayi Ling, Minghui Hou performed the experiment. Liting Chen performed the ELISA analysis, Liting Chen, Ruilan Liang, Minglin Ou analyzed data. Shengxiang Liang and Liting Chen drafted the manuscript. Min Yang also supervised the study and critically revised the manuscript. All the authors read and approved the final manuscript.

Funding. This study was supported by the National Natural Science Foundation of China (No. 81560274), Project of the Guilin Scientific Research and technology development program (20190218–5-3), Guangxi medical and health appropriate technology development and application of the project (S2022128) and Innovation Project of Guangxi Graduate Education (JGY2021153). No funding was received for publication of this article, the journal's Rapid Service Fee was funded by the authors.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest. Shengxiang Liang, Liting Chen, Ruilan Liang, Jiayi Ling, Minghui Hou, Song Gao, Minglin Ou and Min Yang have nothing to disclose.

Ethical Approval. The study was approved by the ethics committee of the Affiliated Hospital of Guilin Medical University (approval number YJSLL202151) and performed in accordance with the Helsinki Declaration of 1964 and its later amendments. Each participant signed an informed consent form. No

identifying information of participants was included in the manuscript.

Open Access. This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License, which permits any non-commercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc/4.0/>.

REFERENCES

1. Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis*. 2010;69(9):1580–8.
2. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet*. 2016;388(10055):2023–38.
3. Zeng S-F, Zhu S-L, Tan A-C, et al. Systematic evaluation of rheumatoid arthritis disease burden and quality of survival studies in China. *Chin J Evid Based Med*. 2013;13(03):300–7.
4. Zanguo Li. The low awareness and high disability rate of rheumatoid arthritis in China should not be ignored. *Chin Med J*. 2009;27:1873–5.
5. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmun Rev*. 2014;13(6):668–77.
6. Lin YJ, Anzaghe M, Schülke S. Update on the pathomechanism, diagnosis, and treatment options for rheumatoid arthritis. *Cells*. 2020;9(4):880.

7. Jang S, Kwon EJ, Lee JJ. Rheumatoid arthritis: pathogenic roles of diverse immune cells. *Int J Mol Sci.* 2022;23(2):905.
8. Schett G, Stach C, Zwerina J, Voll R, Manger B. How antirheumatic drugs protect joints from damage in rheumatoid arthritis. *Arthritis Rheum.* 2008;58(10):2936–48.
9. Baum R, Gravallese EM. Impact of inflammation on the osteoblast in rheumatic diseases. *Curr Osteoporos Rep.* 2014;12(1):9–16.
10. Bensen JT, Dawson PA, Mychaleckyj JC, et al. Identification of a novel human cytokine gene in the interleukin gene cluster on chromosome 2q12-14. *J Interferon Cytokine Res.* 2001;21(11):899–904.
11. Xie L, Huang Z, Li H, Liu X, Zheng S, Su W. IL-38: a new player in inflammatory autoimmune disorders. *Biomolecules.* 2019;9(8):345.
12. Wang M, Wang B, Ma Z, et al. Detection of the novel IL-1 family cytokines by QAH-IL1F-1 assay in rheumatoid arthritis. *Cell Mol Biol (Noisy-le-grand).* 2016;62(4):31–34.
13. Pei B, Chen K, Zhou S, et al. IL-38 restrains inflammatory response of collagen-induced arthritis in rats via SIRT1/HIF-1 α signaling pathway. *Biosci Rep.* 2020;40(5):BSR20182431.
14. Boutet MA, Bart G, Penhoat M, et al. Distinct expression of interleukin (IL)-36 α , β and γ , their antagonist IL-36Ra and IL-38 in psoriasis, rheumatoid arthritis and Crohn's disease. *Clin Exp Immunol.* 2016;184:159–173.
15. Boutet MA, Najm A, Bart G, et al. IL-38 overexpression induces antiinflammatory effects in mice arthritis models and in human macrophages in vitro. *Ann Rheum Dis.* 2017;76:1304–1312.
16. Takenaka SI, Kaieda S, Kawayama T, et al. IL-38: a new factor in rheumatoid arthritis. *Biochem Biophys Rep.* 2015;4:386–91.
17. Xu WD, Su LC, He CS, et al. Plasma interleukin-38 in patients with rheumatoid arthritis. *Int Immunopharmacol.* 2018;65:1–7.
18. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum.* 1988;31(3):315–24.
19. Singh JA, Furst DE, Bharat A, et al. 2012 update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. *Arthritis Care Res (Hoboken).* 2012;64(5):625–39.
20. Möller I, Janta I, Backhaus M, et al. The 2017 EULAR standardised procedures for ultrasound imaging in rheumatology. *Ann Rheum Dis.* 2017;76(12):1974–9.
21. Szkudlarek M, Court-Payen M, Jacobsen S, et al. Interobserver agreement in ultrasonography of the finger and toe joints in rheumatoid arthritis. *Arthritis Rheum.* 2003;48(4):955–62.
22. Qiang W, Linna Ma, Caiping An, et al. The role of IL-38 in intestinal diseases - its potential as a therapeutic target. *Front Immunol.* 2022;13:1051787.
23. Takenaka SI, Kaieda S, Kawayama T, et al. IL-38: a new factor in rheumatoid arthritis. *Biochem Biophys Rep.* 2015;4:386–91.