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LETTER TO THE EDITOR

Bipolar Disorder Associated With Systemic Lupus Erythematosus: A Case–Control Study in the All of Us Research Program

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Dear Editor,

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease where antibodies target multiple auto-antigens, affecting multiple organ systems [1]. Bipolar disorders (BD) are chronic and severe conditions, with bipolar I involving manic episodes and bipolar II characterized by hypomanic episodes alongside major depressive episodes [2]. The association between BD and SLE is known, but studies investigating the association between the two in a nationwide sociodemographic diverse cohort solely conducted in the United States (US) have yet to be done [3, 4]. We examined the association of BD and SLE using the All of Us (AoU) research database, a National Institutes of Health (NIH) initiative created to increase biomedical research in historically underrepresented groups with a cohort of 410,361 US participants and counting [5]. By leveraging a nationally heterogeneous cohort, this study sheds light on the mental health impact of SLE across populations with distinct sociodemographic profiles in the US.

This nested case–control study included individuals aged 18 years and older and was enrolled in the AoU cohort between May 6, 2018, and January 20, 2025. We followed the STROBE guideline.

Chi-square tests or the Fisher exact test were used for categorical variables such as sex, race, ethnicity, and smoking status. For continuous variables like age, we used means and standard deviations. Logistic regression was used to estimate the association between SLE and BD.

Electronic health record (EHR) data in AoU was used to identify BD and SLE by obtaining the systematized nomenclature of medicine (SNOMED) codes (detailed in the eMethods section of the supplemental). Every patient was matched to four individuals who served as a control based on age, sex, race, ethnicity, and smoking status. Two-sided P-value < 0.05 was considered statistically significant. All statistical analyses were performed using Python and utilizing the Pandas, NumPy, SciPy, Statsmodels, and Seaborn libraries.

We found 3799 individuals with SLE (mean [SD] 57.4, [14.5] years) and 15,173 matched controls. Age, sex, ethnicity, race, and smoking status were matched 1:4 between cases and controls. Participants with SLE were more likely to have a diagnosis of BD compared to the control group (356 [9.4%] vs. 589 [3.9%]; $p < 0.001$) (Table 1). In our univariable and multivariable analysis, the association between SLE and BD was found to be statistically significant (OR, 2.56; 95% CI, 2.23–2.94) and (OR, 1.77; 95% CI, 1.52–2.06), respectively (Table 2). We adjusted for systemic corticosteroid use (prednisone, methylprednisolone, prednisolone, dexamethasone), substance use disorder, marital status, and hypothyroidism in our multivariate analysis.

There was a statistically significant association with SLE and BD in our study. Participants with SLE had a 1.65- fold increase in odds of being diagnosed with BD compared to the control group. While our study does not establish a causal relationship between SLE and BD, it provides additional insights into the

Abbreviations: AoU, all of us; EHR, electronic health record; SLE, systemic lupus erythematosus.

TABLE 1 | Clinical characteristics of patients with SLE and control individuals matched by age, sex, race, ethnicity, and smoking status.

Characteristic	Participants, No. (%)[a]		
	Patients with SLE, ($n = 3799$)	Matched controls, ($n = 15173$)	p
Age, mean (SD), y	57.4 (14.5)	57.4 (14.5)	0.97
Sex			0.99
Female	3307 (87.1)	13219 (87.1)	0.91
Male	404 (10.6)	1616 (10.7)	0.98
Other[b]	88 (2.3)	338 (2.2)	0.88
Race and ethnicity			1.00
Not hispanic or latino			
Asian	90 (2.4)	360 (2.4)	
Black	1020 (26.9)	4077 (26.9)	
White	1560 (41.1)	6240 (41.1)	
Hispanic or latino			
Asian	< 20	< 20	
Black	20 (0.5)	80 (0.5)	
White	72 (1.9)	286 (1.9)	
Other[c]	1033 (27.2)	4116 (27.1)	
Ever Smoker			0.99
No	143 (3.8)	565 (3.8)	
Yes	1231 (32.4)	4913 (32.4)	
NA	2425 (63.8)	9697 (63.9)	
Bipolar disorder	356 (9.4)	613 (4.0)	< 0.001
Asian	< 20	< 20	
Black	111 (2.9)	194 (1.3)	
White	160 (4.2)	242 (1.6)	
Other[d]	83 (2.2)	150 (1.0)	
Hypothyroidism	1091 (28.7)	1581 (10.4)	< 0.001
Substance use disorder	686 (18.1)	1240 (8.2)	< 0.001
Marital status	3799 (100.0)	15173 (100.0)	< 0.001
Divorced	708 (18.6)	2446 (16.1)	
Living with partner	194 (5.1)	931 (6.1)	
Married	1395 (36.7)	6264 (41.3)	
Never married	928 (24.4)	3326 (21.9)	
Separated	171 (4.5)	651 (4.3)	
Widowed	248 (6.5)	947 (6.2)	
Other[e]	155 (4.1)	608 (4.0)	
Systemic steroid use[f]	958 (25.2)	1459 (9.6)	< 0.001

[a]In line with the data and statistics dissemination policy of the NIH All of Us Research Program, any values involving fewer than 20 individuals are presented in this manner to ensure participant privacy.
[b]Includes participants who either had no matching concept or who did not identify as male or female, preferred not to answer, or skipped the question.
[c]Includes participants who did not indicate Hispanic or Latino, skipped PMI, identified with multiple populations, chose not to answer, or belonged to single non-specified or Hispanic populations.
[d]Another single population, more than one population, none indicated, none of these, pmi, and I prefer not to answer.
[e]Prefer not to answer, skip.
[f]Dexamethasone, methylprednisolone, prednisolone, prednisone.

TABLE 2 | Association of SLE with BD.

	Univariable analysis odds ratio (95% CI)	Multivariable analysis odds ratio (95% CI)
Bipolar disorder	2.56 (2.23–2.94)	1.65 (1.42–1.92)

growing body of literature supporting a potential link [3, 4, 6]. Given that systemic steroid use is independently associated with mania, hypothyroidism with depression, and marital status and substance use disorder with psychiatric manifestations, we included these factors as covariates and adjusted for them in our logistic regression [7–10].

Using data from a health service organization in Israel, Tiosano et al. published a cross-sectional study demonstrating that SLE patients had an increased rate of BD compared to controls [3]. Our results confirm this association in a large, ethnically diverse dataset in the US, and show a relatively higher percentage of patients with SLE and BD. Our paper expands on existing research by employing a distinct and inclusive national cohort, addressing limitations in generalizability and representation noted in prior studies.

Indeed, mania can be a presenting symptom of SLE, suggesting a possible role of autoantibodies in the onset of symptoms of BD. Increasing evidence suggests that immune dysregulation and systemic inflammation play a critical role in both BD and SLE. One potential link is the antimicrobial peptide cathelicidin LL-37, which is elevated in both conditions, indicating an immunomodulatory bridge between autoimmune and neuropsychiatric diseases. In BD, increased circulating LL-37 levels have been reported in euthymic patients [11] while in SLE, LL-37 has been implicated in autoantibody-mediated inflammation, a key driver of lupus immunopathology [12]. Additionally, antiphospholipid antibodies have been associated with mood disorders in SLE patients, with anti-cardiolipin (ACL) and anti-β2 glycoprotein I (anti-β2GP1) antibodies present in 27.3% and 18.2% of SLE patients with mood disturbances, respectively [13].

BD patients exhibit elevated levels of TNF-α, IL-6, IL-1β, and CRP, reflecting chronic low-grade inflammation [14]. Similarly, SLE patients with serositis show increased levels of IL-6, IL-1β, and TNF-α, cytokines known to drive inflammation in both psychiatric and autoimmune conditions [15]. IL-6 and TNF-α have been linked to both mood disorders and lupus-related serositis, reinforcing a shared inflammatory mechanism. While hypocomplementemia is noted as a biomarker for SLE flares, it was found to be increased in BD patients [16, 17]. This paradoxical difference in C3 regulation between BD and SLE highlights the complexity of immune system involvement in neuropsychiatric and autoimmune conditions, warranting further investigation into complement dysregulation as a shared or divergent pathway in both conditions.

Acneiform eruptions have been reported in both SLE and BD, highlighting a potential shared dermatological feature between these conditions. In SLE, acneiform lesions are a rare but documented cutaneous manifestation, often mistaken for primary acne vulgaris, leading to diagnostic delays. A study examining cutaneous features of SLE found that acneiform eruptions, while uncommon, were present alongside other lupus-specific dermatologic findings, suggesting a broader spectrum of skin involvement in the disease. While acne in BD appears to be more commonly associated with lithium therapy rather than BD itself [18, 19].

Our dataset has limitations, including the inability to capture SLE disease severity or duration, the inability to determine the sequence of diagnoses, reliance on EHR data, and it may not be representative of the entire US population. The association of SLE and BD may be accredited to the presence of neurotropic autoantibodies, family history of mental disorders, or stress associated with disease morbidity [20].

The complex nature of SLE highlights the importance of a comprehensive approach that prioritizes both physical and mental health. Healthcare providers should routinely screen for BD and facilitate referrals to mental health specialists when necessary. Timely identification and management of BD can significantly improve the quality of life and overall outcomes for individuals with SLE. Further research should explore underlying mechanistic causes influencing this relationship.

Author Contributions

N.A.F.: conceptualization, methodology, validation, data curation, writing – original draft, writing – review and editing, project administration, prepared figures, supervision. F.D.: methodology, software, validation, formal analysis, data curation, writing – review and editing, prepared figures. R.O.: conceptualization, methodology, validation, writing – review and editing, project administration, supervision. All authors have approved the final version for submission.

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Ethics Statement

The University of California, San Diego (UCSD) Institutional Review Board has deemed our study (809009) exempt from human subjects research.

Consent

The authors have nothing to report.

Conflicts of Interest

R.O.–Blueprint Medicine, Novartis.

Data Availability Statement

The data used in this article were obtained from the All of Us database.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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LETTER TO THE EDITOR

Capillary Leak Syndrome: A Rare Presentation of ANCA-Associated Vasculitis

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Systemic capillary leak syndrome (SCLS) is a rare condition characterized by episodes of increased vascular permeability, resulting in the extravasation of plasma proteins across the endothelium [1]. Acute episodes typically present with the clinical triad of hypotension, hemoconcentration, and paradoxical hypoalbuminemia [1]. The syndrome can be classified as either primary (Clarkson's disease) or secondary [2].

Potent inflammatory stimuli cause endothelial cell separation that leads to the accumulation of fluid in the interstitial space manifested clinically as systemic pitting edema in mild cases, through noncardiogenic pulmonary edema and acute respiratory distress syndrome (ARDS) [3].

Secondary capillary leak syndrome (SCLS) has been documented in association with various autoimmune conditions, including Sjögren's syndrome and inflammatory myositis, though these associations are rarely reported in the literature [4].

Patient Description

An 84-year-old male with a past medical history of dyslipidemia, essential hypertension, and cataracts presented to the Geriatric Ward with progressive generalized edema of 2 months duration, affecting the lower extremities, abdomen, upper extremities, and face. Associated symptoms included fever, constitutional symptoms, significant weight gain of 10 kg, fatigue, diarrhea, and dyspnea.

On physical examination, marked pitting edema of the legs, sacrum, and abdominal wall was noted (+4, in a grading scale 1–4 that measures how quickly the dimple goes back to normal after gentle pressure on swollen area of skin. Grade 4 indicates rebound between 2 and 3 min with an 8 mm pit).

Otherwise, the rest of the physical examination was unremarkable.

Laboratory investigations revealed marked inflammation with elevated C-reactive protein (CRP) of 7.5 mg/dL and erythrocyte sedimentation rate (ESR) of 61 mm/h. Complete blood count demonstrated microcytic anemia with hemoglobin of 8.0 g/dL and MCV of 78 fL. Peripheral blood smear showed no evidence of blasts or dysplastic changes. Additional hematologic findings included thrombocytosis (534 000/μL), leukocytosis (13 000/μL) with neutrophilia (10 300/μL).

Hepatic panel showed mild transaminitis with AST 52 U/L, ALT 56 U/L, GGT 99 U/L, and LDH 405 U/L. Renal function tests revealed serum creatinine of 1.04 mg/dL and marked hypoalbuminemia (2.0 g/dL). Serum protein electrophoresis was negative for monoclonal gammopathy, and urinalysis showed no proteinuria.

High-resolution computed tomography (HRCT) of the chest revealed bilateral ground-glass opacities, pleural effusions, subcutaneous edema, and interstitial septal thickening. Transthoracic echocardiography demonstrated preserved cardiac function with mild aortic and mitral valve regurgitation. An extensive malignancy workup was negative. Endoscopic intestinal biopsy showed no evidence of amyloid deposition on Congo red staining. Colonoscopy and CT enterography demonstrated subdermal tissue edema throughout the gastrointestinal tract. Bone marrow

biopsy revealed no significant pathology, and genetic analysis was negative for the UBA1 somatic mutation associated with VEXAS (Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic) syndrome.

Initial treatment was directed at presumed pneumonia with broad-spectrum antibiotics and diuretic therapy. However, pulmonary infiltrates persisted despite appropriate antimicrobial coverage. During hospitalization, the patient developed mild proteinuria (urinary protein-to-creatinine ratio 598 mg/g) without hematuria. Renal Doppler ultrasonography excluded renal artery stenosis and renal vein thrombosis.

The patient remained persistently hypotensive throughout admission, with systolic blood pressure consistently below 100 mmHg. Laboratory values showed sustained hypoalbuminemia (2.0 g/dL) and worsening inflammation, with CRP elevation to 10 mg/dL.

Serologic workup for systemic autoimmune disease revealed significantly elevated anti-myeloperoxidase (MPO) antibodies at 354 AI (Antibody Index, reference range: 0.00–0.99 AI). Antiproteinase 3 (PR3) and antiglomerular basement membrane (GBM) antibodies were negative. Antinuclear antibody (ANA) testing showed a titer of 1:80.

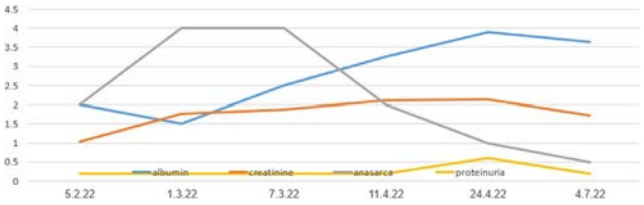
A kidney biopsy showed in immunofluorescence that one of five glomeruli demonstrated a fibrocellular crescent without immune complex deposition, and Electron microscopy showed two glomeruli with signs of necrotic lesions of the glomerulus.

The signs were compatible with pauci-immune glomerulonephritis, and he was diagnosed with anti-MPO-associated microscopic polyangiitis.

The patient was initiated on pulse methylprednisolone therapy (1000 mg) and cyclophosphamide (7.5 mg) administered on two occasions at 2-week intervals, followed by extension to 3-week intervals. Treatment followed the EUVAS vasculitis protocol with dose adjustments based on the patient's age (> 60 years) and reduced renal function (eGFR 27 mL/min/1.73m^2 at diagnosis).

Due to inadequate treatment response, evidenced by persistent laboratory abnormalities, clinical manifestations including proteinuria, hypoalbuminemia, and anasarca, therapy was changed to rituximab. The patient received induction therapy with rituximab 1000 mg administered intravenously at weeks 0 and 2, followed by maintenance therapy of 500 mg every 6 months. This treatment regimen has been maintained for approximately 2 years.

The patient's clinical manifestations showed gradual resolution, accompanied by improvement in serum albumin and CRP levels. At 6-month follow-up after the initial presentation, there was complete resolution of proteinuria and peripheral edema.



Discussion

We present a case of microscopic polyangiitis (MPA) with antimyeloperoxidase (MPO) antibody positivity in a patient who initially presented with anasarca and capillary leak syndrome. The diagnosis was confirmed by renal biopsy demonstrating pauci-immune glomerulonephritis and positive MPO-ANCA serology [5].

Systemic capillary leak syndrome is a rare life-threatening disorder characterized by recurrent episodes of hypotension, systemic inflammation, acute kidney failure, and hypoalbuminemia [1–3]. It manifests as a failure of the endothelium to act as a barrier between the intravascular spaces. Inflammatory stimuli increase endothelial permeability. Hypercytokinemia is believed to be the underlying cause of capillary leak syndrome [3].

Systemic capillary leak syndrome (SCLS) is associated with diverse etiologies, including hematologic malignancies, hemophagocytic lymphohistiocytosis (HLH), infectious diseases (notably arboviruses, brucellosis, and sepsis), drug-induced causes, and idiopathic forms such as Clarkson's disease [3].

Systemic capillary leak syndrome has rarely been documented in autoimmune conditions, including Kawasaki disease, antiphospholipid syndrome, Sjögren's syndrome, systemic sclerosis, and polymyositis [3, 4].

Capillary leak syndrome with elevated inflammatory markers in the absence of marked proteinuria is a rare presentation of systemic vasculitis.

To the best of our knowledge, our case of ANCA-associated vasculitis clinically presented with capillary leak syndrome as the first reported case.

Our case report highlights the importance to of rule-out systemic vasculitis as a cause of capillary leak syndrome.

Author Contributions

Dr. Yarden Assabag, conceptualized the case report, collected data, performed the literature review, and wrote the manuscript in its entirety. Both, Dr. Yarden Assabag and Prof.' Yair Molad were involved in the clinical management of the patient of the patient. All authors reviewed and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Yarden Assabag
Yair Molad

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LETTER TO THE EDITOR

Case Report: A Novel JAK-1 Inhibitor, Upadacitinib, Successfully Treated 1 Case of Chronic Nonbacterial Osteomyelitis in Children

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Dear Editor,

Chronic recurrent multifocal osteomyelitis (CRMO) is a rare, chronic, aseptic autoinflammatory disease that occurs primarily in childhood and falls under the umbrella of spinal arthritis (SpA). The most common bone involved site is the long bones of the limbs, followed by the pelvis, clavicle, and spine, etc. Clinical manifestations are accompanied or not by bone pain and/or joint pain [1]. Due to the rarity of CRMO, there is currently no treatment consensus, and bisphosphonates can be used as a treatment option for CRMO [2]. In this case report, we report for the first time that a pediatric CRMO patient with thigh pain that remained after treatment with bisphosphonates successfully resolved after treatment with upadacitinib.

The patient was a 13-year-old female child who presented with right leg pain with limited mobility at 12 years of age one year ago. [99m]TcMDP whole body bone scan revealed radioactive concentration of the right distal tibia-fibula and abnormal concentration of the right proximal femur (Figure 1A). Histopathological examination of the pathological biopsy showed a small amount of inflammatory cell infiltration, no Langerhans cell histiocytosis, and no microbial growth in the biopsy specimen culture. Immunohistochemical staining showed CDK4 (-), MDM2 (+), SATB2 (+) and Ki67 (positive rate 30%), S-10000 (-), CD1a (-) and P16 (-), CD207 (-), H3F3B (K36M) (-), and CD68 (-); she was diagnosed as CRMO [3]. Intravenous bisphosphonate therapy, Padedidronic acid 4mg/d, was given for 3 consecutive days, and the femur pain improved slightly. The femur pain recurred 6 months later, and the patient was again treated with intravenous bisphosphonate, Padedidronic acid 4mg/d, for 3 consecutive days. However, the patient still had intermittent pain in the femur. Re-examination of the femur MRI showed high signal of the right femur lipid-pressure image (Figure 1B). Due to concerns that tumor necrosis factor (TNF) alpha antagonists might induce skin lesions, the child was subsequently started on monotherapy with upadacitinib (15mg qd). Three months later, the pain in the right thigh disappeared, and reexamination MRI showed that the bone marrow edema in the right middle and upper femur was significantly reduced (Figure 1C).

The pathogenesis of CRMO syndrome is not clear, and current studies have found that infection, genetic, immune, and environmental factors may contribute to the development of the disease. The clinical treatment options for CRMO are very limited, and there is no recognized standardized treatment at present. In general, non-steroidal anti-inflammatory drugs (NSAID) are the first-line treatment for most patients with CRMO [4]. Bisphosphonates can be used as a treatment option for CRMO, and there have been case series demonstrating that bisphosphonates are effective and well tolerated in the treatment of CRMO [5, 6]. In recent decades,

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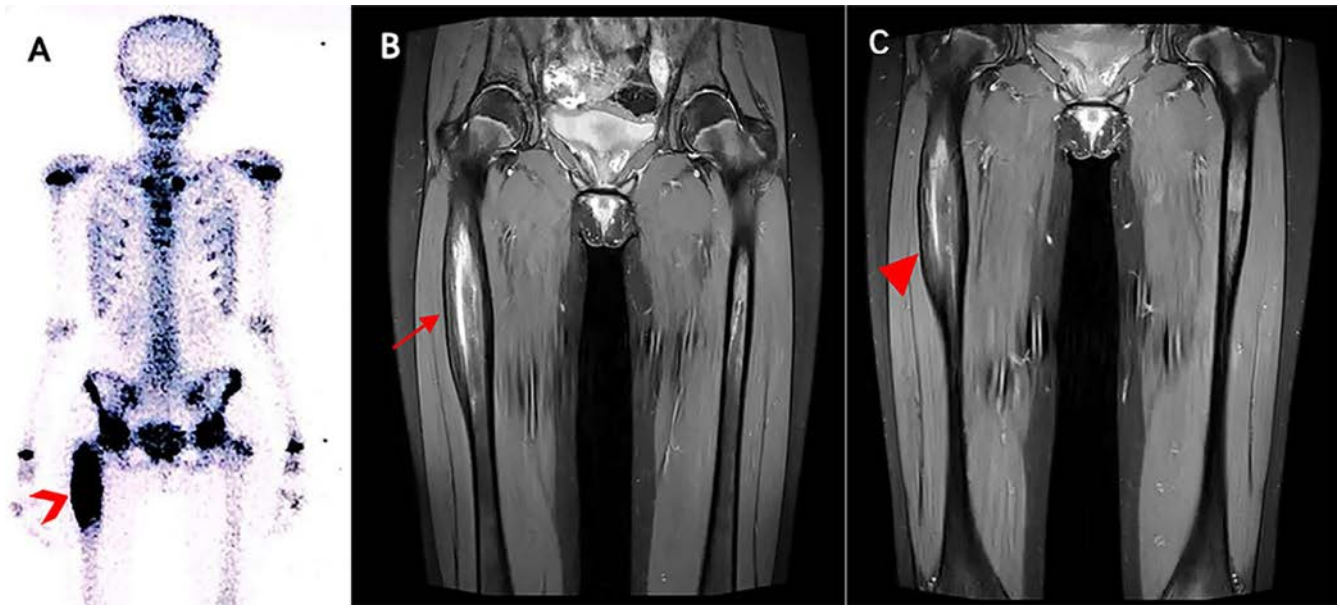

FIGURE 1 | Radiological image of a CRMO patient. (A) [99 m]TcMDP 9 mCi whole body bone scan showed abnormal concentration of her right proximal femur (arrow). (B) MRI of the femur showed high signal of the femur (arrow) lipid-pressing sequence. (C) Abnormal signs of hypertrophy, thickening, and stripy bone marrow edema in the middle and upper femur of the right thigh (triangular arrow). Compared with (B), the bone marrow edema of the right middle and upper femur was significantly reduced in (C).

biologic agents such as tumor necrosis factor (TNF) -α antagonists have been used in the treatment of patients with CRMO syndrome [7], such as adalimumab or Infliximab, which have achieved good results in the treatment of bone inflammation and joint symptoms [8]. However, there are side effects such as contradictory psoriasis, aggravation of primary palmoplantar impetigo, and pyoderma gangrenosa [9–11].

Studies have shown that Tofacitinib, the first-generation JAK inhibitor, can effectively improve skin injury and alleviate bone pain in patients with CRMO [12], and upadacitinib is a novel small-molecule JAK inhibitor. Compared with tofacitinib, upadacitinib can inhibit a single JAK protein with high selectivity, without affecting other cytokines, potentially reducing adverse reactions [13]. Upadacitinib has been approved for the treatment of adults over 12 years of age and adolescents with arthritis associated with inflammatory disease [14]. Some real-world experience has been published on the safety and efficacy of upadacitinib in rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and atopic dermatitis [15], but there are currently no reports of upadacitinib in the treatment of CRMO.

In conclusion, we first report the short-term clinical efficacy of upadacitinib in the treatment of CRMO. Janus kinase inhibitors, including upadacitinib, may be a promising treatment for CRMO that does not respond to bisphosphonates.

Author Contributions

All authors contributed to writing it and approved the final manuscript.

Consent

Informed consent to publication was obtained from the patient.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets analyzed for this study are available from the corresponding author, Chen Li (casio1981@163.com) upon reasonable request.

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ORIGINAL ARTICLE

Burden of Musculoskeletal (MSK) Pain and Arthritis in India: A Community Oriented Program for Control of Rheumatic Diseases (COPCORD—Bone and Joint Decade (BJD)) India Project

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ABSTRACT

Background: Several countries have participated in WHO COPCORD. The Global Disease Burden program (GBD) reports selected MSK disorders. We used a COPCORD India protocol to estimate the national burden of MSK disorders.

Materials and Methods: Trained paramedics used standard questionnaires to screen the population and identify respondents with current and/or past MSK pain (non-traumatic) in 12 survey sites (8 rural); cross-sectional design and prospective data. Several standard measures were recorded; MSK pain was self-reported (on human manikin). The site rheumatologist examined each respondent and provided a clinical diagnosis. Pooled data (anonymized) from all sites was analyzed using standard statistical software. Standardized point prevalence rates (adjusted to Indian Census) and odds ratios (risk factors) were calculated: 95% confidence intervals in parentheses.

Results: 56 548 population (60% rural, response rate > 70%) was screened; 10 273 respondents (18%, 65% women). The prevalence of MSK pain was 16.14 (14.2, 18.3) and higher in the rural population (20% vs. 10.3%); rheumatoid arthritis 0.34%, undifferentiated inflammatory arthritis 0.22%, spondyloarthritis 0.23%, osteoarthritis 4.39%, Gout 0.05%, chikungunya arthritis 1.2%. Non-specific arthralgias, soft tissue pains, and degenerative arthritis were dominant disorders; 12% of respondents reported inflammatory arthritis. Significant risk factors associated with MSK pain included female gender, poor literacy, non-vegetarian diet, chronic non-MSK illness, past trauma, and tobacco use. Limitations included non-random

Abbreviations: AS, ankylosing spondylitis; CCQ, COPCORD core questionnaire; CDD, crystal deposition disorder; CI, confidence interval; CTD, connective tissue disorder; DALY, disability adjusted life years; GBD, global burden of disease; IA-U, undifferentiated inflammatory arthritis; IDS, ill-defined aches and pains; JIA, juvenile idiopathic arthritis; MCTD, mixed connective tissue disorder; MSK, musculoskeletal; OA, osteoarthritis; OIA, other inflammatory arthritis; OR, odds ratio; PCAR, post-Chikungunya arthritis and rheumatism; PGOA, primary generalized osteoarthritis; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SpA, spondyloarthritis; SR, symptom related diagnosis; STR, soft tissue rheumatism.

selection, clinical diagnosis, and limited investigations. However, in comparison to GBD, the COPCORD outcome seemed all-inclusive and clinically meaningful.

Conclusion: The high prevalence of MSK pain and arthritis indicates a huge disease burden in India and prioritizes the need for a national control program.

1 | Introduction

Musculoskeletal (MSK) pain and arthritis contributed to 5.9% of the global DALY (Disability adjusted life years) burden and were a leading cause of disability and impaired quality of life [1]. The MSK disease burden increased several fold during the period 1990–2019 [2]. The contribution of arthritis to mortality remained underestimated. Non-communicable diseases (NCD) were increasingly recognized, but MSK pain and arthritis remained neglected in India and the developing World [3–6].

The WHO and International League of Associations for Rheumatology (ILAR) launched a community-oriented program for the control of rheumatic diseases (COPCORD) in Jan 1981. It was focused on MSK pain, disability, and arthritis in developing economies [3]. COPCORD had three stages: population survey (Stage I), health education and risk factors (Stage II), and prevention and control strategies (Stage III). The program encouraged optimum utilization of local resources and a clinical approach to diagnosis [3] Several countries in the Asia Pacific, Latin America, and Africa completed COPCORD surveys [5].

The maiden India COPCORD was carried out in village Bhigwan (district Pune) in 1996 and continued to date [6, 7]. The Bhigwan survey unraveled a huge burden of MSK pain and arthritis, and the data was used by the WHO to project RA and OA in Southeast Asia [6, 8]. This prompted an unmet need to assess MSK pain and arthritis in the Indian population. Further impetus was provided by the global Bone and Joint Decade program 2000–2010 (BJD) in India [9]. The previously validated COPCORD India Bhigwan model was used in the current study to carry out surveys at predetermined sites with a uniform protocol (Figure 1). Some of these surveys were published and contributed data to the Global Burden of Disease (GBD) India project (2015) [4, 10–12].

In this report, we present standardized prevalence rates of MSK pain and arthritis in the Indian population. Some important risk factors are also described. We also present a brief overview of the comparison between the current study and other selected COPCORD surveys (India and global) and also highlight some important features of non-COPCORD surveys (European) and the WHO-Global Burden Disease (GBD) project.

2 | Materials and Methods

2.1 | Study Design

This was an observational study with a cross-sectional design and prospective data. There were multiple study sites (8 rural and 4 urban, Figure 1) selected in a non-random manner. The project was completed during the period 2006–2013.

The COPCORD India Bhigwan model (1996) suitable for the Indian situation, was used [6, 10]. It contained the standard COPCORD Core Questionnaire (CCQ) (Stage I survey) which is enclosed (Data S1) [5]. Each site survey was carried out in 3 concurrent phases: screening the population to identify respondents (Phase 1), self-reported MSK pain, joint swelling, and function, and other relevant data (Phase 2), and clinical evaluation (Phase 3).

Although a formal approval was not required, the principal investigator (PI) at each site presented a project overview and protocol to the local ethics committee and administration prior to beginning the survey. Salient aspects, including the use of coded (anonymous) individual data, were orally explained to each study participant.

2.2 | Survey Sites

The local site PI was responsible for the selection of the site. Some important considerations were a well-defined geographical area, a non-migrant population of about 4–5000 residents, local support (administration and public) and easy road access.

Figure 1 shows the sites and screened population sample size. The urban sites were in the metropolis cities of Pune, Jammu, Chennai and Bikaner. The rural/village sites were Naora-Pargana (Kolkata), Atal-Ballabgarh (Delhi), Kanniparamba-Cheruppa (Calicut), Ottoor-Varkala (Thiruvananthapuram), Kandakur-Rangareddy (Hyderabad), Keinou-Bishnupur (Manipur), Ralegan-Siddhi (Ahmednagar) and Sarpara-Mirza (Guwahati); henceforth referred by the district shown in parenthesis.

2.3 | Participants

The primary inclusion criteria for the Phase 1 population screen were (i) adults aged > 16 years and (ii) bona fide residence in the study site. Exclusions were children, non-resident (site) individuals, and those with migrant/temporary residence.

2.4 | Study Site Team

A dedicated rheumatologist (PI or co-PI) was identified a priori for each survey site. Other members included physician assistants, paramedics (including trained voluntary subjects from the local population), nurses, laboratory technicians, and medical social workers.

Summary

- Musculoskeletal pain (MSK) is a common ailment in a community and can infrequently lead to a moderate to severe impact on daily life.
- Important causes of MSK pain are non-specific arthralgias, soft tissue rheumatism, osteoarthritis, and inflammatory arthritis.
- Some important risk factors that can be controlled and prevented are tobacco use, nature of work, lack of education, and other chronic concurrent illnesses.

2.5 | Respondents

The primary screening question in Phase 1 was 'Have you suffered from pain/swelling/stiffness in the joints or musculoskeletal soft tissues within the last 7 days (current) or sometime in the past?' Participants answering affirmatively were classified as respondents (Phases 2 and 3) [5, 6, 10].

3 | Procedures

3.1 | Training

Study paramedics, physicians, and rheumatologists attended pre-survey training workshops to standardize survey procedures. Rheumatologists discussed the suitable application of standard diagnostic and standardized a clinical approaches for diagnosis based on the earlier Bhigwan experience [5, 6, 13].

3.2 | Survey

The population was systematically screened and monitored. Trained paramedics supervised Phase 1 and Phase 2 surveys. Although self-reported, they provided suitable guidance and assistance to the participants. They also arranged and coordinated the medical examination of respondents by the assistant physicians and rheumatologists in an easy-to-access facility in a central location (Phase 3). Home visits were made to examine subjects with severe disabilities. After three failed attempts (spread over 4 weeks) to contact and screen, the residents/respondents were declared non-respondent.

3.3 | Documents

Standard paper COPCORD India questionnaires (suitably translated) and rheumatology case record forms were used (Appendix 1: Data S1).

The Phase 1 questionnaire included the primary screening question (see above) and the recording of demographics and other health information especially relevant to expected risk factors and self-reported co-morbidity (e.g., diabetes). In Phase 2 questionnaire, respondents recorded their pain sites on a human mannequin and completed a validated India version of the modified Stanford Health Assessment Questionnaire (HAQ) for functional assessment (data not shown) [7]. The case record form (Phase 3) included a standard format for joint examination (68 joints as recommended by the ACR), spine, and soft tissues.

3.4 | Diagnosis and Classification

The diagnosis was essentially clinical. Several apparently non-distinct symptom-based entities, such as non-specific arthralgias (NSA), ill-defined symptoms (IDS), and soft tissue rheumatism (STR) were diagnosed/classified according to the earlier COPCORD Bhigwan experience [5, 6, 8].

3.5 | Statistics and Data

The minimum population of 4000 (non-random sampling) at each site was recommended to be screened and assessed (MSK pain and arthritis) in 24 weeks. A population sample size of 51 741 subjects was finally used to calculate prevalence. Phase 3 data from the Chennai site was disqualified because of the low response (36%) rate in Phase 2 evaluation (respondents).

A central electronic database (CRD, Pune) was used to enter data from each site after comprehensive manual checks. An independent senior DEO and COPCORD investigators (RG, AV, MS) carried out random checks of the electronic database for correctness and consistency. The database program was indigenously designed and created using certified original software programs (Microsoft Visual Basic platform 2004) and had inbuilt checks to identify errors.

Each site data was standardized (direct) for age-gender using India census 2001 [14]. The adjusted data was aggregated (and pooled) into a single master MS Excel file. In the case of an overlap/multiple diagnosis, each diagnosis was counted as a separate entity (prevalence data). Anonymized individual participant data was used for analysis. The final analysis was carried out by a senior biostatistician (SS) and COPCORD investigators (RG, AV).

Both crude and adjusted point prevalence rates (percentage, 95% confidence interval/CI, shown in parenthesis) are shown in the current report. The prevalence was calculated using an exact binomial method (Epi Info 6 version 6.04d -January 2001, Stat Software).

Standard odds ratio (univariate) was shown for the risk factors; data mined from completed Phase 1 questionnaires. Chronic illness (risk factor) included diabetes, hypertension, ischemic heart disease, and stroke. Multivariable logistic regressions were carried out to identify predictors of MSK pain.

Standard statistical software package was used; significant $p < 0.05$, two-tailed.

Check lists showing compliance with the standard STROBE and GATHER statements are enclosed in the Data S1.

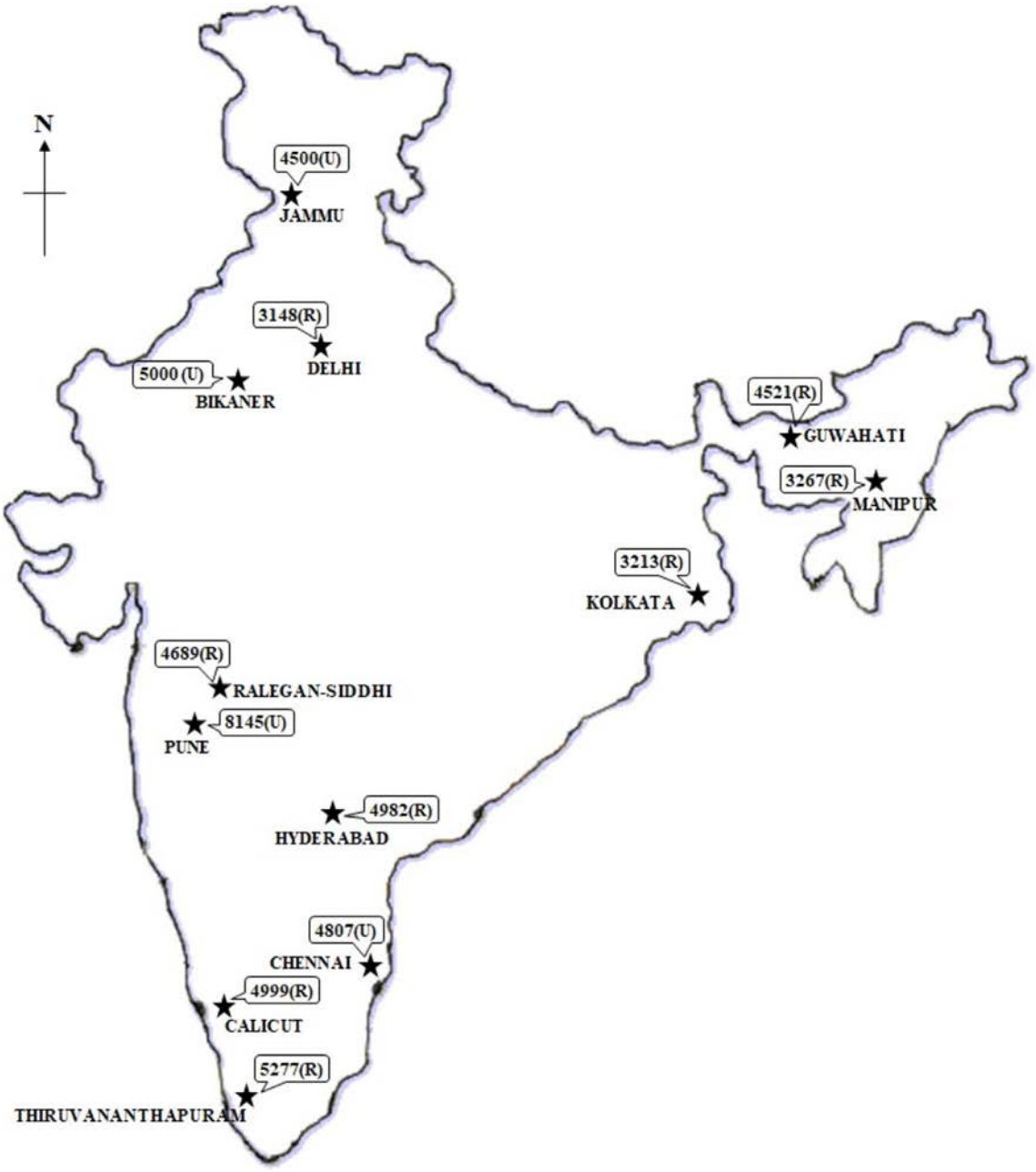

FIGURE 1 | Individual study sites and sample size (Survey Population $N = 56\,548$) in All India COPCORD Survey. R, rural; U, urban.

4 | Results

56 548 population (60% rural, response rate > 70%) was screened (Phase 1). 10 273 respondents with non-traumatic MSK pain (18%, 65% women) were identified; 26.9% urban and 73.1% rural. The site sample size varied from 3148 (Delhi rural) to 8145 (Pune urban; Figure 1).

A minimum of 74% response in Phase 1 and 71% response in Phases 2 and 3 was achieved at each qualified site.

The distribution of age seemed matched with that of the Indian census 2001; the dominant age group was 25–44 years in both populations, but the proportion of 65+ was higher (Figure S1). The median age of the India population sample was 37 years (range 16–103 years) and that of the respondents was 48 years (range 16–100 years; Table S1).

Table 1 shows the gender distribution of the participants and respondents (Phase 1 and 2). There were more women amongst the respondents with 66.6% and 64.5%, respectively, in urban and rural populations.

The adjusted prevalence of MSK pain in the current India population sample was 16.14 (14.2–18.3) respectively (Table 1). The rate was higher in the rural population (20.04% vs. 10.34%), highest in rural Calicut (25.91%) and least in urban Bikaner (6.40%). The frequency of MSK pain sites (human manikin, Phase 2) is shown in Figure S2. The three most frequent pain sites were knees, low back, and ankle-foot in urban and rural

TABLE 1 | COPCORD Stage I, Phases 1 and 2- Distribution of number and frequency (percent) of study subjects reporting musculoskeletal pain at individual sites, in urban and rural areas and all India; Crude and Adjusted point prevalence of MSK pain (95% confidence interval) at each study site shown.

	Phase-1					Phase-2					MSK pain	
	Male		Female		Total	Male		Female		Total		
Site	*n*	%	*n*	%	*n*	*n*	%	*n*	%	*n*	Crude prevalence % (95% CI)	Adjusted prevalence % (95% CI)*
Urban												
Bikaner (*N* = 5000)	2551	51.0	2449	49.0	5000	103	28.9	253	71.1	356	7.12 (6.42–7.87)	6.40 (5.10–7.85)
Chennai (*N* = 4807)	2377	49.4	2430	50.6	4807	235	31.8	505	68.2	740	15.39 (14.38–15.45)	14.95 (13.02–16.98)
Jammu (*N* = 4500)	2460	54.7	2040	45.3	4500	183	35.1	339	64.9	522	11.60 (10.68–12.57)	12.24 (10.53–14.19)
Pune (*N* = 8145)	4010	49.2	4135	50.8	8145	403	35.0	749	65.0	1152	14.14 (13.39–14.92)	11.15 (9.52–13.04)
Rural												
Calicut (*N* = 4999)	2467	49.3	2532	50.7	4999	560	38.0	915	62.0	1475	29.51 (28.24–30.79)	25.91 (23.58–28.44)
Delhi (*N* = 3148)	1780	56.5	1368	43.5	3148	210	37.1	356	62.9	566	17.98 (16.65–19.37)	20.65 (18.49–22.99)
Guwahati (*N* = 4521)	2325	51.4	2196	48.6	4521	101	21.6	366	78.4	467	10.33 (9.46–11.25)	10.23 (8·65–12.04)
Hyderabad (*N* = 4982)	2711	54.4	2271	45.6	4982	391	56.0	307	44.0	698	14.01 (13.06–15.01)	13.33 (11.55–15.35)
Kolkata (*N* = 3213)	1538	47.9	1675	52.1	3213	288	37.4	482	62.6	770	23.97 (22.50–25.48)	23.40 (21.10–25.80)
Manipur (*N* = 3267)	1584	48.5	1683	51.5	3267	266	37.8	438	62.2	704	21.55 (20.15–23.00)	20.44 (18.26–22.74)
Ralegan-Siddhi (*N* = 5000)	2221	47.4	2468	52.6	4689	399	32.4	834	67.6	1233	26.30 (25.03–27.58)	22.21 (19.98–24.59)
Thiruvananthapuram (*N* = 5277)	2188	41.5	3089	58.5	5277	449	28.2	1141	71.8	1590	30.13 (28.89–31.39)	23.60 (21.32–26.04)
Urban total (*N* = 22 452)	11 398	50.8	11 054	49.2	22 452	924	33.4	1846	66.6	2770	12.34 (11.91–12.77)	10.34 (8.79–12.07)
Rural total (*N* = 34 096)	16 814	49.3	17 282	50.7	34 096	2664	35.5	4839	64.5	7503	22.01 (21.61–22.44)	20.04 (17.86–22.37)
Total (All India; *N* = 56 548)	28 212	49.9	28 336	50.1	56 548	3588	34.9	6685	65.1	10 273	18.17 (17.85–18.49)	16.14 (14.19–18.29)

*$p < 0.05$, 2-tailed.

TABLE 2 | COPCORD Stage I, Phases 1 and 2- Distribution of crude and adjusted point prevalence (95% CI) of rheumatic disorders at each study site.

	RA		IA-U		SpA		OA		STR		Back pain	
Site	Crude% (95% CI)	Adjusted* % (95% CI)	Crude% (95% CI)	Adjusted* % (95% CI)	Crude% (95% CI)	Adjusted* % (95% CI)	Crude% (95% CI)	Adjusted* % (95% CI)	Crude% (95% CI)	Adjusted* % (95% CI)	Crude% (95% CI)	Adjusted* % (95% CI)
Urban												
Bikaner	0.82 (0.59–1.11)	0.72 (0.32–1.32)	0.24 (0.12–0.42)	0.25 (0.05–0.68)	NIL	NIL	4.72 (4.15–5.34)	4.17 (3.17–5.43)	0.20 (0.10–0.37)	0.18 (0.02–0.56)	3.60 (3.10–4.15)	3.23 (2.36–4.39)
Jammu	0.31 (0.17–0.52)	0.32 (0.08–0.79)	NIL	NIL	0.04 (0.00–0.16)	0.04 (0.00–0.43)	4.89 (4.28–5.56)	5.41 (4.26–6.82)	0.62 (0.41–0.90)	0.62 (0.27–1.22)	3.80 (3.26–4.40)	3.88 (2.90–5.09)
Pune	0.45 (0.32–0.63)	0.32 (0.24–0.40)	0.37 (0.25–0.53)	0.32 (0.25–0.41)	0.27 (0.17–0.41)	0.27 (0.20–0.35)	6.46 (5.93–7.01)	4.01 (3.74–4.29)	1.30 (1.07–1.57)	1.20 (1.05–1.36)	6.41 (5.89–6.96)	5.19 (4.06–6.56)
Rural												
Calicut	0.20 (0.10–0.37)	0.16 (0.02–0.56)	0.02 (0.00–0.11)	0.02 (0.01–0.03)	0.62 (0.42–0.88)	0.54 (0.22–1.12)	4.48 (3.92–5.09)	3.55 (2.63–4·74)	2.44 (2.03–2.91)	2.14 (1.45–3.13)	6.22 (5.57–6.93)	5.64 (4.47–7.08)
Delhi	0.16 (0.05–0.37)	0.18 (0.02–0.56)	0.13 (0.03–0.33)	0.14 (0.02–0.56)	0.03 (0.00–0.18)	0.03 (0.02–0.04)	9.40 (8.41–10.48)	11.23 (9.59–13.12)	0.25 (0.11–0.50)	0.27 (0.05–0.68)	11.05 (9.98–12.20)	12.75 (10.97–14.69)
Guwahati	0.38 (0.22–0.60)	0.38 (0.13–0.90)	0.22 (0.11–0.41)	0.22 (0.05–0.68)	0.18 (0.08–0.35)	0.17 (0.02–0.56)	2.52 (2.08–3.02)	2.51 (1.71–3.49)	0.73 (0.50–1.02)	0.72 (0.32–1.32)	2.81 (2.35–3.33)	2.78 (1.97–3.85)
Hyderabad	0.32 (0.18–0.52)	0.29 (0.08–0.79)	0.18 (0.08–0.34)	0.16 (0.02–0-56)	0.06 (0.01–0.18)	0.04 (0.03–0.05)	4.38 (3.82–4.98)	3.63 (2.70–4.83)	1.04 (0.78–1.37)	0.94 (0.48–1.62)	0.12 (0.04–0.26)	0.09 (0.00–0.43)
Kolkata	0.40 (0.22–0.69)	0.40 (0.37–0.44)	0.03 (0.00–0.17)	0.03 (0.03–0.05)	0.56 (0.33–0.88)	0.53 (0.22–1.12)	5.23 (4.48–6.06)	5.24 (4.06–6.56)	3.49 (2.88–4.18)	3.37 (2.43–4.47)	7.03 (6.17–7.97)	6.87 (5.52–8.36)
Manipur	0.21 (0.09–0.44)	0.22 (0.05–0.68)	0.15 (0.05–0.36)	0.15 (0.02–0.56)	0.09 (0.02–0.27)	0.10 (0.00–0.43)	3.31 (2.72–3.98)	3.02 (2.16–4.12)	1.81 (1.38–2.32)	1.72 (1.07–2.58)	12.40 (11.29–13.58)	11.74 (10.02–16.62)

(Continues)

TABLE 2 | (Continued)

Site	RA		IA-U		SpA		OA		STR		Back pain	
	Crude% (95% CI)	Adjusted* % (95% CI)	Crude% (95% CI)	Adjusted* % (95% CI)	Crude% (95% CI)	Adjusted* % (95% CI)	Crude% (95% CI)	Adjusted* % (95% CI)	Crude% (95% CI)	Adjusted* % (95% CI)	Crude% (95% CI)	Adjusted* % (95% CI)
Ralegan-Siddhi	0.30 (0.16–0.50)	0.25 (0.04–0.68)	0.87 (0.63–1.18)	0.72 (0.32–1.32)	0.34 (0.19–0.55)	0.32 (0.08–0.79)	7.25 (6.52–8.03)	4.65 (3.58–5.96)	0.62 (0.41–0.89)	0.60 (0.27–1.22)	17.72 (16.64–18.85)	15.53 (13.60–17.64)
Thiruvananthapuram	0.45 (0.29–0.68)	0.38 (0.13–0.90)	0.27 (0.15–0.44)	0.19 (0.02–0.56)	0.40 (0.25–0.61)	0.47 (0.17–1.01)	7.88 (7.17–8.64)	4.96 (3.85–6.31)	4.30 (3.77–4.88)	3.43 (2.49–4.56)	14.06 (13.13–15.03)	11.16 (9.52–13.04)
Urban total	0.52 (0.42–0.64)	0.42 (0.01–0.94)	0.24 (0.17–0.32)	0.22 (0.04–0.63)	0.14 (0.09–0.20)	0.12 (0.02–0.52)	5.57 (5.23–5.91)	3.83 (2.89–4.99)	0.82 (0.69–0.96)	0·72 (0.35–1.33)	4.30 (4.04–4.57)	3.67 (2.76–4.82)
Rural total	0.31 (0.25–0.38)	0.29 (0.09–0.82)	0.25 (0.20–0.31)	0.22 (0.05–0.70)	0.30 (0.24–0.36)	0.28 (0.09–0.82)	5.53 (5.29–5.77)	4.66 (3.54–5.95)	1.88 (1.74–2.03)	1.75 (1.10–2.65)	8.79 (8.49–9.09)	7.97 (6.55–9.63)
Total (All India)	0.38 (0.30–0.44)	0.34 (0.08–0.79)	0.25 (0.20–0.29)	0.22 (0.05–0.68)	0.24 (0.20–0.28)	0.23 (0.05–0.68)	5.54 (5.34–5.74)	4.39 (3.30–5.61)	1.52 (1.42–1.63)	1.39 (0.83–2.20)	7.00 (6.80–7.22)	6.23 (4.95–7.67)

*$p < 0.05$, 2-tailed.

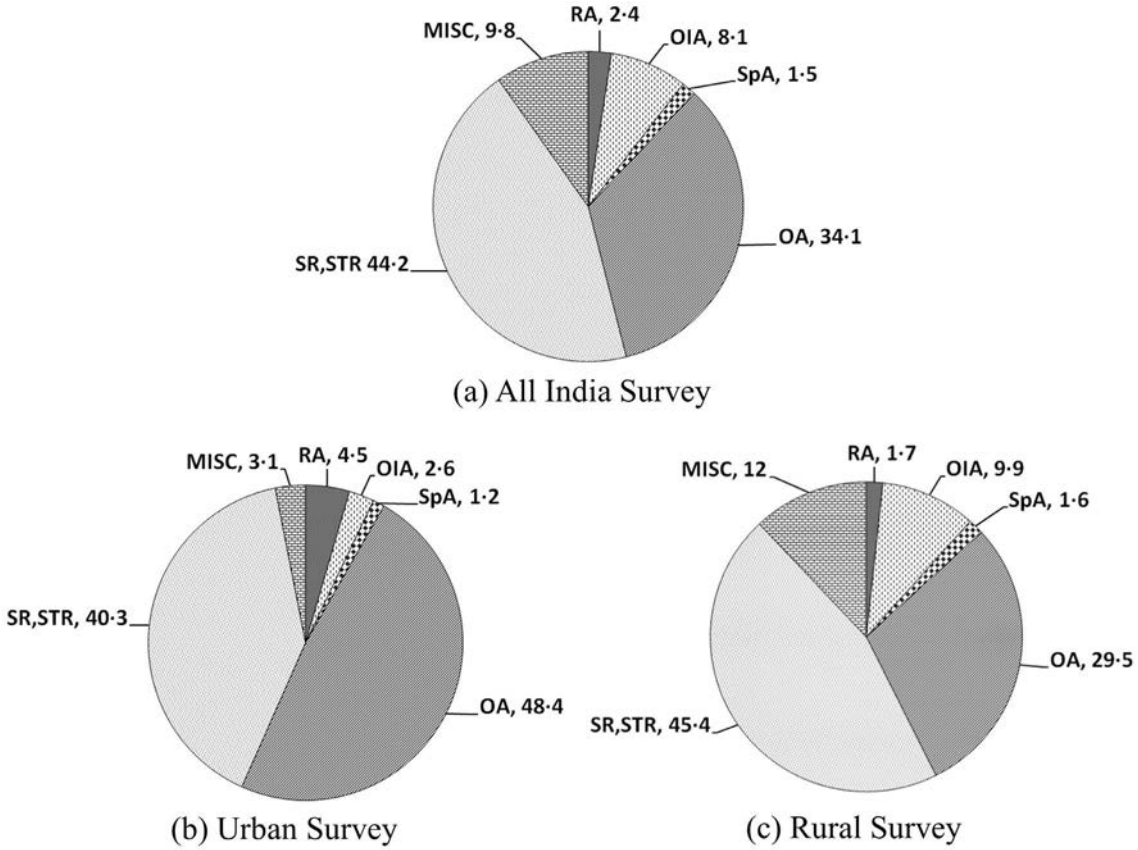

FIGURE 2 | Distribution of broadly classified musculoskeletal and rheumatic disorder in the study cohort: All India, Rural and Urban survey. Misc, miscellaneous; OA, osteoarthritis; OIA, other inflammatory arthritis; RA, rheumatoid arthritis; SpA, spondyloarthritis; SR, symptom related; STR, soft tissue rheumatism.

populations. The adjusted prevalence of back pain (any site; Table 2) was 6.23 (4.95–7.67); higher in the rural population (7.97 vs. 3.67).

4.1 | Phase 3

Figure 2 shows the proportion (percent) of broadly classified diagnosis categories. The pattern was comparable in rural and urban populations. Ill-defined SR disorders/STR were the predominant group (44.2%): urban 40.3%, rural 45.4%. OA was classified in 34.1% of subjects (urban 48.4%, rural 29.5%). Inflammatory arthritis (RA, SpA, OIA) was classified in 12% of subjects (urban 8.3%, rural 13.2%).

A detailed breakup of OIA and the miscellaneous groups (Figure 2) was shown (Table S2). The latter included a large proportion of undifferentiated forms of inflammatory arthritis and which was several fold higher in the rural population. A substantial proportion of post-Chikungunya arthritis (Calicut and Thiruvanthapuram survey sites) was also included.

Table 2 shows the prevalence of rheumatic disorders. The adjusted prevalence was RA 0.34% (0.08–0.79), IA-U 0.22% (0.05–0.68), SpA 0.23% (0.05–0.68), OA 4.39% (3.30–5.61) and STR 1.39% (0.83–2.20); prevalence of OA Knees was 3.34% (2.43–4.47).

The adjusted prevalence of RA varied from 0.16% in rural Calicut to 0.72% in urban Bikaner; 0.42% in the urban and 0.29% in the rural population. 60.9% of RA cases were seropositive for rheumatoid factor (Rural 50%, Urban 68.5%). The male: female ratio was 1:5. The adjusted prevalence (per 100 000 population) of RA in women was 263, 967, and 826, respectively, in the age bands 16–34, 35–54, and 55 years plus; in the age band 25–44 years, the prevalence was 714 in rural and 640 in urban India.

The adjusted prevalence of SpA varied from 0.03% in rural Delhi to 0.54% in rural Calicut.

The adjusted prevalence of OA varied widely from 2.51% in rural Guwahati to 11.23% in rural Delhi: 3.82% urban and 4.66% rural population.

A high prevalence of STR was shown in the rural sites in Kolkata (3.37%), Calicut (2.14%) and Thiruvananthapuram (3.43%).

The adjusted point prevalence of several other MSK disorders is shown in Table S3—gout 0.05% (0.04–0.07), AS 0.03% (0.02–0.05), and PsA 0.01% (0.00–0.02).

4.2 | Risk Factors

Table 3 shows the odds ratio (OR) of the selected risk factors of MSK pain in a univariate and Multiple logistic regression

TABLE 3 | Risk factor analysis of MSK pain using selected variable.

Risk factor variable	Respondent with exposure %	Non respondent with exposure %	Univariate analysis	Multivariate logistic regression	
			OR (95% CI)	OR (95% CI)	Role of the 'predictor'
Age	—	—	—	1.03 (1.03, 1.03)*	Risk
Female gender	63.1	47.0	1.93 (1.85, 2.01)	2.12 (2.00, 2.24)*	Risk
Literacy	73.8	83.4	0.56 (0.53, 0.59)	1.05 (0.98, 1.13)	—
Non-Vegetarian Diet	72.5	65.7	1.38 (1.31, 1.45)	1.14 (1.33, 1.50)*	Risk
Farm work	23.5	16.4	1.56 (1.46, 1.67)	—	—
Heavy nature of work	17.5	16.2	1.10 (1.03, 1.17)	1.01 (0.93, 1.09)	—
Chronic-illness (HTN, Diabetes, Heart, Paralysis)	16.8	9.7	1.87 (1.73, 2.02)	1.91 (1.73, 2.10)*	Risk
Trauma	6.4	1.8	3.77 (3.16, 4.49)	—	Risk
Alcohol-ever	6.7	7.1	0.94 (0.86, 1.02)	0.85 (0.76, 0.95)*	Protective
Tobacco any form-ever	25.1	18.0	1.53 (1.45, 1.61)	1.34 (1.23, 1.42)*	Risk

*Significant $p < 0.05$.

analysis. Female gender, poor literacy (unable to read and write), heavy work, chronic non-MSK illnesses (diabetes, hypertension, ischemic heart disease and stroke), past trauma, and tobacco use were significant adverse risk factors; vegetarian diet and alcohol seemed protective. Some form of tobacco was used by 19.5% of the study population and 25.1% of MSK respondents, as described in Table S4. The highest OR (4.33, 95% CI 3.42–5.48) of tobacco use was among urban women respondents; correspondingly, urban men respondents had 2.61, rural men population 1.77, and rural women population 1.48.

5 | Discussion

The current multisite COPCORD India survey (Figure 1) of 56 548 population sample showed a strikingly high point prevalence (Tables 1 and 2, Figure 2) of MSK pain and arthritis. Non-specific MSK pains (NSA and STR) and OA were the most common disorders in the community. Twelve percent of respondents suffered from RA and other inflammatory arthritis including Post Chikungunya arthritis and rheumatism (PCAR). Several preventable/modifiable risk factors such as tobacco use, poor literacy, heavy manual work, and certain chronic diseases (e.g., diabetes, hypertension) were identified.

Based on the current study, prevalence rates and the last India census in 2010 (1.2 billion 195.29 million people (127.13 million women) or roughly one-sixth of the India population suffer from MSK pain and several million from various arthritis (Table 2)—4.22 million RA, 54.44 million OA, and 17.24 million STR. Notably, even low prevalence rates (e.g., gout and AS) in the current study would mean a relatively large disease burden. The new India census (delayed by the COVID pandemic) has not yet begun.

COPCORD was designed as a 'total study of the population' and meant to reveal 'any hidden reservoir of rheumatic complaints' [3]. Towards this end, COPCORD surveys have served well but remain unrecognized (Table 4) [6, 10–12, 15–38]. In addition to specific disorders COPCORD India surveys have consistently demonstrated a huge burden of non-specific MSK soft tissue and joint pains and IA-U.

Chronic MSK pain was estimated to affect 20%–33% of the global population [39]. MSK pain in the current study was assessed in a sizable population sample (non-random) at each study site. This was a challenging task, and MSK pain was likely to be confounded by several factors, including individual perceptions and recall bias [40, 41]. We used experienced and validated COPCORD methods [5, 6, 10]. MSK pain was the dominant self-reported ailment in the community in the current study as compared to other ailments (data not shown) and was similar to earlier COPCORD India surveys [6, 10, 11]. Non-specific MSK pain (and STR) also impacted function (as measured by the Health Assessment Questionnaire) adversely [6, 10, 11]. In our long-drawn experience (COPCORD and community practice), MSK pain was mostly self-managed, and only those with moderate to severe intensity and experiencing difficulty in daily living and livelihood seek organized medical care [6, 7, 40]. MSK pain and arthritis were often neglected in the community and primary care setting [6, 10, 38, 40]. Undoubtedly, more research is required to understand the nuances of MSK pain in the community. Meanwhile, education and awareness need to be imparted to doctors and the community. The emphasis should be on the first medical post

TABLE 4 | Prevalence rates reported by selected COPCORD survey across the World.

Country	Publication year	Sample size	MSK pain	RA	OA	STR	FM	GOUT	SpA	AS	Back pain
Southeast Asia											
India (R)—Current study	Current	34096	20.04	0.29	4.66	1.75	NR	0.07	0.28	0.04	7.97
India (U)—Current study	Current	22452	10.34	0.42	3.83	0.72	NR	0.02	0.12	0.03	3.67
All India (U + R)—Current study	Current	56548	16.14	0.34	4.39	1.39	NR	0.05	0.23	0.03	6.23
India—Bhigwan (R) [6, 10]	2001	4092	17.9	0.67	5·8	3.2	0	0.1	0.25	0.09	13.1
India—Lucknow (R) [15]	2018	5118	15.1	0.16	4.5	NR	4.12	0	0	0	10.66
India—Lucknow (U) [15]	2018	5053	34.1	0.47	10.6	NR	3.24	0.09	0	0	3.65
Bangladesh (R) [16]	2005	2601	26.2	0.7	7·5	2.7	4.4	0	NR	0.04	20.1
Bangladesh (U) [16]	2005	1307	24.9	0.4	9.2	2.5	3.2	0	NR	0.08	18.1
Malaysia (SR) [17]	2007	2594	21.1	0.15	2.97	NR	0.92	NR	NR	0.15	11.6
Vietnam (U) [18]	2003	2119	14.9	0.28	4.1	3.4	NR	0.14	NR	NR	11.2
West Asia											
Iran—Tehran (U) [19]	2008	10291	41.9	0.33	16.6	4.6	0.69	0.13	0.23	0.12	13.4
Iran—Tuyserkan (R) [20]	2009	1565	66.6	0.19	20.5	2.2	0.06	NR	1.1	1.1	23.4
Kuwait (U) [21]	2004	7670	26.8	0.04	0.99	1.69	0.13	0.03	NR	0.01	9.48
Lebanon (R) [22]	2012	3530	32.9	1	4	5.8	1	0.01	0.3	0.1	3.0
East Asia											
China—Shanghai (U) [23]	2003	6584	13.3	0.28	4.1	3.4	NR	0.22	0.11	0.11	5.6
Australia											
Australia Aboriginal (R) [24]	2004	847	33	0	5.5	7.4	0.11	3.8	0.5	0.5	12.5
Africa											
DR Congo (R) [25]	2017	1500	49.5	1.4	36.8	5.2	NR	0.06	3.8	3.8	13.4
Nigeria (PU) [26]	2022	3056	58	0.03	6.1	1.7	0.6	0.3	NR	NR	3.1
Latin America											
Argentina—Rosario (U) [27]	2016	1656	31.2	2.4	4	2.9	0.1	0	NR	NR	19.3
Brazil (U) [28]	2004	3038	30.9	0.46	4.14	NR	2.5	NR	NR	NR	NR
Mexico (U + R) [29]	2011	19213	25.5	1.49	10.24	3.8	0.68	0.35	NR	0.15	5.8
Colombia (U) [30]	2018	6693	48	1.49	10.81	NR	0.72	0.56	0.28	0.11	7.24
Cuba (U) [31]	2009	3155	43.9	1.24	20.4	6.4	0.22	0.38	0.19	0.1	11.6
Ecuador—Cuenca (U + R) [32]	2016	4877	32.5	0.8	9.8	NR	2	0.4	NR	0.08	9.3
Ecuador—Saraguro (R) [33]	2020	2687	46.3	1.3	6.5	5.8	1.8	0.01	NR	0.03	9.3
Guatemala—San Juan (R) [34]	2012	4000	14.45	0.85	30.95	2.13	NR	0.03	NR	NR	0.48
Guatemala—City (U) [34]	2012	4000	9.3	0.53	1.65	1	NR	NR	NR	NR	0.50
Peru (U) [35]	2018	1095	31.7	1.27	4.75	8.86	1.09	0.64	0	0	6.76
Venezuela (U) [36]	2015	3973	22.4	0.4	15	NR	0.2	0.3	0.1	0.05	2.8
Venezuela (R) [37]	2016	1537	32.9	1.1	14.1	NR	0.5	0.3	0.4	0.05	12.4

Abbreviations: AS: ankylosing spondylitis; FM: fibromyalgia; NR: prevalence rates are not reported in publication; OA: osteoarthritis; PU: peri-urban; R: rural; RA: rheumatoid arthritis; SpA: spondyloarthritis; SR: semi-rural; STR: soft tissue rheumatism; U: urban.

(primary care physicians) and the rheumatologists ought to be more proactive.

Although limited by the screening questionnaire, the current study assessed several risk factors of MSK pain (Table 3). Several risk factors such as lack of literacy and tobacco use were modifiable and amenable to change in the future prevention program (COPCORD Stage 3). The current findings (risk factors) were consistent with the earlier COPCORD and GBD reports [2, 4, 6, 10]. However, the supposedly protective roles of vegetarian diet and alcohol (Table 3) need validation and further research. Several other risk factors (MSK pain) such as genetics, environment (including infections), socioeconomics, and individual personality traits ought to be assessed.

It seems reasonable to compare the point prevalence rates of different COPCORD surveys and explore the background information of the population and survey site; rates may not be standardized (Table 4). A uniform standardized community approach and a validated CCQ were used by COPCORD surveys (Table 4) [3, 5, 10, 38]. COPCORD Mexico showed good validation (sensitivity and specificity for specific arthritis) of the CCQ using current MSK pain as the primary variable in a very large population sample [42]. Although not random, COPCORD site/population selection was driven by epidemiology concerns (such as sample size, valid residence, community participation, and a good response rate (> 70%)) [5, 10].

Comparing COPCORD India Surveys (Tables 2 and 4)-Additional Insights: The COPCORD Bhigwan (1996) was followed by COPCORD Lucknow. Altogether, 22 COPCORD surveys were completed from 1996 to 2012. There were 13 sites in the current study (Figure 1) [5, 6, 10–12, 15]. An independent randomized survey (30 000 population in urban–rural sites in 3 regions) based on the COPCORD Bhigwan model was sponsored by the Indian Council of Medical Research (ICMR) and supervised and coordinated by the first author (AC; not published).

The adjusted prevalence of RA in the maiden rural COPCORD Bhigwan (0.67) was reported to be strikingly high. However, a lower rate was found in the subsequent COPCORD Lucknow and evident in the current India study (Table 2). A substantial number of young women seemed to suffer from RA, and this was considered unique to the Indian population. Data from COPCORD India Bhigwan showed a high prevalence (876/100 000 population) of RA in the young women (age band 15–44 years); some corresponding rates reported were USA 308, Norway 150, China 237, South Africa 1042 [8]. A similar RA trend was also observed in the current study (see results). This warrants public health intervention and elaborate research. We speculate that the cause may be related to a gene–environment interaction.

The adjusted prevalence of OA in the current study sites ranged from 2.5% to 5.24% (except higher in the Delhi site) which was less compared to Bhigwan and Lucknow surveys. Substantial IA-U was reported from several current sites (especially rural) and Bhigwan but was not reported in the Lucknow study. The prevalence of SpA/AS in rural (0.28) and urban (0.12) India was lower than that in Bhigwan (≤ 0.3) and not reported from Lucknow. Interestingly, a high prevalence of SpA (0.5) was reported from the two sites in South India in the current study, which were surveyed 18 months after the Chikungunya epidemic [11, 12]. The prevalence of gout was uniformly low (≤ 0.15%.) in COPCORD India surveys.

In the ICMR randomized COPCORD study, the crude prevalence of RA, OA, and gout was 0.17–0.62, 3.28–6.5, and 0.03–0.13, respectively (unpublished). Intriguingly, the latter results were not remarkably different from those of the current non-randomized population study.

NSA, STR, and OA were the predominant disorders in the current study (Figure 2) and were much higher in the rural sites, similar to the COPCORD Bhigwan survey. The latter was in sharp contrast to COPCORD Lucknow. The distinction between urban and rural regions is an important consideration in public health in India [10, 40].

IA-U was a heterogeneous group of inflammatory arthritis that could not be diagnosed distinctly as RA or SpA, or PsA. Several cases (IA-U) were believed to be post-infective, and in recent times, PCAR seems to have increased the burden [10, 43, 44]. The current study surveys in Thiruvendran and Calicut (Figure 2) were carried out about 18–24 months after the epidemic and captured a high prevalence and disability (DALY) of PCAR (standardized prevalence 1.17 (0.67, 1.97)) [11, 12, 45]. Several cases of IA-U mimicked seronegative RA and were an important diagnostic dilemma [44].

COPCORD Bhigwan reported an unusually high consumption of tobacco, both in the population and MSK respondents, and it was an important risk factor [6, 40]. The latter was also evident at all current sites (data not shown) and in the pooled current data (Table 3); more in rural sites (24.2% vs. 10.4% urban, Table S4). Several other risk factors in the current study (Table 3) such as female gender, trauma, illiteracy, heavy manual work, and co-morbidity, were also reported in the Bhigwan survey. This encourages a more focused program of prevention in rural India.

Although the current study population and site selection were not randomized, a large population sample from several sites distributed all over India (in no order) was likely to address the issues connected with diversity and representation of the Indian population. India is well known for multifaceted diversity and in particularly related to ethnicity, culture and traditions, and socioeconomics. The rapid modernization and migration of people from villages to towns and cities are bound to influence the epidemiology of MSK and several other diseases. The previous COPCORD India studies (Bhigwan and Lucknow) were based on single-site survey, which cannot be representative of the Indian population. All in all, the current study is likely to be more contemporaneous and a better reflection of the national landscape of MSK pain and arthritis.

5.1 | Comparing Selected Global COPCORD Surveys (Table 4)

Country data (classified into geographical regions) was shown for eyeball comparison, and the reader is encouraged to assess

the country-specific publication for more definitive information [3, 6, 10–12, 16–37]. Several publications prior to 2000 were not included (Philippines, China, Pakistan, Egypt, Indonesia and Australia) and may be accessed on the website [5]. The MSK pain rate in West Asia, Africa, and Latin America seemed higher than in Southeast Asia and China. The prevalence of RA was mostly less than 1% except for Lebanon, DR Congo, Mexico, Colombia, Cuba, Peru, and Argentina. Intriguingly, RA was not reported by the Australian Aboriginal survey. The prevalence of symptomatic OA was strikingly high in Iran (16.6%–20.5%), rural DR Congo (36.8%) and rural Guatemala (30.95%); elsewhere, it was much less. The rate for STR exceeded 10% in several countries. Few COPCORD surveys classified FM, and a prevalence > 1% was reported from India (Lucknow site), Lebanon, Australian Aborigines, DR Congo, Ecuador, and Peru. The prevalence of gout was exceptionally high in the Australian Aboriginals (3.8%) and much less in Asian and other COPCORD surveys; some Latin American countries showed prevalence of 0.3%–0.65%.

Several factors were likely to contribute to the variation in the prevalence of MSK pain and arthritis among COPCORD surveys. The brief description about diversity in India (see above) was also relevant to several developing countries and ancient and ethnic civilizations.

5.2 | Selected European MSK Surveys

Unlike COPCORD, these surveys were mostly retrospective and used electronic data based on different resources such as medical/insurance records, tertiary care hospitals, telephonic surveys, and registries [39, 46–48]. They were selective in nature (target disorders) and some were self-reported. However, except for neck and back pains, several other common community ailments were not identified, as shown in COPCORD surveys (Table 4).

Almost one-third (32%) respondents (more women) reported current MSK pain with wide variation (e.g., Ireland 18% and Finland 44%) [48]. On superficial comparison, most of the European rates seemed higher than those reported by COPCORD surveys (Table 4). 31.9% of adults (≥ 35 years) reported high-impact non-inflammatory chronic MSK pain [47]. The latter was higher in people of African and Asian ethnicity compared to white residents, but the difference was considerably reduced when data was adjusted for income, occupation, and adverse life events [46].

The recent comprehensive Europe MSK Health report (v5) described a wide spectrum of MSK pain and arthritis with a striking variation in prevalence rates like that shown by the COPCORD surveys (Table 4). The RA prevalence varied from 0.2% in North France to 0.66% in South France and was reported nationwide as 0.31%, which otherwise seemed unusually low. The RA prevalence varied from 0.3% (Italy) and 0.66% (Spain) to 0.83% (UK) and 3.14% (The Netherlands). The reason for such a remarkable difference is not clear. A similar situation existed for symptomatic knee OA (Italy 5.39%–29.8%, UK 6.5%, Spain 40.39%). Symptomatic OA hip showed a high prevalence in several European countries but was infrequently reported in COPCORD surveys [10]. The lesser prevalence in the latter Indian survey was speculated to be due to traditional lifestyles of squatting and sitting cross-legged. The prevalence of gout was high in several countries and was in sharp contrast to that shown by COPCORD surveys (except Australia Aboriginals).

GBD: Based on the GBD data, chronic pain and disability was reported as greater in developing countries compared to developed countries [1, 49]. This may not be entirely true. GBD does not obtain chronic MSK pain in totality for analysis as done in the COPCORD (Table 4) [50]. Except for neck and back pain, GBD primarily targets RA, OA, and gout, and everything else was categorized as 'Other MSK' [1, 49, 50]. It is prudent to note that although providing invaluable global MSK disease burden estimates, a major limitation of the GBD project is the heterogeneity and lack of verification of the source data [1, 2, 4, 50, 51].

Ever since inception, the focus of WHO-GBD was on incidence, disability (DALY) and mortality [52]. COPCORD surveys provide prevalence data, and several (in particular India) have used HAQ to record functional disability (Table 4). Recently, the WHO and GBD reports described a shift towards prevalence data (rather than incidence) which is much more meaningful to health policy makers [52]. Some COPCORD data was used recently by the GBD project [4, 50, 51]. Overall, despite being a major source of MSK data in the developing countries, COPCORD remains under-recognized and neglected for global relevance and use [52, 53].

'Other MSK' has been listed amongst the top 20 contributors to global daily and the incidence increased by 123.4% during the period 2009–2019 [50, 51]. Currently, "Other MSK" is a heterogeneous category lacking any meaningful clinical or otherwise description. The emerging huge burden of PCAR in Asian and Latin American countries ought to be quickly recognized and addressed suitably or else it will be another 'Other MSK' (GBD) and escape appropriate attention and addressal [43, 45, 54]. In fact, the method for calculation (Other MSK) was indirect and somewhat uncertain [50]. The latter report stated that in view of the lack of comprehensiveness and quality, the MSK data may be an underestimate. The authors also expressed concern about resetting the GBD analytic process for 'Other MSK' outcome [50]. All in all, this is indeed worrisome from a rheumatology and public health perspective.

5.3 | Other Limitations

Several non-standardized clinical descriptives were used to classify MSK disorders. Although rheumatologists examined every respondent, the lack of laboratory and radiology investigations was often an impediment to making a definite diagnosis in difficult clinical situations such as early arthritis or ill-defined MSK pain. Community surveys and data collection was likely to be biased in several ways—observer and information. There were several unexpected delays due to problematic logistics, deficient skilled manpower, lack of funds, and unanticipated encounters with the Chikungunya epidemic (2006–2009) and Covid pandemic (2020–2022). This was a "prevalence rate" study and we did not calculate the DALY burden except in the case of PCAR [12]. Admittedly, COPCORD was not meant to measure

uncommon rheumatic disorders such as lupus and dermatomyositis, and childhood onset arthritis.

5.4 | Future Direction

There is an urgent need to enhance the COPCORD website and establish an online data repository [54]. The current author (AC) is aware of the ongoing efforts by the APLAR towards the latter project. Wigley et al. (2009) opined on the likelihood of improvement in the standard of living in developing countries if rheumatic diseases are well controlled and proposed that a socioeconomic evaluation (COPCORD Stages II and III) be carried out of the ongoing COPCORD India Bhigwan [55]. A 25-year COPCORD Bhigwan resurvey was completed in 2022–2023 and preliminary data were presented in the APLAR Congress 2023 (unpublished).

All in all, the current COPCORD study outcome remains relevant to the current Indian context of MSK pain and arthritis. Perhaps it is also an incentive for developing countries.

6 | Conclusion

This WHO COPCORD population survey demonstrated a high prevalence of MSK pain and arthritis in the Indian population, and particularly in the rural sites. A wide spectrum of MSK disorders, including the recently recognized post-Chikungunya pain and arthritis, was described. Several risk factors of MSK pain were reported, and several, such as tobacco use, were potentially modifiable. Non-specific arthralgias, soft-tissue rheumatism, and OA were the dominant community ailments, although the burden of inflammatory arthritis was substantial. An alarming proportion of RA was described in young women. Our study calls upon the Government of India to launch a national control program for MSK pain and arthritis as a priority.

Author Contributions

Concept and Plan: A.C., A.V., M.S., K.M. First Draft: A.C., S.S., G.R.P., K.M. Draft revisions and Final Draft: A.C., A.J.M., R.H., A.V., M.S., K.M. Data input and site revisions: V.L.-J., L.G., R.A., B.P., D.K., S.P., C.S., T.B., A.M., R.S., A.G., K.T., C.P.R. All the authors read and approved the final draft (current revision). The authors of the first and final drafts vouch for the veracity and correctness of the data and other references used in the preparation of the manuscript.

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Disclosure

Role of the Funding Source: The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Role of Public and Study Participants: No member of the public or any of the study survey resident participants was ever involved in the current study at any stage—planning, protocol, surveys, data processing and analysis, report writing, and manuscript submission for publication.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The electronic database of the current study data is available for conditional access at the Centre for Rheumatic Diseases, Pune, India. However, a personal application can be made to the corresponding author of the current paper for further processing of the request to access the latter database. The application should be accompanied by the CV, current and past 5-year employability, and the purpose of data access. The study database is not available for any commercial use.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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LETTER TO THE EDITOR

A Study of the Diagnostic Value of Doppler Ultrasound Score in a Single Wrist Joint to Assess the Activity of Rheumatoid Arthritis

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Dear Editor,

Rheumatoid arthritis (RA) is a generalized, chronic inflammatory disease involving multiple joints, characterized by joint swelling, tenderness, tendonitis, tenosynovitis, bone destruction, and it usually affects the surrounding joints, mainly including the small joints of the hands and feet [1]. The 2018 Chinese Guidelines for the Diagnosis and Treatment of Rheumatoid Arthritis recommend the clinical use of 28 disease activity scores in 28 joints (DAS28) to evaluate patients' mobility [2]. Ultrasound, on the other hand, is more sensitive and reproducible than clinical assessment of joint inflammation [3]. Power Doppler ultrasound (PDUS) can capture the changes in blood flow signals, and on the basis of gray scale ultrasound (GSUS), determine whether there is inflammation at the hyperplasia site, and can diagnose RA more accurately and early through the joint examination of the wrist joint by GSUS and PDUS [4]. Nowadays, many studies at home and abroad use multi-joint ultrasound scores to diagnose and assess RA mobility, but the establishment of single-wrist ultrasound score as a standard for evaluating RA mobility is rarely reported. This study was approved by the Research Institution Review Committee of the First People's Hospital of Nantong, and the subjects voluntarily participated in the clinical study and signed the informed consent form.

RA patients who were admitted to the Department of Rheumatology and Immunology of Nantong First People's Hospital from 2021 to 2022 were randomly selected, a total of 100 RA patients were enrolled and divided into two groups based on disease activity: low/medium mobility and high mobility. Musculoskeletal ultrasonography was performed by using the French acoustic AIXPLORER ultrasound instrument and probe SL15-6 (frequency 6~15 MHz). Scan the mid-wrist, radial, and ulnar wrist of both wrist joints for diagnosis of synovitis, and scan the volar wrist flexor carpi radialis tendon, flexor pollicis longus tendon, flexor pollicis longus tendon, and dorsal carpal 1st to 6th chamber tendon for tendonization/tenosynovitis. Scan of the single wrist joint with an overall score, bone erosion, and joint effusion. The operator took pictures of all the scanned parts, and two sonographers with 5 years of experience in musculoskeletal imaging diagnosis could not obtain other clinical indicators of the patient, and the semi-quantitative score of the more severe lateral wrist joint under ultrasound conditions was completed. Synovitis (S) and tendon/tenosynovitis (T) were assessed with gray-scale score (GS) and energy Doppler score (PD), respectively, and bone erosion and joint effusion were assessed with GS. The Verio 3.0T high-field magnetic resonance imaging instrument of Siemens was used to perform MR noncontrast scan + enhanced examination on the wrist joint. The wrist with the higher VAS score was scanned by the VAS score [1]. The MR noncontrast scanning sequence selected the coronal turbo spin echo (TSE) and fat suppression (FS). TI-TSE sequence: repetition time (TR) 500 ms; echo time (TE) 24 ms; Layer thickness 2 mm; Layer spacing 2 mm; Field of view (FOV): 180 mm × 180 mm. T2-de3d sequence: TR 12.79 ms; TE 4.57 ms； Layer thickness 0.5 mm; Layer

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spacing 0 mm; FOV 180 mm × 180 mm. Axial Pd-FS sequence: TR 2500 ms; TE 9.8 ms； Layer thickness 2 mm; Layer spacing 2 mm; FOV 120 mm × 120 mm [2]. MR enhancement was injected with a dose of 0.2 mL/kg and a flow rate of 2.0 mL/s Gadolinium glumethylamine acetate (Gd-DTPA), and the injection site was the elbow vein, and the coronal T1-vibe-FS image was obtained. T1-vibe-FS sequence: TR 14.7 ms; TE 6.06 ms； Layer thickness 0.5 mm; Layer spacing 0 mm; FOV 180 mm × 180 mm. Two radiologists with 5 years of experience in RA osteoarthritic imaging will perform a semi-quantitative score of the patient's magnetic resonance images, and if there is a disagreement, the agreement will be completed. Scored according to the RAMRIS system criteria [5], synovitis scores for the distal ulnar-radial, radial wrist, and interosseous-metacarpophalangeal joints of the wrist; Bone marrow edema and bone erosion scores were performed on the distal ulna, distal radius, base of metacarpal bone, and carpal bone, and the scores were added to obtain RAMRIS. The RA uni-wrist ultrasound score, unilateral hand and wrist ultrasound score, and RAMRIS were consistent among the two observers, and the ICC values were 0.8, 0.8, and 0.7, respectively ($p < 0.001$).

In our study, including 47 in the high activity group (DAS 28 > 5.1) and 53 in the low and intermediate activity group (DAS-28 ≤ 5.1). The incidence of RA synovitis was 58 cases, and the incidence rate was 58%, among which the midline of the wrist was the most common (45/100, 45%), and the radial side of the wrist (30/100, 30%) and the ulnar side of the wrist (29/100, 20%) were the most common incidences. There were 48 cases of tendon/tenosynovitis, with an incidence rate of 48%, among which the extensor tendon of the carpi radialis was the most common (16/100, 16%), followed by the flexor tendon of the third finger (15/100, 15%) and the flexor tendon of the second finger (14/100, 14%). There were 7 cases of bone erosion, with an incidence rate of 7%; There were 2 cases of joint effusion, with an incidence rate of 2%. There were statistically significant differences between the synovitis scores, mid-wrist synovitis, wrist radial synovitis, wrist ulnar synovitis, tendon/tenosynovitis scores, 3rd finger flexor tendon tenosynovitis, 4th finger flexor tendon tenosynovitis, extensor carpi radialis breviferum tendon and extensor carpi radialis longus tenosynovitis, and total ultrasound scores, as shown in Table 1. The ultrasound performance of the RA high activity group is shown in Figure 1. The RA patient had a CRP of 55.77 mg/L, an ESR of 53 mm/h, five painful joints, five swollen joints, a DAS28 score of 5.5, synovitis, tenosynovitis, and bone erosion in the ultrasound image of the patient, and a single-wrist ultrasound score of 14. The synovium and tendon of the wrist joint of RA patients were examined by GSUS, PDUS, and magnetic resonance imaging, respectively, and the differences of S, T, GS, PD, total ultrasound score, synovitis score (RAMRIS), bone marrow edema score (RAMRIS), bone erosion

TABLE 1 | Comparison of ultrasound image characteristics between the RA high-mobility group and the low and intermediate mobility group.

Image features	Medium to low activity group	High mobility group	*Z*	*p*
Synovitis score (S) (points)	1 (0, 2)	6 (0, 11)	−4.609	< 0.001[a]
Midline of the wrist	0 (0, 2)	2 (0, 4)	−3.037	0.002[a]
Radial side of the wrist	0 (0, 0)	2 (0, 4)	−5.018	< 0.001[a]
Ulnar side of the wrist	0 (0, 0)	2 (0, 4)	−5.212	< 0.001[a]
Tendon/tenosynovitis score (T) (points)	0 (0, 2)	2 (0, 6)	−2.985	0.003[a]
Wrist flexor tendons	0 (0, 0)	0 (0, 0)	−1.032	0.302
Flexor pollicis longus tendon	0	0 (0, 0)	−1.509	0.131
Flexor tendon of the 2nd finger	0 (0, 0)	0 (0, 0)	−1.012	0.311
Flexor tendon of the 3rd finger	0 (0, 0)	0 (0, 1)	−2.788	0.005[a]
Flexor tendon of the 4th finger	0 (0, 0)	0 (0, 1)	−2.907	0.004[a]
Flexor tendon of the 5th finger	0 (0, 0)	0 (0, 0)	−1.546	0.122
Extensor pollicis brevis tendon and abductor pollicis longus tendon	0 (0, 0)	0 (0, 0)	−0.151	0.880
Extensor carpi radialis brevis tendon and extensor carpi radialis longus tendon	0 (0, 0)	0 (0, 1)	−2.137	0.033[a]
Extensor pollicis longus tendon	0 (0, 0)	0 (0, 0)	−0.666	0.505
Extensor tendons of the index fingers and extensor tendons of the fingers	0 (0, 0)	0 (0, 0)	−0.984	0.325
Ulnar carpi extensor tendon	0 (0, 0)	0 (0, 0)	−1.661	0.097
Bone erosion score (score)	0 (0, 0)	0 (0, 0)	−1.288	0.198
Joint effusion score (points)	0 (0, 0)	0 (0, 0)	−0.100	0.921

[a]$p < 0.05$ was statistically significant.

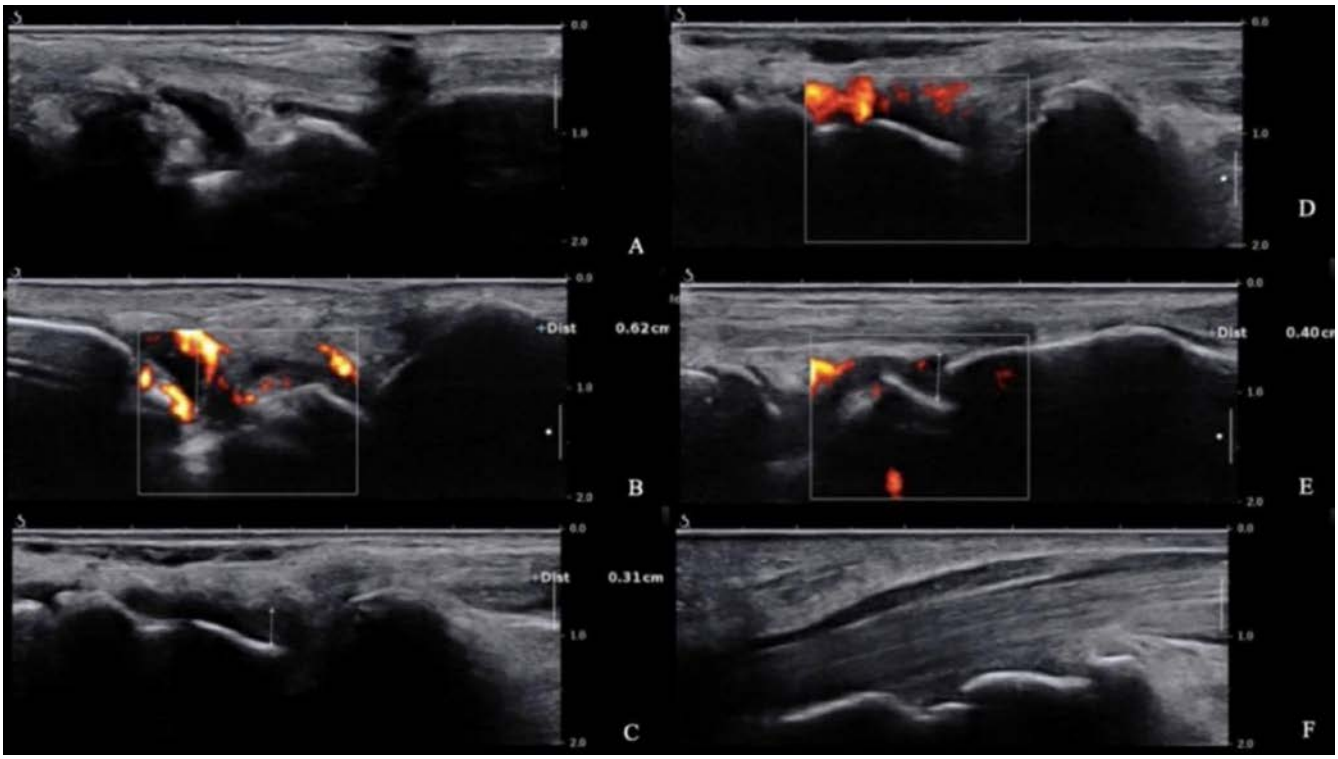

FIGURE 1 | Male, 72-year-old with confirmed high-mobility RA.

score (RAMRIS) and RAMRIS between the two groups were statistically significant ($p < 0.05$). The total ultrasound score, S score, T score, GS score, and PD score of RA were positively correlated with DAS28, and the correlation was statistically significant (rho = 0.447, 0.404, 0.221, 0.391, 0.461, $p < 0.05$). Within the ultrasound scores, the S score was positively correlated with the GS score and PD score (rho = 0.564, 0.822), the T score was positively correlated with the GS score and PD score (rho = 0.816, 0.550), and the GS score was positively correlated with the PD score (rho = 0.783), and the correlation was statistically significant. The DAS28 score of RA was positively correlated with wrist RAMRIS (rho = 482, $p < 0.001$). The area under the ROC curve of the unilateral wrist ultrasound score and the unilateral hand and wrist ultrasound score to determine different RA mobility and 95% CI were 0.814 (0.724–0.903) and 0.842 (0.762–0.921), respectively, and there was no significant difference between them ($p = 0.396$). The single wrist joint ultrasound score and RAMRIS areas under the ROC curve were 0.814 and 0.852, respectively, and there was no significant difference between them ($p = 0.513$).

RA is characterized by persistent synovitis [6], Synovial inflammation can be used as an indicator or predictor of systemic inflammatory response [7]. In this study, it was found that the incidence of synovitis is higher than that of tenosynovitis, bone erosion, and joint effusion, and the incidence of tendon/tenosynovitis is lower in the low- to medium-active group (37%). This suggests that the use of ultrasound to assess synovial inflammation and assess disease activity is of important clinical value. In this study, the diagnostic performance of ultrasound scores and RAMRIS in assessing the mobility of RA patients was compared, and the area under the ROC curve of single wrist ultrasound score and RAMRIS was 0.814 and 0.852, respectively, and the diagnostic performance of RAMRIS was higher, and there was no significant difference between the two. Although the diagnostic power of the two scores is similar, the composition of the scores is different; the wrist ultrasound score is composed of synovitis, tenosynovitis, bone erosion, and joint effusion and is quantitatively counted for tendon/tenosynovitis, while RAMRIS is the sum of synovitis, bone erosion, and bone marrow edema scores, and only qualitative statistics are used for tendon/tenosynovitis. On the one hand, ultrasound is cheap, real-time, and convenient, while magnetic resonance is expensive and less available. On the other hand, bone marrow edema is a reliable predictor of arthritic disease progression [8], bone marrow edema cannot be detected by ultrasound, whereas magnetic resonance imaging is sensitive in assessing joint inflammation and can detect bone marrow edema [9]. Due to the difference in imaging ability between the two and the different focus of detection, ultrasound cannot be used to completely replace MRI, so the two can be combined, and their respective advantages can be used to diagnose ultrasound and magnetic resonance when conditions permit, so as to obtain better disease evaluation results. In this study, we also compared the single wrist score, unilateral hand, and wrist scores, and there was no significant difference between them, with an area under the ROC curve of 0.814 and 0.842, respectively ($p = 0.396$), which further confirmed the possibility of reducing the hand joint score, shortening the examination time without reducing the diagnostic rate of mobility. DAS28 can be affected by external factors such as environment and psychology, and while DAS28 does not include imaging assessments, ultrasound provides a visual picture of disease progression and is more meaningful than laboratory results. Patients with RA often undergo ultrasound of both hands and wrists, and the vast majority of RA ultrasound scores are multi-articular; for example, Nam J et al. successfully predicted the progression of RA using a US32-joint protocol

[10]. In this study, considering the cumbersomeness of US examination of multiple joints, we performed US examination of both wrists and selected unilateral wrist joints with more severe US images for scoring, which simplified the ultrasound scoring and saved the time and cost of examination of PIP and MCP in both hands. Disease activity can be assessed more rapidly, and screening for active RA can be facilitated.

Our study also has certain limitations. First, the sample size of this study is small, and there may be selection bias. Secondly, there is a lack of a healthy control group in this study. Thirdly, other joint scores were not included in this study. Fourth, pathological results were not used as the gold standard to evaluate the diagnostic efficacy of different methods.

In conclusion, our study demonstrated that the ultrasound score of a single wrist joint is closely related to rheumatoid arthritis activity and has diagnostic potential for RA activity.

Author Contributions

Zijing Chu, Zhixing Zhou, Muhammad Asad Iqbal contributed to the conception and design of the study. Zhongxin Zhang, Xian Wang wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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ORIGINAL ARTICLE

Fourteen-Year Retrospective Cohort Study on the Impact of Climatic Factors on Chronic Musculoskeletal Pain: A Spanish Primary Care Analysis

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ABSTRACT

Introduction: Chronic musculoskeletal pain, often affected by environmental factors such as temperature, humidity, and atmospheric pressure, can influence pain perception and increase the number of healthcare visits.

Objective: This study examined the link between climate variables and referral rates for chronic musculoskeletal pain in Spanish primary care over 14 years and evaluated the impact of climatic factors on rehabilitation referrals based on variations in pain type, age, and sex.

Methods: A retrospective cohort of 44 212 adults diagnosed with chronic musculoskeletal pain (2010–2023) across three primary care centers was analyzed. The inclusion criteria were CIAP2 (International Classification of Primary Care, second edition) diagnostic codes, with ethical clearance from the Puerta de Hierro Majadahonda Hospital (PI 70/24). This study adhered to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for observational reporting. Climatic data, including temperature, precipitation, wind speed, hours of sunlight, and barometric pressure, were obtained from (Agencia Estatal de Meteorología). Statistical analyses used ARIMAX (AutoRegressive Integrated Moving Average with eXternal regressors) and ETSX (Exponential Smoothing State Space Model with eXternal regressors) models, optimizing model fit through root mean squared error (RMSE), mean absolute percentage error (MAPE), and mean absolute scaled error (MASE).

Results: Significant associations were found between climate factors and referral rates. Higher minimum temperatures reduced shoulder/arm pain referrals by −0.019 (95% CI: −0.036, −0.002; $p < 0.05$). Male patients were more likely to consult, with age being inversely linked to thoracic/lumbar pain (−0.044; 95% CI: −0.071, −0.018; $p < 0.05$) and positively associated with shoulder/arm pain (0.038; 95% CI: 0.024, 0.052; $p < 0.05$). ARIMAX was optimal for most pain types, except for cervical pain, for which ETSX was better.

Conclusions: Climatic factors, such as temperature and pressure, affect referral patterns, highlighting the need for climate-sensitive healthcare planning to aid resource management and patient guidance on pain in varying weather conditions.

1 | Introduction

Human beings interact with the environment as part of their nature [1]. Biometeorology is defined as the study of the impact of climate and the environment on living beings [2]. Meteoropathy is defined as the study of the effects of climatic conditions on the human body [3]. It is estimated that 30% of the population has weather-related disorders [4] and almost three-quarters of patients with chronic pain report pain fluctuations related to climatic factors [5]. Climatic variables that can affect health include atmospheric pressure, humidity, wind, rain, temperature [3], and solar radiation [6]. Studies in both animal models [7] and human clinical trials [8] relate these environmental factors to the alteration of endocrine and neurophysiological processes, especially in the vestibular nucleus, which is associated with the appearance of psychological disorders, and alterations in physical function with the appearance of musculoskeletal pain [9, 10]. Studies indicate that these symptoms tend to increase when environmental factors change abruptly with increases in humidity > 70% and temperature > 30°C [11], or with prolonged exposure to cold [12], which can worsen pain [1, 13]. Patients with arthritis, chronic joint pathologies [14], headaches [8, 15], fibromyalgia [16], neuropathic pain [13], and back pain, especially cervical and lower back pain, with and without irradiation [12], are the most affected [17]. These patients are often repeatedly referred for physiotherapy, which is considered the most effective treatment [18, 19], in combination with pharmacological treatment [14]. Studies exploring the relationship between environmental factors and musculoskeletal problems are scarce; it is not common to stratify patients into subgroups [5], and the samples are relatively small [6], especially if we consider that for predictive models to be reliable, they require the processing of a large amount of data [20].

To our knowledge, no previous study has examined the relationship between the number of consultations in the primary care of patients with chronic musculoskeletal problems and environmental temperature, precipitation, wind speed, hours of sunshine, and barometric pressure, since previous studies usually include one or two meteorological variables [6]. Variations in precipitation, wind speed, hours of sunshine, and barometric pressure were associated with changes in the number of referrals from patients with chronic pain of musculoskeletal origin to primary care physician offices.

This study hypothesizes that climatic variables significantly impact chronic musculoskeletal pain referral rates and that patient age, sex, and type of musculoskeletal disorder further influence this association.

This study aimed to analyze the association between climatic variables and the number of referrals for patients with chronic pain of musculoskeletal origin. The secondary objectives were to analyze the differences in this association according to the type of musculoskeletal disorder, explore the effects of age and sex on this association, and analyze the temporal evolution of the referrals of these patients as a function of climatic variables.

2 | Methods

2.1 | Data Source and Study Population

A retrospective cohort study was conducted using data extracted from the electronic health records of patients from three primary care centers: "El Abajón" in Las Rozas, "Cerro del Aire" in Majadahonda, and "San Juan de la Cruz" in Pozuelo de Alarcón, Spain. The study period was from January 1, 2010 to December 31, 2023 and included all patients aged 18 years and older who were diagnosed with chronic musculoskeletal pain. Diagnoses were identified using International Classification of Primary Care (ICPC-2) codes. Meteorological data were obtained from the Spanish Meteorological Agency (AEMET) station in Pozuelo de Alarcón. The AEMET station in Pozuelo de Alarcon is located 15 km from Las Rozas de Madrid AND The 12 km from Majadahonda (Spain) a. The three health areas covered in this study have a total area of 140 km^2, recorded from January 1, 2010 to January 1, 2024 (ID3194Y, latitude 40°26′54″ N, longitude 3°48′ 48″ ′W).

The study protocol was approved by the Research Ethics Committee of the Puerta de Hierro Majadahonda Hospital (PI 70/24, Act 06/2024). All the procedures complied with the principles of the Declaration of Helsinki and ensured patient anonymity and confidentiality throughout the study. The procedures were conducted following the STROBE statement and checklist [21].

2.2 | Inclusion and Exclusion Criteria

Patients were eligible for inclusion if they had been diagnosed with the following codes according to the CIAP2 (International Classification of Primary Care second edition) [22]: cervical pain [L01 (neck signs/symptoms), L83 (neck syndromes]), thoracic/lumbar pain [L02 (dorsal signs/symptoms), L03 (lumbar signs/symptoms), L84 (lumbar/thoracic syndromes without pain irradiation), L86 (lumbar/thoracic syndromes with pain irradiation]), shoulder/arm pain [L08 (shoulder signs/symptoms), L92 (shoulder syndromes), L09 (arm signs/symptoms]), and hip/thigh pain [L13 (hip signs/symptoms), L14 (thigh and leg signs/symptoms]).

According to the international definition of chronic pain [23], we selected patients who attended consultations for the same reason (CIAP2 code) at least twice, with an interval of at least 3 months. Patients aged < 18 years were excluded from this study.

2.3 | Follow-Up Process for Consultations

To capture patterns in chronic pain-related healthcare usage, patients were monitored for the frequency of referrals over consecutive visits to primary care centers. This follow-up approach enabled the analysis of repeated consultations for each type of

musculoskeletal pain, providing insights into recurring healthcare needs based on specific pain categories.

2.4 | Sample Size

Based on the criteria established by Burmeister and Aitken [24], a minimum of 240 patients were required for a multiple linear regression model with 11 continuous variables and one dichotomous variable. However, we estimated a total sample size of approximately 40 000 patients over the 14-year study period.

2.5 | Study Procedures

Data on each patient's age, sex, and ICPC-2 diagnosis were anonymized and extracted from medical records. These data were then linked to daily meteorological variables, including average, maximum, and minimum temperatures; precipitation; wind direction; wind speed; maximum gust speed; hours of sunlight; and barometric pressure [25].

2.6 | Outcome Measures

The primary outcome was the number of referrals to physiotherapy for chronic musculoskeletal pain categorized by the ICPC-2 code. The secondary outcomes included variations in referral numbers based on different musculoskeletal conditions and the influence of age, sex, and climatic factors on these variations.

The diagnostic criteria for each pain category were consistently applied using CIAP2 codes, with variables defined according to the AEMET standards for climatic factors.

3 | Statistical Analysis

Statistical analysis was performed using R Ver. 4.1.3 program (R Foundation for Statistical Computing, Institute for Statistics and Mathematics, Welthandelsplatz 1, 1020 Vienna, Austria).

The level of significance was set at $p < 0.05$. Quantitative variables are described as mean ± standard deviation, and qualitative variables are described as absolute and relative values (%). The number of cases from January 1, 2010 to December 31, 2023 was analyzed through a time series analysis with both the total number and cases of cervical, thoracic/lumbar, shoulder, and hip/pelvis pain grouped by week. As external regressors, age; gender; average, maximum, and minimum temperature; wind direction; average and gust speed; and maximum and minimum barometric pressure were included, eliminating variables with a variance inflation factor (VIF) > 10. The selection of the model, ETSX (exponential smoothing state space model with eXternal regressors) or ARIMAX (AutoRegressive Integrated Moving Average with eXternal regressors) was carried out by analyzing the forecasting accuracy of a training set (75% of the sample) on a test set (25% of the sample) and comparing the root mean squared error (RMSE), the mean absolute percentage error (MAPE) and the mean absolute scaled error (MAS), the lower the value the better. The stationarity of the series was tested using the augmented Dickey–Fuller test, as well as compliance with the assumptions using the Ljung–Box test (autocorrelation of the residuals) and the Kolmogorov–Smirnov test with Lilliefors correction (normality of the residuals).

4 | Results

A total of 44 212 cases were included in the study, with an age of 59.34 ± 5.30 years, mostly men (65.79%) (Table 1). Missing data for patient demographics and climatic variables were minimal (< 5%) and were handled using list-wise deletion to maintain analytical rigor. (Table 1).

It is observed how the number of cases is stable until 2020, the period of the Covid-19 pandemic is evidenced by the sharp drop in the number of cases and, starting in the post-pandemic period, a marked increase is observed, especially in cervical, shoulder/arm, thoracic/lumbar (Figure 1).

The best fit was that of the ARIMAX model, except for the cervical pain series, in which the ETSX model was the most accurate (Table S1). All series were stationary, and the residuals did not exhibit autocorrelation, although their distribution was non-normal, except in the thoracic/lumbar, shoulder/arm (Table S2). As in all the models, being male significantly increased the probability of more cases appearing. Increasing age predicted fewer thoracic/lumbar vertebrae (−0.044, 95% CI [−0.071, −0.018]) and a greater number of shoulders/arms (0.038, 95% CI [0.024, 0.052]). Finally, an increase in the minimum temperature decreased the probability of more shoulder/arm (−0.019, 95% CI [−0.036, −0.002]) (Table 2).

The 4-year forecasting models show stabilization in the number of cases in line with the general trend; only the cases of excessively wide cervical confidence intervals indicate lower model accuracy (Figure 2).

5 | Discussion

This study aimed to evaluate the impact of climatic variables on referral patterns for chronic musculoskeletal pain in primary care in Spain, focusing on age, sex, and specific pain types, over a 14-year period. We examined associations between weather conditions, such as temperature, humidity, and barometric pressure, and referral rates for pain regions, including cervical, thoracic/lumbar, and shoulder/arm pain.

Key findings revealed a complex relationship between climatic factors and healthcare demands for chronic pain management. ARIMAX and ETSX statistical models were used, with ARIMAX being more effective for most pain types. However, for cervical pain, the ETSX model provided a better fit, indicating distinct climate-related sensitivities based on the pain location.

Given the low average barometric pressures observed, primarily influenced by the elevation of the Iberian Plateau, it is important to note that elevation affects human physiology. Lower atmospheric pressure at higher altitudes can lead to physiological changes, such as reduced oxygen availability, which may

TABLE 1 | Sample characteristics and atmospheric conditions.

	Overall	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Number of cases															
Overall	44212	1764	1980	1960	2106	2227	2506	2749	2867	3198	3687	2950	4127	5105	6986
Cervical, *n* (%)	6962 (15.75)	252 (14.29)	240 (12.12)	296 (15.1)	276 (13.11)	266 (11.94)	310 (12.37)	413 (15.02)	477 (16.64)	536 (16.76)	680 (18.44)	432 (14.64)	602 (14.59)	882 (17.28)	1300 (18.61)
Shoulder/arm, *n* (%)	10574 (23.92)	523 (29.65)	516 (26.06)	549 (28.01)	605 (28.73)	652 (29.28)	707 (28.21)	698 (25.39)	692 (24.14)	709 (22.17)	800 (21.7)	582 (19.73)	914 (22.15)	1084 (21.23)	1543 (22.09)
Hip/thigh, *n* (%)	5164 (11.68)	140 (7.94)	177 (8.94)	164 (8.37)	222 (10.54)	288 (12.93)	353 (14.09)	342 (12.44)	384 (13.39)	406 (12.7)	478 (12.96)	374 (12.68)	568 (13.76)	556 (10.89)	712 (10.19)
Thoracic/lumbar, *n* (%)	21512 (48.66)	849 (48.13)	1047 (52.88)	951 (48.52)	1003 (47.63)	1021 (45.85)	1136 (45.33)	1296 (47.14)	1314 (45.83)	1547 (48.37)	1729 (46.89)	1562 (52.95)	2043 (49.5)	2583 (50.6)	3431 (49.11)
Demographic characteristics															
Age	59.34 ± 5.30	62.20 ± 5.98	61.65 ± 4.23	62.95 ± 5.58	61.92 ± 6.15	60.37 ± 4.63	61.22 ± 4.95	60.51 ± 4.53	60.08 ± 4.16	57.41 ± 4.45	57.29 ± 4.33	56.19 ± 5.40	56.86 ± 4.05	55.33 ± 3.63	56.82 ± 2.96
Gender (Female), *n* (%)	15126 (34.21)	597 (33.84)	691 (34.9)	650 (33.16)	709 (33.67)	701 (31.48)	882 (35.2)	878 (31.94)	1047 (36.52)	1079 (33.74)	1242 (33.69)	1024 (34.71)	1440 (34.89)	1829 (35.83)	2357 (33.74)
Gender (Male), *n* (%)	29086 (65.79)	1167 (66.16)	1289 (65.1)	1310 (66.84)	1397 (66.33)	1526 (68.52)	1624 (64.8)	1871 (68.06)	1820 (63.48)	2119 (66.26)	2445 (66.31)	1926 (65.29)	2687 (65.11)	3276 (64.17)	4629 (66.26)
Atmospheric conditions															
Average temperature (°C)	14.99 ± 7.42	14.74 ± 8.23	15.53 ± 7.24	14.13 ± 7.67	13.77 ± 7.53	14.91 ± 6.57	15.28 ± 7.53	14.73 ± 7.48	15.28 ± 7.66	14.57 ± 7.60	15.12 ± 7.18	15.07 ± 7.19	14.95 ± 7.10	16.14 ± 7.77	15.67 ± 7.61
Maximum temperature (°C)	21.86 ± 8.52	20.21 ± 9.56	21.98 ± 8.37	21.43 ± 8.61	20.60 ± 8.68	21.55 ± 7.87	22.28 ± 8.43	21.62 ± 8.84	23.02 ± 8.53	21.21 ± 8.83	22.36 ± 7.95	21.76 ± 8.51	21.93 ± 8.14	23.09 ± 8.79	22.98 ± 8.53
Minimum temperature (°C)	8.13 ± 6.68	9.27 ± 6.98	9.08 ± 6.43	6.81 ± 7.10	6.93 ± 6.67	8.27 ± 5.63	8.29 ± 6.96	7.83 ± 6.42	7.55 ± 7.04	7.93 ± 6.61	7.88 ± 6.92	8.39 ± 6.18	7.98 ± 6.39	9.19 ± 7.09	8.36 ± 7.14
Average rainfall (L/m^2)	1.43 ± 2.51	2.02 ± 2.96	1.26 ± 1.91	1.09 ± 2.58	1.41 ± 2.37	1.48 ± 2.31	0.92 ± 1.36	1.84 ± 2.56	0.85 ± 1.60	1.94 ± 3.11	1.26 ± 2.20	1.40 ± 2.53	1.28 ± 2.21	1.48 ± 2.27	1.79 ± 4.02
Wind direction (degree dec.)	22.03 ± 8.47	18.83 ± 4.04	18.66 ± 4.15	18.65 ± 5.54	18.46 ± 5.29	18.79 ± 4.55	18.60 ± 4.31	18.55 ± 4.68	18.97 ± 4.86	22.35 ± 6.82	24.34 ± 8.84	26.12 ± 9.52	30.05 ± 11.68	26.72 ± 10.46	29.34 ± 11.38
Average wind speed (m/s)	2.87 ± 1.14	2.71 ± 1.00	2.54 ± 0.96	2.91 ± 1.14	2.90 ± 1.28	3.09 ± 1.29	2.68 ± 1.09	2.73 ± 1.42	2.55 ± 1.03	3.01 ± 1.06	3.29 ± 1.18	2.96 ± 1.15	2.94 ± 0.95	2.83 ± 0.96	3.02 ± 1.17
Wind gusts (m/s)	10.03 ± 2.33	10.09 ± 1.96	9.67 ± 1.93	10.26 ± 2.38	10.52 ± 2.31	10.60 ± 2.52	9.72 ± 2.44	9.89 ± 2.55	9.94 ± 2.24	10.10 ± 2.15	10.58 ± 2.69	9.75 ± 2.40	9.90 ± 2.16	9.92 ± 2.14	9.56 ± 2.56
Sunshine (h)	8.20 ± 3.05	7.79 ± 3.18	8.30 ± 3.12	8.47 ± 2.96	8.16 ± 2.99	8.10 ± 3.19	8.31 ± 2.83	7.97 ± 3.30	8.75 ± 2.62	8.05 ± 3.10	8.82 ± 3.02	7.89 ± 3.34	7.86 ± 2.83	7.93 ± 3.48	8.44 ± 2.69
Maximum pressure (hPa)	940.67 ± 4.50	937.96 ± 4.04	941.09 ± 4.47	940.83 ± 4.69	940.26 ± 4.88	939.39 ± 3.81	942.62 ± 4.97	941.17 ± 4.74	941.93 ± 3.47	939.56 ± 5.02	940.48 ± 4.39	941.25 ± 4.52	940.58 ± 3.55	941.08 ± 4.18	941.12 ± 4.53
Minimum pressure (hPa)	936.29 ± 4.97	933.01 ± 4.83	936.98 ± 5.01	936.62 ± 4.96	935.66 ± 5.51	934.96 ± 4.50	938.26 ± 5.03	936.65 ± 4.83	937.47 ± 3.68	935.00 ± 5.67	935.91 ± 5.20	936.98 ± 4.89	936.47 ± 3.64	936.94 ± 4.55	937.04 ± 5.07

Note: Data expressed with mean ± standard deviation, median [interquartile range] or with absolute and relative values (%).

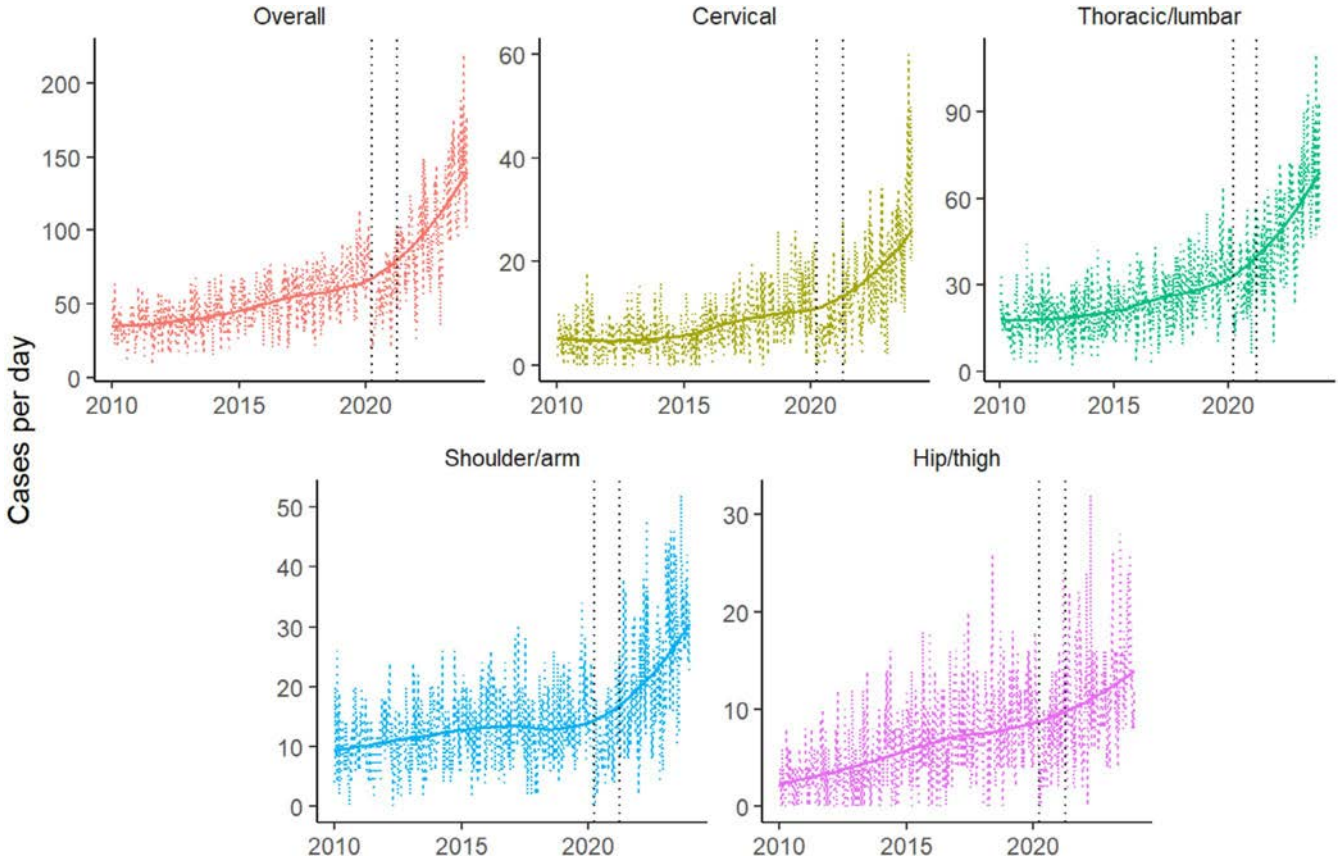

FIGURE 1 | Reported cases time series. Dotted lines represents start and end dates of Covid-19 pandemic.

exacerbate chronic conditions. Although this study did not find specific variations in pain sensitivity due to pressure alone, the role of elevation warrants consideration in future research to better understand its impact on chronic musculoskeletal pain and patient outcomes.

Temperature was a significant predictor, particularly for shoulder and arm pain. Higher minimum temperatures were linked to decreased referrals for shoulder and arm pain, suggesting that warmer temperatures may reduce the demand for care. This trend varied across pain types, highlighting different responses of the musculoskeletal regions to climatic factors.

Patient demographics, particularly age and sex, significantly influenced referral patterns. Male patients had higher referral rates across all pain types, possibly due to a higher incidence of musculoskeletal conditions or differing care-seeking behaviors compared with females. Age-related patterns varied by pain type: younger patients were more likely to be referred for thoracic and lumbar pain, while older age was associated with higher referral rates for shoulder and arm pain.

Horvath et al. reviewed the existing literature on how factors such as atmospheric pressure, wind, humidity, precipitation, temperature, and geomagnetic and cosmic ray activity influence pain, highlighting inconsistencies across studies. Variability is often attributed to sample size, sampling frequency, and study duration [26]. Our study, encompassing a substantial sample of 44.212 subjects over a 14-year period, helps address these inconsistencies by providing robust evidence that fluctuations in temperature, barometric pressure, and humidity significantly affect chronic pain. This aligns with the findings of Yimer et al. [27], who, using a Bayesian multilevel regression analysis with over 13000 subjects, identified that approximately 10% of patients with chronic pain were sensitive to temperature changes, with smaller percentages showing sensitivity to humidity, pressure, and wind speed. The identification of these sensitivity subgroups underscores the complex relationship between atmospheric changes and chronic pain, emphasizing the need for individualized predictive models.

Jiang et al. [28] examined the role of humidity in chronic pain sensitivity across regions of China. Their study found significant humidity-related pain sensitivity among patients with arthritis, particularly those with advanced conditions. This highlights the need for regionally specific and pain-type focused research to better understand variations in climatic sensitivity. Similarly, Matsui et al. [29] explored the barometric pressure effects in patients with fibromyalgia and identified a subgroup with heightened sensitivity. These findings underscore our results and support the call for predictive models that consider unique patient profiles, potentially improving clinical outcomes by adapting treatments to weather variations.

The influence of climatic factors on chronic pain does not appear to equally apply to acute pain. Duong et al. [30] found that acute pain lacks the same level of sensitivity to weather changes as chronic pain. For chronic conditions, however, it is critical to consider the broader impact of weather on patients' mood and cognition, as Keller et al. [31] demonstrated that climate can alter psychological states, potentially increasing healthcare

TABLE 2 | Final time series models summary.

		Coefficient (SE)	95% CI	*Z* value (*p*[a])
ARIMAX models				
Overall (2, 1, 3)	Age	0 (SE = 0.006)	−0.013, 0.012	$Z = -0.039$, $p = 0.969$
	Gender (male)	0.114 (SE = 0.002)	0.109, 0.118	$Z = 49.903$, $p < 0.001$
	Minimum temperature (°C)	−0.003 (SE = 0.007)	−0.017, 0.01	$Z = -0.489$, $p = 0.625$
	Average rainfall (L/m^2)	−0.01 (SE = 0.016)	−0.042, 0.022	$Z = -0.605$, $p = 0.545$
	Wind direction (degree dec.)	0 (SE = 0.004)	−0.008, 0.009	$Z = 0.04$, $p = 0.968$
	Average wind speed (m/s)	−0.022 (SE = 0.068)	−0.155, 0.111	$Z = -0.324$, $p = 0.746$
	Wind gusts (m/s)	0.013 (SE = 0.034)	−0.054, 0.079	$Z = 0.375$, $p = 0.708$
	Sunshine (h)	−0.004 (SE = 0.016)	−0.036, 0.029	$Z = -0.225$, $p = 0.822$
	Maximum pressure (hPa)	0.006 (SE = 0.01)	−0.012, 0.025	$Z = 0.665$, $p = 0.506$
Thoracic/lumbar (2, 1, 2)	Age	−0.044 (SE = 0.014)	−0.071, −0.018	$Z = -3.271$, $p = 0.001$
	Gender (Male)	0.139 (SE = 0.004)	0.13, 0.148	$Z = 31.161$, $p < 0.001$
	Minimum temperature (°C)	−0.003 (SE = 0.013)	−0.029, 0.023	$Z = -0.244$, $p = 0.808$
	Average rainfall (L/m^2)	−0.025 (SE = 0.035)	−0.093, 0.043	$Z = -0.726$, $p = 0.468$
	Wind direction (degree dec.)	0.012 (SE = 0.009)	−0.005, 0.03	$Z = 1.381$, $p = 0.167$
	Average wind speed (m/s)	−0.17 (SE = 0.141)	−0.447, 0.107	$Z = -1.206$, $p = 0.228$
	Wind gusts (m/s)	0.047 (SE = 0.07)	−0.091, 0.185	$Z = 0.668$, $p = 0.504$
	Sunshine (h)	0.013 (SE = 0.032)	−0.05, 0.077	$Z = 0.41$, $p = 0.682$
	Maximum pressure (hPa)	−0.016 (SE = 0.019)	−0.054, 0.022	$Z = -0.841$, $p = 0.4$
Shoulder/arm (1, 0, 1)	Age	0.038 (SE = 0.007)	0.024, 0.052	$Z = 5.316$, $p < 0.001$
	Gender (male)	0.044 (SE = 0.002)	0.039, 0.048	$Z = 20.264$, $p < 0.001$
	Minimum temperature (°C)	−0.019 (SE = 0.009)	−0.036, −0.002	$Z = -2.218$, $p = 0.027$
	Average rainfall (L/m^2)	−0.001 (SE = 0.018)	−0.036, 0.035	$Z = -0.031$, $p = 0.976$
	Wind direction (degree dec.)	−0.003 (SE = 0.005)	−0.013, 0.006	$Z = -0.693$, $p = 0.488$
	Average wind speed (m/s)	−0.034 (SE = 0.08)	−0.192, 0.123	$Z = -0.427$, $p = 0.669$
	Wind gusts (m/s)	0.029 (SE = 0.039)	−0.048, 0.107	$Z = 0.741$, $p = 0.458$
	Sunshine (h)	0.006 (SE = 0.02)	−0.033, 0.046	$Z = 0.319$, $p = 0.75$
	Maximum pressure (hPa)	0 (SE = 0.001)	−0.001, 0.001	$Z = -0.513$, $p = 0.608$
Hip/thigh (0, 1, 1)	Age	0.006 (SE = 0.013)	−0.019, 0.031	$Z = 0.484$, $p = 0.629$
	Gender (male)	0.054 (SE = 0.004)	0.046, 0.062	$Z = 12.54$, $p < 0.001$
	Minimum temperature (°C)	0.005 (SE = 0.013)	−0.02, 0.03	$Z = 0.401$, $p = 0.689$
	Average rainfall (L/m^2)	0.004 (SE = 0.032)	−0.059, 0.068	$Z = 0.138$, $p = 0.89$
	Wind direction (degree dec.)	−0.004 (SE = 0.008)	−0.021, 0.012	$Z = -0.519$, $p = 0.604$
	Average wind speed (m/s)	−0.024 (SE = 0.133)	−0.285, 0.237	$Z = -0.182$, $p = 0.855$
	Wind gusts (m/s)	−0.015 (SE = 0.066)	−0.145, 0.115	$Z = -0.224$, $p = 0.823$
	Sunshine (h)	0 (SE = 0.031)	−0.061, 0.061	$Z = 0.003$, $p = 0.998$
	Maximum pressure (hPa)	0.012 (SE = 0.018)	−0.024, 0.048	$Z = 0.654$, $p = 0.513$
ETSX model				

(Continues)

TABLE 2 | (Continued)

		Coefficient (SE)	95% CI	*Z* value (p^a)
Cervical (A, N, N)	Age	0.062 (SE = 0.058)	−0.051, 0.175	$Z = 1.074$, $p = 0.283$
	Gender (male)	−0.222 (SE = 0.032)	−0.284, −0.16	$Z = -7.018$, $p < 0.001$
	Minimum temperature (°C)	−0.061 (SE = 0.062)	−0.183, 0.06	$Z = -0.986$, $p = 0.324$
	Average rainfall (L/m^2)	0.004 (SE = 0.15)	−0.29, 0.299	$Z = 0.028$, $p = 0.978$
	Wind direction (degree dec.)	0.001 (SE = 0.04)	−0.077, 0.08	$Z = 0.032$, $p = 0.974$
	Average wind speed (m/s)	0.03 (SE = 0.625)	−1.195, 1.255	$Z = 0.048$, $p = 0.962$
	Wind gusts (m/s)	−0.066 (SE = 0.312)	−0.678, 0.546	$Z = -0.211$, $p = 0.833$
	Sunshine (h)	0.073 (SE = 0.148)	−0.217, 0.363	$Z = 0.495$, $p = 0.621$
	Maximum pressure (hPa)	−0.007 (SE = 0.087)	−0.177, 0.163	$Z = -0.079$, $p = 0.937$

Note: Models notation in parenthesis: ETSX (error, trend, seasonality), letters A: additive and N: non-present, ARIMAX (autoregressive order, differencing order, moving average order); SE: standard error; 95% CI: 95% confidence interval.
[a]Significant if $p < 0.05$ (shown in red).

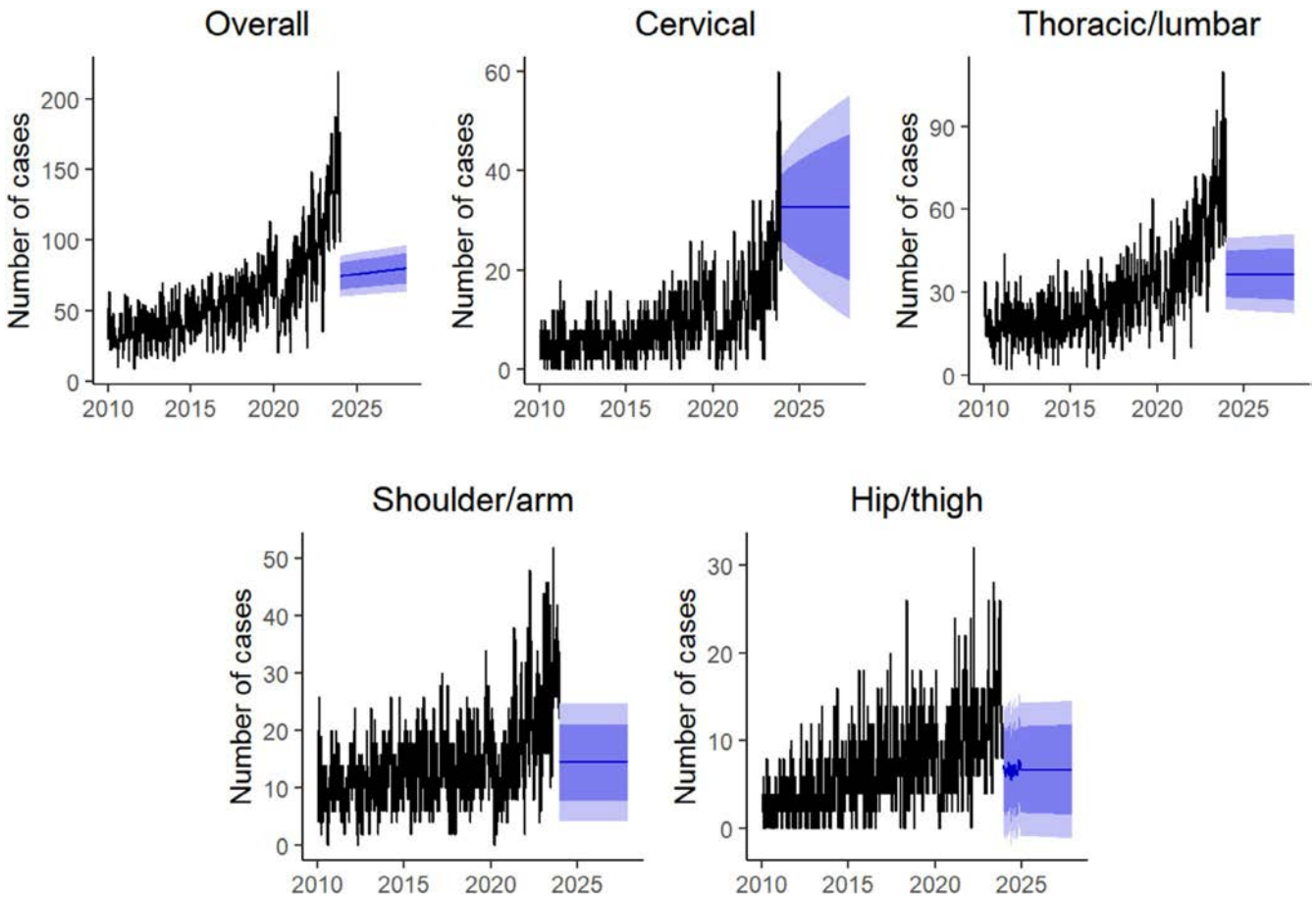

FIGURE 2 | Time series forecast.

demand as patients experience exacerbation of symptoms. This multifactorial nature of chronic pain is consistent with the characteristics of chronic primary pain, as defined by the IASP Task Force for ICD-11 classification, which involves complex interactions of biological, psychological, and social factors [32]. This perspective suggests that healthcare resources should adapt to climate-driven fluctuations in patient demand, similar to seasonal adjustments for respiratory infections [33]. However, the impact of climate on chronic pain-related healthcare utilization has rarely been integrated into health planning.

Thakur et al. [34] reinforced this perspective by showing that climate extremes significantly increase healthcare demand among patients with chronic pain, emphasizing the need for health systems to incorporate climate forecasting into resource planning. Our findings support this idea, because developing predictive

models based on climate data could help forecast surges in consultations, allowing for optimal resource allocation in primary care. Such a model could also serve as a tool for patient education, helping individuals with chronic pain anticipate and better manage pain fluctuations associated with weather conditions [35].

5.1 | Limitations and Future Directions

This study had several limitations. First, the retrospective design relies on the accuracy and completeness of the data extracted from electronic health records, which could introduce bias owing to missing or misclassified information. Additionally, meteorological data were collected from a single weather station, potentially limiting the capture of microclimatic variations across geographical areas covered by primary care centers. This geographical limitation could affect the generalizability of our findings to regions with different climate patterns. Furthermore, although the study covered a 14-year period, other environmental and socioeconomic factors, such as pollution or lifestyle variations, were not included in the analysis but may have influenced chronic pain symptoms. Finally, the study did not incorporate patient-reported outcomes on pain severity or quality of life, restricting our understanding of how climatic factors affect patients' subjective experience of pain.

To address these limitations, future studies should integrate data from multiple weather stations across regions to enhance their representativeness. Additionally, using longitudinal designs with patient-reported outcomes would provide more nuanced insights into pain severity and QoL.

5.2 | Generalizability

Our results highlight the need for caution when generalizing these findings to other populations. Regional climate variations could lead to different pain sensitivity responses, suggesting that these findings may not be universally applicable. Future research should consider assessing chronic pain sensitivity to climatic factors in various geographic settings to provide region-specific insights that enhance the applicability of climate-informed healthcare planning.

Although these findings are specific to the Spanish climate, similar effects can be observed in other regions with comparable weather patterns. This suggests that climate-sensitive healthcare planning can benefit populations across diverse climatic zones.

5.3 | Clinical Implications

The findings of this study suggest that climatic factors such as temperature fluctuations, barometric pressure, and humidity are associated with increased referrals to physiotherapy for chronic musculoskeletal pain. These results have important clinical implications for the primary care management of patients with chronic pain. Clinicians should consider environmental factors when assessing and treating musculoskeletal pain, particularly during periods of extreme weather. This could also influence patient education, emphasizing lifestyle adaptations and early interventions during specific climatic changes. Additionally, the development of predictive models based on climatic data could help healthcare providers anticipate surges in consultations and optimize resource allocation in primary care settings.

6 | Conclusions

This 14-year retrospective study highlighted a significant association between climatic factors, such as temperature, wind speed, humidity, and barometric pressure, and the frequency of primary care referral for chronic musculoskeletal pain.

These findings suggest that healthcare providers could benefit from integrating climate-sensitive strategies such as predictive modeling to anticipate increased referrals and optimize resource allocation during adverse weather conditions.

Future research should explore multi-regional data, include patient-reported outcomes, and address additional environmental factors such as air pollution.

Incorporating these elements may strengthen our understanding and management of climate-related pain exacerbations, ultimately enhancing the care of patients with chronic musculoskeletal pain across diverse climates.

Author Contributions

E.A.S.-R. and J.N.C.-Z.: conceptualization. E.A.S.-R. and J.N.C.-Z.: methodology. J.N.C.-Z.: software. J.N.C.-Z. and E.A.S.-R.: formal analysis. J.N.C.-Z., C.d.C.-V., S.G.-T., R.A.-Z., and P.G.-P.: investigation. J.N.C.-Z.: resources. J.N.C.-Z.: data curation. J.N.C.-Z. and E.A.S.-R.: writing – original draft preparation. J.N.C.-Z., P.M.-L., and E.A.S.-R.: writing – review and editing. J.N.C.-Z. and E.A.S.-R.: visualization. J.N.C.-Z., P.M.-L., and E.A.S.-R.: supervision. J.N.C.-Z.: project administration, funding acquisition, no funding. All authors: validation. All the authors have read and agreed to the published version of the manuscript.

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Ethics Statement

The study was approved by the Ethics Committee for Research with Medicines (CEIM) of the Hospital Puerta de Hierro Majadahonda (PI 70/24 min 06/2024). The principles of the Declaration of Helsinki were followed in this study.

Consent

Informed consent was obtained from all the subjects involved in the study. Written informed consent for publication was obtained from all participating patients (including the patients). Written informed consent was obtained from the patients (s) for publication of this paper.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data presented in this study are available upon request from the corresponding authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

International Journal of Rheumatic Diseases

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LETTER TO THE EDITOR

Case Report: Isolated Pulmonary Arteritis in a Young Woman With Chest Pain

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Dear Editor,

Systemic vasculitis is a term that covers a group of rare conditions characterized by inflammation of blood vessels, which leads to organ ischaemia and damage [1, 2]. Less frequently, vasculitis may also manifest in a localized fashion, reflecting a limited expression of a systemic vasculitis (e.g., localized granulomatosis with polyangiitis) or a vascular inflammation restricted to only one organ or organ system [3, 4]. Localized, single-organ vasculitides have been reported to affect the skin, kidneys, central nervous system, peripheral nerves, genitourinary tract, calf muscles [5], aorta, coronary arteries, retina, or gastrointestinal tract [4, 6]. Isolated pulmonary vasculitis is a very rare entity [7]. Here we describe a young woman with isolated pulmonary arteritis presented as unspecified fever and chest pain. A literature review of cases of localized pulmonary vasculitis was also conducted.

1 | Case Presentation

A 18-year-old woman presented with a 1-month history of chest pain and fever, admitted to our hospital on October 18th, 2024. She resided as a senior-high school student every Monday to Friday in Haining county, Zhejiang province. A cat and a hamster were kept as her pets at home on the weekends. Fever was moderate, up to 38°C, accompanied by dull chest pain. The past history indicated neither traveling outside nor contact with infected people in infected areas. A routine blood test displayed that the white blood cell count was 10.04×10^9/L with 72.8% in neutrophils, hemoglobin was 127 g/L, and platelet count was 378×10^9/L. CRP was 46.6 mg/L. Serum antibodies to some pathogens were all negative. It was given of azlocillin(6 g/day, for 4 days), for suspecting of acute upper respiratory tract infection. The chest pain relieved, and the peak temperature dropped to normal. Antibiotics were stopped without any further examination. Five days later, the recurrence of fever and chest pain made her return to the local clinic. The patient was given penicillin (1000 mg/day, for 3 days) and cephalosporin (500 mg/day, for 3 days) sequentially. Yet the main symptoms did not relieve but were accompanied by loss of appetite and fatigue.

Then the patient was admitted to our hospital for further consultation. Physical examinations revealed that: the temperature was 38°C; cardiac and pulmonary auscultation showed no murmurs or abnormal breath sounds; Blood pressure was symmetrical in both upper and lower limbs; No tenderness was found on the abdomen, costovertebral angle, sternum, spine, peripheral joints, and muscles; Superficial lymph nodes were not palpable for enlargement. Neural system examinations were negative. No tenderness or vascular murmurs were found in the area where superficial arteries ran.

Laboratory blood tests showed that the white blood cell count was 8.9×10^9/L with 62.8% in neutrophils, hemoglobin was 113 g/L, and platelet count was 394×10^9/L; Fecal egg aggregations for many parasites were negative, including hookworm, roundworm, protozoan amoeba, and whipworm. C-reactive protein (CRP) was 112.2 mg/L; Interleukin-6 (IL-6) was 38.9 pg/mL. Antinuclear antibody profiles, antineutrophil cytoplasmic antibodies (ANCA) profiles, and HLA-B27 were negative. Complement 3 was 1.68 g/L; IgG4 was 1.44 g/L. Analysis of Arterial Blood Gas (ABG) was normal in saturation of oxygen and carbon dioxide. Lipid protein A was 77.3 mg/dL. Anti-Typhoid O antibody was 1:160 (negative, 5 days later); IgM-type

autoantibodies to respiratory pathogens were all negative, such as mycoplasma pneumoniae, chlamydia, human respiratory syncytial virus, adenovirus, human para-influenza virus, and influenza A/B virus. Multiple blood cultures were negative. Total T cell ($CD3^+$) was 86.8%, $CD3^+CD4^+$T Cell was 54.89%, $CD3^+CD8^+$T Cell was 24.13%, b lymphocytes ($CD19^+$) were 8.86%, NK lymphocytes (CD15/56+) were 4.23%, and $CD3^+CD4^+CD8^+$T Cell was 0.68%; Electrocardiogram (ECG) scan showed sinus rhythm. Cardiac ultrasound showed mild regurgitation in the tricuspid valve. Computed Tomography (CT) scan of the lung and aorta showed a benign ground-glass nodule in the lower lobe of the left lung (Diameter:3-6 mm). Ultrasound scan showed no apparent abnormalities in superficial arteries, such as bilateral carotid arteries, upper and lower limb arteries, and renal arteries.

Levofloxacin was prescribed intravenously for 6 days. But the patient still had a low-grade fever and mild chest pain. During the process, she denied recurrent ulcers, dryness in mouth and eyes, joint pain, and rash. There were also no complaints of chills, coughing up sputum, abdominal pain, urinary irritation, joint and muscle pain, and other discomforts.

With a suspicion of an underlying malignancy and insidious infection, the whole-body 18F-fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET-CT) scan was performed. The scan revealed high uptake in the main trunk and branches of the pulmonary artery (Figure 1).

Then we performed a series of differential diagnoses in detail. Firstly, our patient had no history of oral ulceration, intermittent claudication of limbs, and negative results of RF, ANCA, and ANA profiles. The systemic vasculitis and connective tissue diseases were being ruled out. Secondly, the lesions were widely distributed in the pulmonary artery and had no occupying effects. There was no proof of pulmonary sarcomas or metastatic tumors, even though the biopsy was inaccessible. Thirdly, the infectious diseases were being excluded by negative results of pathogenic microorganisms and bad therapeutic effects to antibiotics. Later on, a diagnosis of isolated pulmonary vasculitis was made. By re-evaluation of the enhanced CT scan again, the radiologists discovered the abnormal lesions in pulmonary arteries (Figure 2).

Then treatments with high-dose intravenous glucocorticoids (GCs, 40 mg/day) and tocilizumab (TCZ, 480 mg/month) were started. The chest pain and fever alleviated rapidly. During the follow-up in 3 months, the dosage of GC was tapered to 20 mg/day, and TCZ was kept at 480 mg every month. She had no complaints of fever and chest pain again. The acute reactants, like

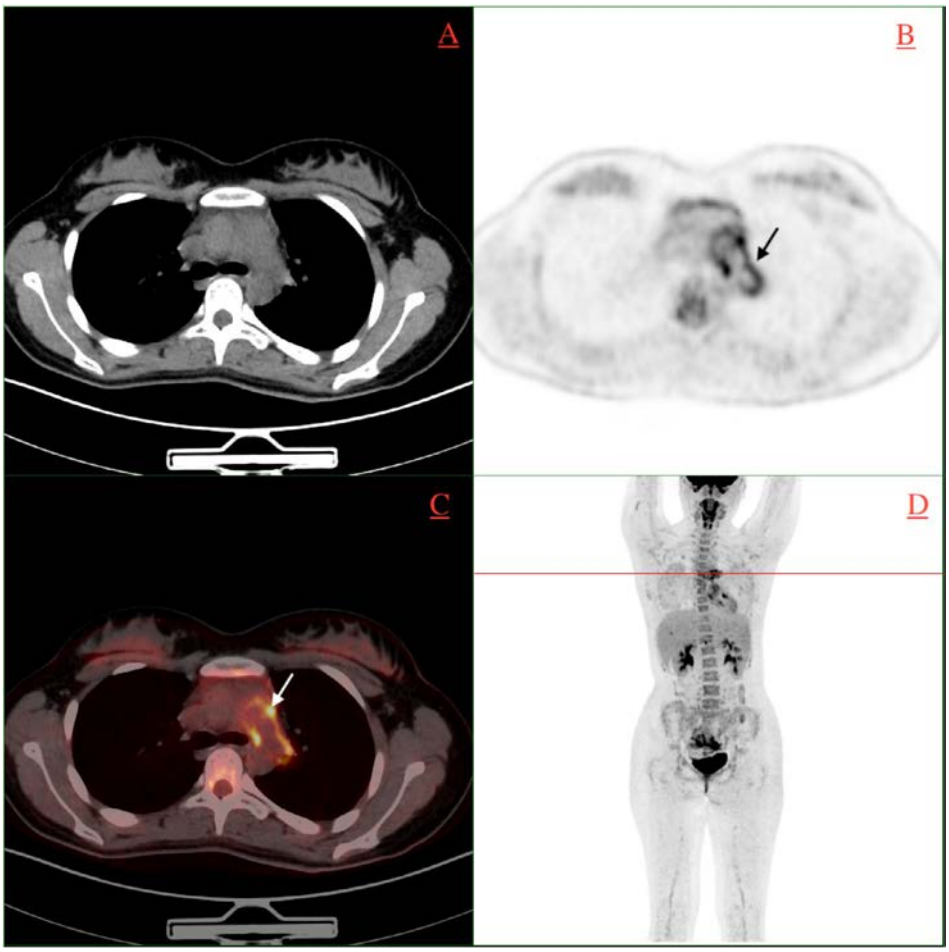

FIGURE 1 | PET-CT scan showed hypermetabolism in the main trunk and branches of the pulmonary artery. The selected axial images [(A) chest mediastinal window CT; (B) PET; (C) fusion; (D) MIP, Minimum Intensity Projection] showed hypermetabolism in the main trunk and branches of the pulmonary artery (arrowhead), which had an SUVmax of 6.33 after injection of 18F-FDG one hour later.

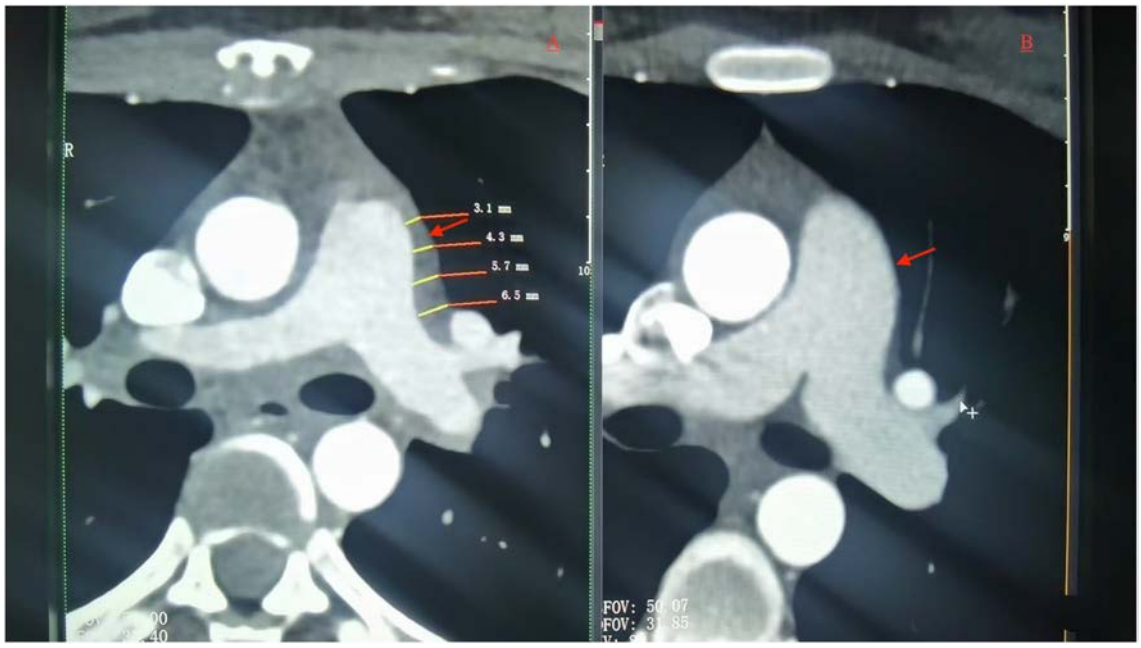

FIGURE 2 | Aorta CT showed abnormal lesions in pulmonary arteries in the young patient (A: Arrowhead) compared with the patient without pulmonary arteritis (B: Arrowhead).

CRP, ESR, and IL-6, remained in the normal range. It was too short to perform the enhanced CT imaging. Overall, the benign process was in favor of an inflammatory etiology, as this type of changes may be observed in patients with pulmonary arteritis, even without the biopsy to confirm the diagnosis.

2 | Discussion

Pulmonary arteritis may involve vessels of different sizes, characterized by vessel wall inflammation [8]. Takayasu's arteritis (TA), Behçet's disease, or giant cell arteritis may affect pulmonary vessels of largesize, preceding the remaining involved vessels by many years [7, 9]. Pulmonary arteritis involvement (PAI) might also be as a manifestation of connective tissue diseases as seen in systemic lupus erythematosus [10], rheumatoid arthritis, or polymyositis [7]. It can be identified by lung biopsy in medium- and small-sized vessels [10]. According to the literature review, isolated pulmonary arteritis was rarely reported. Most reports were derived from case reports and case series [3, 4, 7, 8, 11]. The main symptoms were progressive exertional dyspnea, pulmonary embolism, and pulmonary hypertension (PH). In the case we reported, it was quite difficult to find the lesions in the pulmonary arteries. The young woman had nonspecific symptoms: fever and accompanied chest pain. We know that the most frequent pathologies related to chest pain were cardiovascular disorders, lung disorders, chest wall syndrome, and gastrointestinal disorders [12]. Even though we conducted physical examinations, laboratory tests, and imaging, pulmonary arteritis was identified only after the PET-CT. The scan showed hypermetabolic activity in the main trunk and branches of the pulmonary arteries. Nuclear medicine physicians gave the diagnosis of pulmonary arteritis definitively, by ruling out pulmonary tumors. The latter always presented as a localized mass and made an expansive growth in the pulmonary artery lumen [13]. After re-tracing CT images by radiologists, thickening of the pulmonary artery wall was seen in the lesions discovered by PET-CT. The case reminds us that isolated pulmonary arteritis might be a rare cause of chest pain in young women, especially without the involvement of other organs. And the radiologists should learn to recognize the insidious changes in the pulmonary arteries with unspecified chest pain.

According to the literature review, pulmonary arteritis can be confirmed by cardiac ultrasound, pulmonary ventilation-perfusion imaging, or CT scan [11, 14–16]. Echocardiogram, CTA, ventilation-perfusion lung scan, dual-energy pulmonary angiography, and pulmonary perfusion imaging are highly in accordance with pulmonary angiography to find inflammatory vasculitis in the pulmonary artery. The case we reported had normal images in Echocardiogram, CTA (before re-reading the images), and whole-body MR. The traditional images might have some limitations before visible changes are seen in arteries, like narrowing, obliteration, or thrombus. In recent years, PET-CT has played an increasingly important role in the early diagnosis of arteritis [17]. The PET-CT scan might need more applications in rare arteritis or fever of unknown origin. However, the newly applied imaging technology also has its own shortcomings, due to its high sensitivity. We should carry on more studies to compare the manifestations and prognosis of arteritis diagnosed by pet-ct and traditional images.

Currently, there is no consensus on the treatments of PAI in TA [18], needless to say, on the treatment of isolated pulmonary arteritis. If not promptly treated, pulmonary artery involvements may develop into irreversible vascular damage and life-threatening pulmonary hypertension, which results in poor prognosis and increased risk of mortality [15, 16, 19]. We started the treatments with high-doses of GCs and tocilizumab, referring to the therapy in TA. In the guideline of TA in Japan, tocilizumab is the first-line biologic [20]. Till the time we reported, the patient did not complain of fever and chest pain again. The

physical examination and laboratory tests stayed in a normal state continuously. So we expected that the early intensive treatments may help to better control the lesions in the pulmonary arteries, without progressing to PH. Our case also had an inevitable shortcoming of only 3-month follow-up. It will need a longer follow-up to confirm the therapeutic effect and prognosis.

3 | Conclusion

In our case report, 18F-PET-CT was used to diagnose an early case of pulmonary arteritis. Isolated pulmonary arteritis might be a rare reason for a young woman with chest pain. Early intensive treatments, with glucocorticoids and biological agents, may help to restrain the development of damage in the pulmonary artery, which requires a longer follow-up.

Author Contributions

The authors takes full responsibility for this article.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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LETTER TO THE EDITOR

Case Report: Infection-Associated Vasculitis Mimicking Giant Cell Arteritis Induced by Bacteremia from Bacterial Infection

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Dear Editor,

Vasculitis, a systemic inflammatory disease affecting arteries, vessels, and capillaries across multiple organs, presents a complex diagnostic challenge for clinicians. The Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitides (CHCC) categorizes vasculitis into primary and secondary types [1]. Secondary vasculitis is further classified based on associations with systemic diseases or probable etiologies such as drugs, cancer, or microorganisms. Various pathogens have been linked to different types of vasculitis, including hepatitis C virus with small vessel vasculitis, hepatitis B virus with medium vessel vasculitis, and syphilis with large vessel vasculitis.

Giant cell arteritis (GCA), a primary large vessel vasculitis, typically affects older adults and often involves the temporal arteries, presenting with fever and headache. The 2022 American College of Rheumatology/European League Against Rheumatism Classification Criteria for GCA include elevated inflammatory markers and wall thickening of the bilateral superficial temporal arteries, observable through imaging techniques. These criteria emphasize the importance of excluding alternative diagnoses that mimic vasculitis [2].

We report a case of infection-associated vasculitis mimicking GCA, caused by bacteremia due to bacterial infection. A 66-year-old male presented to our hospital with a 1-week history of fever, headache, and mild sore throat. His medical history included being a past smoker, diverticulitis, postoperative gastric cancer, diabetes mellitus treated with sitagliptin for 14 years, and fatty liver. Initially diagnosed with a viral infection and prescribed non-steroidal anti-inflammatory drugs, he returned 10 days after his initial presentation for further evaluation due to persistent symptoms.

Clinical examination revealed clear consciousness; a body temperature of 37.2°C; blood pressure of 117/76 mmHg; pulse rate of 81 beats per minute; and oxygen saturation of 99%. Heart and respiratory sounds were normal, and the abdomen was flat and non-tender on palpation. No neurological abnormalities were found, but mild dilation of temporal arteries without tenderness or jaw claudication was noted. Laboratory findings showed elevated C-reactive protein (CRP) at 11 mg/dL, erythrocyte sedimentation rate (ESR) at 88 mm/h, procalcitonin at 0.07 ng/mL (normal range: < 0.05 ng/mL), and HbA1c at 7.9%. MPO-ANCA and PR3-ANCA were negative, and urinalysis was normal.

Ultrasonography was performed using a canon Aplio i800 with a linear probe (5–18 MHz), which revealed wall thickening of the bilateral superficial temporal arteries frontal branches, suggesting GCA (Figure 1A). However, contrast-enhanced CT from the neck to the pelvis showed no findings indicating large vessel vasculitis. Blood cultures were obtained, and a temporal artery biopsy (TAB) was performed for differential diagnosis.

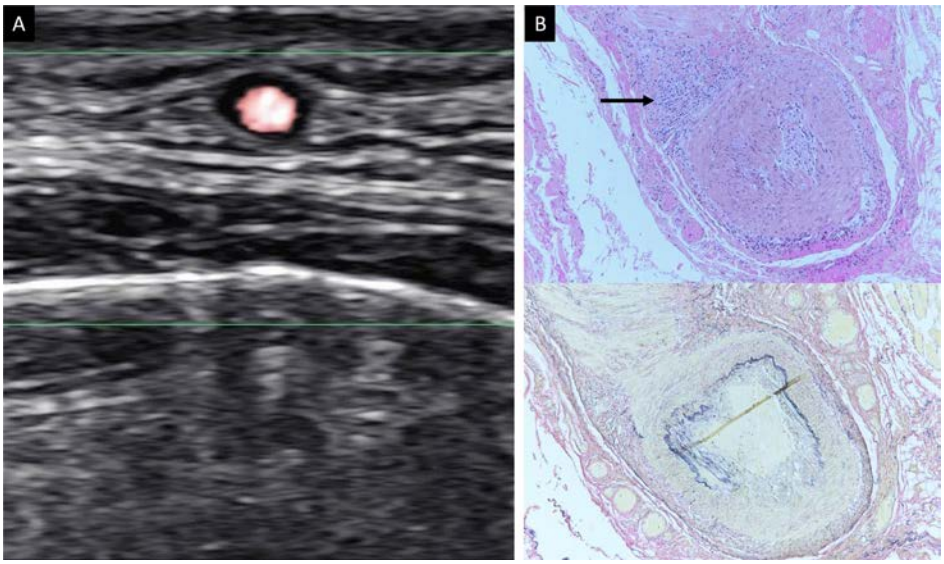

FIGURE 1 | (A) Temporal ultrasonography image in transverse view shows hypoechoic wall thickening ("halo" sign) of right superficial temporal arteries frontal branches. (B) Histopathological findings of temporal artery dissection revealed mild intimal hyperplasia, partial fragmentation of the internal elastic lamina, and mild to moderate inflammatory cell infiltration of the intima and adventitia (arrow). Hematoxylin and eosin stain, elastic van Gieson stain, low-power field view.

On the fourth day after specimen collection, blood cultures grew *Cronobacter sakazakii*, leading to a diagnosis of bacteremia. Intravenous ceftriaxone was administered, resulting in the resolution of fever and headache 3 days later and a decrease in CRP levels to 0.62 mg/dL 4 days after treatment. TAB results showed mild intimal hyperplasia, partial fragmentation of the internal elastic lamina, and mild to moderate inflammatory cell infiltration of the intima and adventitia (Figure 1B). The dominance of neutrophil infiltration in the intima without multinucleated giant cells was inconsistent with GCA.

Based on the clinical course and pathological findings, the patient was diagnosed with infection-associated large vessel vasculitis. The route of infection could not be identified. This case highlights the challenge of differentiating between GCA and infection-associated vasculitis, as the initial presentation with continuous headache, elevated CRP levels, and the detection of a halo sign via ultrasonography suggested GCA. However, the absence of tenderness on palpation, loss of pulse, and jaw claudication were inconsistent with GCA [2]. We diagnosed this patient with bacteremia rather than sepsis because the laboratory tests showed a mild elevation of procalcitonin, and he did not exhibit life-threatening organ failure. The absence of organ failure affecting vital signs may also have made the diagnosis more difficult.

Infection-associated vasculitis can result from direct pathogen invasion or from immune reactions triggered by infection [3]. In this case, while blood cultures were positive for bacteria, no pathogens were detected in histological findings. The neutrophil-based inflammatory response observed in histopathology suggests pathogenesis due to an immune response triggered by pathogens.

Various bacteria can cause infectious large vessel vasculitis [4], with Staphylococci and *Treponema pallidum* being more frequently reported. *Treponema pallidum* has been known to cause temporal arteritis [5]. *Cronobacter sakazakii* is a pathogenic bacterium found in the environment and is a well-known cause of foodborne disease in neonates and infants. Although bacteremia caused by *Cronobacter sakazakii* in immunocompromised adults has been reported [6], this is the first reported case of *Cronobacter sakazakii* causing vasculitis characterized by temporal lesions. Although the pathogen was known, the source of the infection was not identified, and the possibility of a recurrent infection that triggers vasculitis cannot be ruled out.

This case underscores the critical importance of accurate etiological diagnosis in vasculitis management. Misdiagnosis and subsequent administration of immunosuppressive therapy could potentially aggravate the condition in patients with infection-associated vasculitis. Clinicians should maintain a high index of suspicion for infectious causes when evaluating patients with suspected vasculitis. The complexity of vasculitis diagnosis and treatment highlights the need for a comprehensive approach, considering both primary vasculitis and potential infectious etiologies to ensure optimal patient care and outcomes.

Author Contributions

N.D. performed the literature search and wrote the manuscript, supported by K.S., M.H., M.K., and A.K. performed the pathological evaluation. All authors revised and approved the final manuscript.

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The authors have nothing to report.

Conflicts of Interest

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Data Availability Statement

Data sharing does not apply to this article, as no new datasets were generated or analyzed in this case report.

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LETTER TO THE EDITOR

Improvement in Hand Joint Flexibility Following Upadacitinib Treatment of Palmoplantar Pustulosis in Adolescents

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1 | Introduce

Palmoplantar pustulosis (PPP) is a rare, chronic inflammatory dermatological condition characterized by sterile pustules, erythema, and hyperkeratosis on the palms and soles. While PPP mainly occurs in adulthood, a small number of children and adolescents are affected. Kubota et al. reported that the prevalence of PPP in Japan was 0.12% [1–3]. There is currently a lack of unified and effective treatment options for children and adolescents with PPP. Upadacitinib is a new small molecule JAK inhibitor that has shown good efficacy in alleviating the symptoms of sterile pustules, erythema and scale in adults with PPP. However, the complication of hand joint mobility impairment is a rarely observed sequela of PPP [4]. In this case report, we describe a teenager patient afflicted with PPP demonstrating hand joint mobility issues, who exhibited noteworthy improvement following a three-month course of upadacitinib treatment.

2 | Case Reports

A 15-year-old adolescent was diagnosed with PPP at the age of 6, presenting with erythema, scaling, and pustules on his hands and feet. At the age of 11, he began to experience difficulty in flexing and extending his finger joints, which seriously affected the patient's daily life, such as writing, dressing and other common activities. The patient was also unwilling to communicate with others due to repeated episodes of rash, resulting in an inferiority complex. The patient had previously been treated with topical steroid ointments, but the treatment failed. Upon presentation at our institution, the patient exhibited conspicuous desquamation and pustulation on the extremities, coupled with cutaneous thickening and restricted hand joint mobility (Figure 1a–c). When diagnosing the patient, we excluded his relevant medical history and medication history. Upadacitinib therapy was initiated at a dose of 15 mg orally once daily. After 3 months of treatment, the

Qinchen Gu, Chen Zhang, and Wei Song contribute equally to this letter and should be the co-first author.

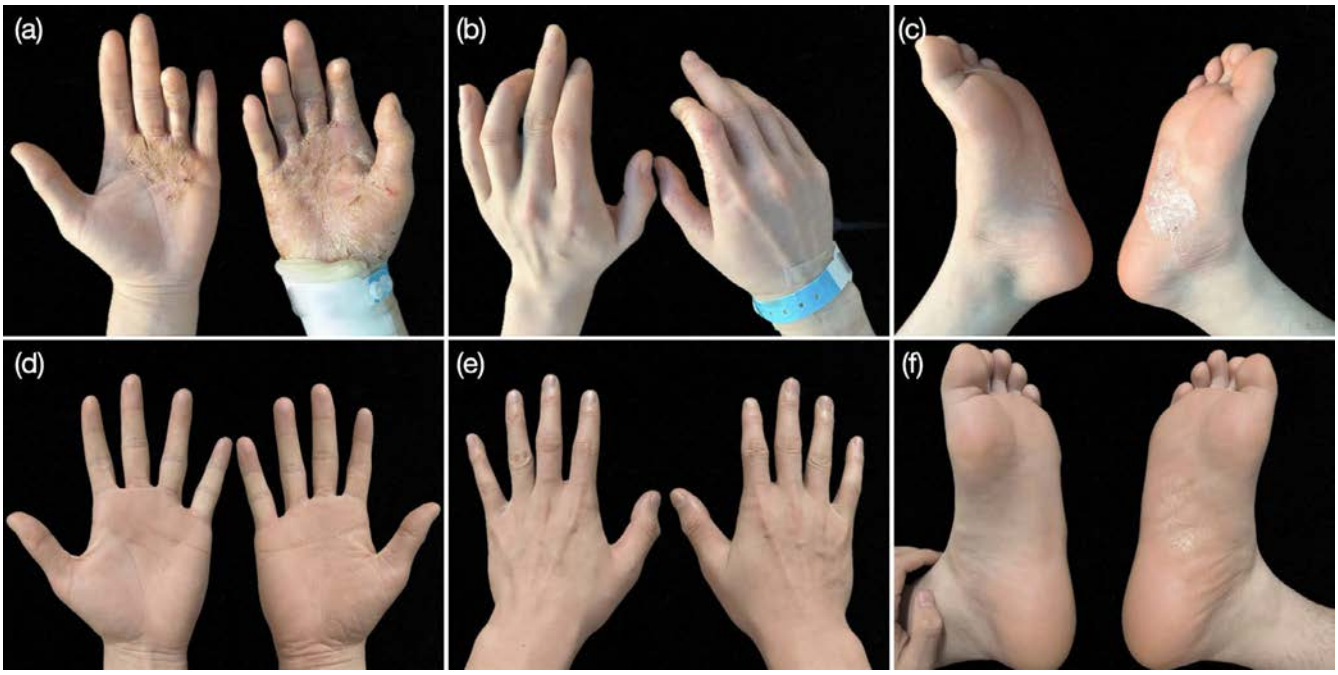

FIGURE 1 | (a) Both palms, (b) backs of both hands, and (c) both soles before upatinib treatment, and (e) both palms, (f) backs of both hands, and (g) both soles after 12 weeks of upadacitinib therapy.

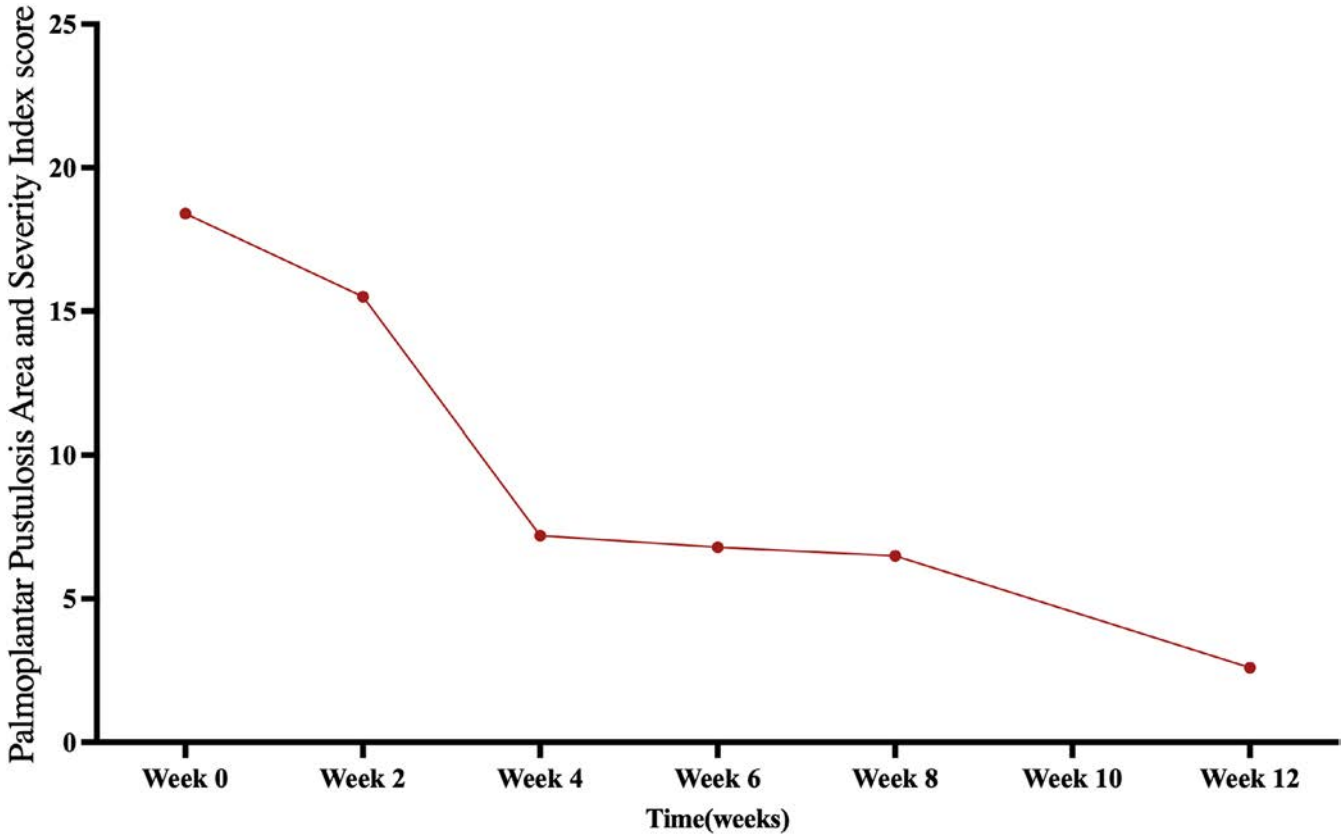

FIGURE 2 | Palmoplantar Pustulosis Area and Severity Index (PPPASI) scores of patients at 0, 2, 4, 6, 8, 10, and 12 weeks.

patient's symptoms improved significantly (Figure 1e–g), with reductions in skin thickening, desquamation, absence of new pustules, and restoration of joint mobility to normal levels. The initial Palmoplantar Pustulosis Area and Severity Index (PPPASI) stood at 18.4, which significantly decreased to 2.6 after 12 weeks of upadacitinib therapy (Figure 2). No untoward events were recorded throughout the course of upadacitinib treatment.

3 | Discussion

Currently, PPP places a great burden on the daily life and mental health of children and adolescent patients who are of school age. Afflictions in the hands, in particular, can severely disrupt their academic performance and daily activities. Tasks that involve the hands, such as writing, turning pages, and engaging in manual activities, become challenging and sometimes even impossible due to the symptoms of PPP. At the same time, it places a great psychological burden on children who are in an important period of mental health [5].

The patient with PPP reported in this article had difficulty flexing and extending the hand joints due to the lack of timely and effective treatment for a long time. However, with the relief of symptoms such as pustules, desquamation, and skin thickening on the hands after treatment, we also found that this difficulty in flexing and extending the hand joints can be significantly improved. We believe that the difficulty in flexing and extending the joints is related to the involvement of the diseased skin. However, there is currently no research on the effect of palmoplantar pustulosis on joint flexion and extension, so we conducted this case report.

Different from the three cases of middle—aged female PPP patients abroad treated with upadacitinib reported by Gaiani et al. in 2023 [6], our patient is a teenage male. In addition to more severe skin lesions, he also has restricted flexion and extension of the hand joints. There are significant differences compared with previous case reports, so we reported this case. Currently, as for palmoplantar pustulosis, there are no studies on the relationship between hand joint flexibility and skin lesions. However, some research [7, 8] has shown that skin lesions are closely linked to joint lesions. Changes in the severity of skin lesions are often accompanied by alterations in joint symptoms. Since the hands are areas where both skin and joints are concentrated, this correlation may imply that skin lesions can affect the flexibility of hand joints. Since effective treatment options for adolescent PPP patients are currently limited, the management of adolescent PPP patients still faces major challenges. Clinical studies of upadacitinib in the treatment of minor patients have focused mainly on the treatment of atopic dermatitis in adolescents, but there are also limited reports on the efficacy and safety of upadacitinib in the treatment of adolescent PPP [9, 10]. It is worth noting that there is still a lack of case studies and safety data on the use of upadacitinib in adolescents for PPP tretment. We found that short-term use of upadacitinib may produce rapid and beneficial effects in adolescent PPP patients grappling with joint flexion and extension impairment. Specifically, short-term treatment with upadacitinib may be a promising treatment option for adolescent PPP patients with impaired joint flexion and extension.

Author Contributions

Qinchen Gu: research design, data analysis, manuscript writing (primary contributor). **Chen Zhang:** data collection, manuscript editing. **Wei Song:** data analysis, figure preparation, manuscript editing. **Zhimin Lin:** experimental design, data validation, manuscript review. **Chen Li:** data collection, literature review, manuscript review. **Zhenhua Ying:** research design, funding acquisition, final approval (corresponding author).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Chen Zhang
Wei Song
Zhimin Lin
Chen Li
Zhenhua Ying

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CLINICAL IMAGE

Limb-Girdle Muscular Dystrophy Type 2 Caused by a Novel Homozygous Mutation in DYSF in a Consanguineous Family

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A 40-year-old man was referred to our clinic due to limb weakness. Around the age of 30, he started experiencing limb muscle soreness and weakness. Over time, his bilateral lower extremities had visibly thinned, and this manifestation had exacerbated in the last 4 months. On physical examination, atrophy was detected in the bilateral biceps brachii, quadriceps, and calves, as well as in the left pectoralis major (Figure 1A,B). The patient denied having a rash, shortness of breath, or cough. He had been diagnosed with dermatomyositis positive for antimelanoma differentiation-associated gene 5 (anti-MDA5) antibody by immunoblot assay in another hospital based on a weak positive anti-MDA5 antibody result. He was treated with glucocorticoids, intravenous immunoglobulin, and tacrolimus, but no clinical or biochemical improvement was observed. Hematological examinations indicated that the serum creatine kinase level increased markedly, reaching 3273 U/L (normal range, 21–190 U/L). Upon re-examination, the anti-MDA5 antibody retesting by immunoblot yielded a negative result. Electromyography demonstrated a myopathic pattern. Muscle biopsy revealed scattered necrosis of muscle fibers with occasional signs of muscle fiber regeneration (Figure 1C). Immunohistochemical analysis indicated that the major histocompatibility complex-1 was partially expressed on the membranes of a few muscle fibers. The expression of dystrophin remained normal. In contrast, a partial reduction in the expression of dysferlin was observed (Figure 1D–F). A deeper look into the patient's family history showed that the patient's parents are first cousins, having a third-degree consanguineous relationship. The patient also has two asymptomatic younger brothers. Whole exome sequencing identified a novel homozygous mutation in the DYSF gene (c.1256G>C, [p.Arg419Pro]) (Figure 1G). Although the variant is present in gnomAD at a very low frequency (1.24e-6), its absence in HGMD and homozygous states in population databases supports its novelty in the context of limb-girdle muscular dystrophy type R2 (LGMDR2). Consequently, a diagnosis of LGMDR2 was established [1].

Conclusion

This particular case underscores the crucial importance of adopting a comprehensive diagnostic methodology, incorporating genetic testing and muscle biopsy, for patients suspected of having muscle disorders. Additionally, mild anti-MDA5 antibody positivity on immunoblot should be interpreted with caution, especially in the absence of characteristic clinical features of dermatomyositis.

Author Contributions

Zhen He collected data and wrote the manuscript. Zhen He, Sheng-Ming Dai, and Zhiyong Chen revised the manuscript. Zhiyong Chen managed this patient and conceived this work. All authors have read and approved the final version of the manuscript.

Consent

The informed consent was obtained from the patient.

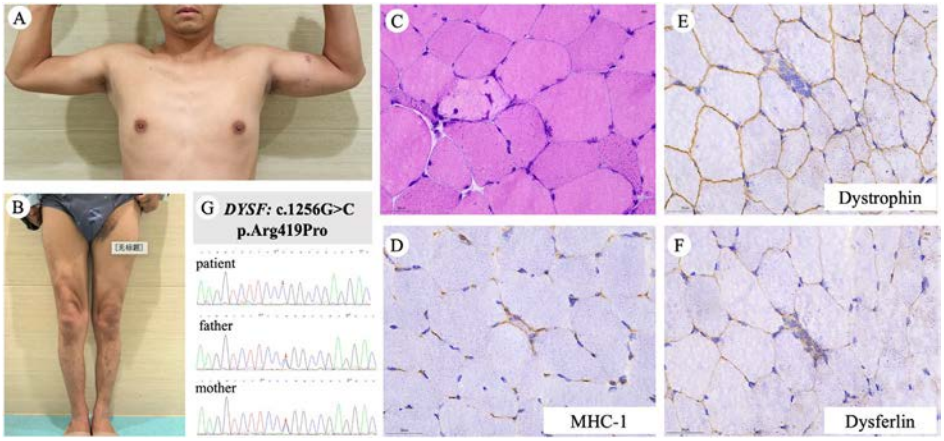

FIGURE 1 | Description of an LGMDR2 patient with a confirmed dysferlin mutation. (A, B) Physical examination revealed symmetric muscle atrophy in the quadriceps, calf muscles, and upper limb musculature. (C) A biopsy of the left biceps muscle demonstrated scattered occurrences of necrosis of both old and fresh muscle fibers (40×). (D) Immunohistochemical staining revealed that in skeletal muscle, partial expression of MHC-1 could be seen on the membranes of several muscle fibers (40×). (E) The expression of dystrophin on the membranes of muscle fibers was normal (40×). (F) The expression of dysferlin on the membranes of some muscle fibers was incomplete or showed weak staining (40×). (G) Direct Sanger sequencing of the patient's family members identified a homozygous mutation in the DYSF gene (c.1256G>C, [p.Arg419Pro]).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Predictive Value of Serum sIL-2R Levels and Th17/Treg Immune Balance for Disease Progression in Patients With Rheumatoid Arthritis-Associated Interstitial Lung Disease

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Keywords: cox regression | interstitial lung disease | prognosis | rheumatoid arthritis | ROC curve | sIL-2R | Th17/Treg

ABSTRACT

Background: This article analyzed the relationship between serum sIL-2R levels and Th17/Treg immune balance in patients with rheumatoid arthritis-associated interstitial lung disease (RA-ILD) and their prognostic value.

Methods: RA patients ($n = 311$) were retrospectively selected for research and then allocated to the RA and RA-ILD groups. Baseline data and 3-year follow-up records of all patients were attained to assess disease progression. Serum sIL-2R levels were examined with ELISA, and Th17 and Treg cell clusters were tested with flow cytometry, followed by the calculation of the Th17/Treg ratio. The correlation of serum sIL-2R with Th17/Treg and related cytokines (IL-17, IL-6, IL-10, and TGF-β1) in RA-ILD patients were analyzed with Spearman's analysis. ROC curves were plotted for analyzing the performance of serum sIL-2R levels and the Th17/Treg ratio for predicting disease progression in RA-ILD patients. A multivariate Cox regression model was developed to screen independent risk factors for disease progression in RA-ILD patients.

Results: RA-ILD patients had elevated serum levels of sIL-2R, Th17 cells, IL-17, and IL-6 and an increased ratio of Th17/Treg, accompanied by a decreased Treg cell population and IL-10 and TGF-β1 levels. Serum sIL-2R levels were correlated positively with IL-17 levels and the Th17/Treg ratio in RA-ILD patients and negatively with IL-10 levels. DAS28 scores, serum sIL-2R levels, and an elevated Th17/Treg ratio were independent risk factors for disease progression in RA-ILD patients, and increased FEV1 and FEV1/FVC were protective factors.

Conclusion: Serum sIL-2R levels in conjunction with Th17/Treg immune balance can assist in predicting 3-year disease progression in RA-ILD patients.

1 | Introduction

Interstitial lung disease (ILD) represents a severe extra-articular complication of rheumatoid arthritis (RA) that dramatically raises mortality [1, 2]. A great deal of studies are centered on multiple markers for the severity and risk of RA-ILD from the radiographic, genetic, molecular, clinical, and serologic perspectives [3, 4]. Few publications identified age, male gender, course of disease, and antibodies to cyclic citrullinated peptides as the most common risk factors for RA-ILD progression, whereas no consensus on risk and prognostic factors for RA-ILD has been reported yet [5]. Accordingly,

more screening tools for RA-ILD are demanded to accelerate the early diagnosis of RA-ILD [6].

Immune dysregulation is an important feature of RA-ILD. For instance, lower proportions of T cells and CD4+ T cells are found in the peripheral blood of RA patients developing ILD [7]. Regulatory T cells (Tregs) refer to a T cell subset functioning as a defender in the immune system, which can affect the activation of effector T cells and dendritic cells when activated [8, 9]. T-helper 17 (Th17) cells, another T cell subset, are IL-17-releasing cells that have shown critical roles in the maintenance of immune homeostasis [10]. Studies have reported the implication of Th17/Treg imbalance in several inflammatory autoimmune disorders. Also, this imbalance is detected in the peripheral blood of RA patients [11, 12]. Additionally, patients with anti-synthetase syndrome-ILD have an elevated proportion of Th17 cells and an increased ratio of Th17/Treg [13]. The lung Treg/Th17 ratio has been regarded as a risk marker for the acute exacerbation of ILD [14]. These findings contribute to the crucial significance of the Treg/Th17 ratio in the prognostic evaluation of immune-associated disorders.

Interleukin (IL)-2 and IL-2 receptor (IL-2R) constitute an important pathway participating in immunity [15]. A soluble form of IL-2R (sIL-2R), which is secreted from activated T cells, is increased in blood samples under many immunological diseases, such as hemophagocytic lymphohistiocytosis and sarcoidosis [16]. Moreover, serum sIL-2R has been revealed to hold the promise as a diagnostic indicator for immune-associated adverse events [17]. Nonetheless, the expression and prognostic value of serum sIL-2R levels in RA-ILD patients are currently unclear. Augmented serum levels of sIL-2R were detected in dermatomyositis patients according to a recent study, showing significant correlations with Th17 cell subsets and the Th17/Treg ratio [18]. It has not been discussed as to whether serum sIL-2R correlates to Th17/Treg immune balance in RA-ILD and thus has certain prognostic value. Hence, this article analyzed the link of serum sIL-2R levels to Th17/Treg immune balance in RA-ILD patients, as well as their prognostic value, to offer new references for the diagnosis and therapy of RA-ILD patients.

2 | Methods

2.1 | Study Subjects

This retrospective research initially enrolled 417 patients diagnosed with RA who visited West China Hospital, Sichuan University, between May 2018 and September 2021. After screening based on inclusion and exclusion criteria, 331 patients were selected for the final analysis, who were allocated to RA ($n=194$) and RA-ILD ($n=137$) groups. The study protocol received approval from the Ethics Committee of West China Hospital, Sichuan University, and adhered to the principles outlined in the *Declaration of Helsinki*, ensuring ethical and humane treatment of all participants. The flowchart of the study is depicted in Figure S1.

2.2 | Inclusion and Exclusion Criteria

Inclusion criteria were as follows: (1) Patients conformed to the diagnostic criteria for RA as defined by the American College of Rheumatology [19]; (2) Patients with ILD were diagnosed based on the criteria set by the American Thoracic Society/European Respiratory Society [20] and confirmed through high-resolution computed tomography (HRCT) and/or lung biopsy; (3) Patients had complete clinical and follow-up data for analysis.

Exclusion criteria were the following: (1) Respiratory diseases: Patients with other respiratory conditions, including tuberculosis, chronic obstructive pulmonary disease, bronchial asthma, bronchiectasis, and acute lung injury; (2) Infections: Patients with acute or chronic infections; (3) Malignancies: Patients with any type of malignant tumor; (4) Non-RA-related ILD: Patients with ILD unrelated to RA (to ensure that the study focused solely on patients with RA-induced ILD); (5) Other connective tissue disorders: Patients who suffer from other connective tissue disorders, such as polymyositis/dermatomyositis, systemic sclerosis, Sjögren's syndrome, and mixed connective tissue disease.

2.3 | Sample Size Estimation

The sample size of this study was estimated using a statistical efficiency-based approach and the software G*Power 3.0.10 (University of Düsseldorf, Düsseldorf, Germany). First, the statistical parameters were set as follows: two-tailed test, $\alpha=0.01$, and 1-$\beta=0.95$, and the predicted effect size was medium. Effect size $d=0.5$ and sample size N1/sample size N2 = 1.5 were set (N1 denoted the RA group and N2 represented the RA-ILD group). The estimation result was as follows: N1 = 121, N2 = 181, and the total sample size $N=302$. Therefore, 417 RA patients were initially enrolled, and 331 were finally screened for the study based on the inclusion and exclusion criteria. This sample size met the requirements of the independent samples *t*-test, Mann–Whitney *U* test, and chi-squared test.

2.4 | Clinical Data Collection

By accessing the electronic medical record system, we collected and organized the data of all participants, including age, gender, BMI, smoking history, duration of disease, current treatment medication, number of swollen joints, and painful joints, Disease Activity Score (DAS28) [21], pulmonary function indices (FEV1, FVC, FEV1/FVC, and DLCO), and patterns of injury (usual interstitial pneumonia [UIP] and nonspecific interstitial pneumonia [NSIP]). For all participants, fasting blood was drawn from the elbow vein on the day of admission; none of the participants had undergone any relevant treatment. The serum was subsequently separated and cryopreserved for subsequent biochemical testing.

2.5 | Peripheral Blood Th17 and Treg Cell Assays

Th17 and Treg cell contents were evaluated with flow cytometry (CytoFLEX, Beckman) as previously described [22], where $CD4^+CD196^+$ was utilized to indicate Th17 cell contents and $CD4^+CD25^+FOXP3^+$ was adopted to indicate Treg cell contents. Cell suspension (1 mL) prepared with PBS was added with 2 μL Cell Activation Cocktail and mixed for 4-h stimulation at 37°C, followed by 5-min centrifugation at 350 g and

resuspending with 100 μL of PBS. Each tube was labeled with CD4 (5 μL) and CD25 (5 μL), and the supernatant was removed after 5-min centrifugation at 350*g* and 4°C in the dark for 30 min. After another 5-min centrifugation at 350*g*, the supernatant was abandoned. Each tube was supplemented with the mixed solution (True-Nuclear 4X Fix Concentrate with True-Nuclear Fix Diluent at 1:3), followed by 50-min incubation at ambient temperature in the dark, 5-min centrifugation at 400*g*, and removal of the supernatant. Subsequently, each tube was resuspended with 1 mL of 1× permeabilization Wash Buffer before centrifugation at 400*g* for 5 min and removal of the supernatant. Upon two repetitions, 100 μL of Perm Buffer working buffer was utilized to resuspend the cells, and CD196 (5 μL) and FOXP3 (5 μL) were appended to each tube for labeling, followed by 30 min of exposure to light at 4°C. The supernatant was discarded subsequent to 5-min centrifugation at 400*g*. Lastly, cells were resuspended in 500 μL of PBS and analyzed with flow cytometry for $CD4^+CD196^+$ and $CD4^+CD25^+FOXP3^+$ cell levels.

The antibodies utilized in the experiment were the following: FITC anti-human CD4 antibody (300505), APC anti-human CD196 (CCR6) antibody (353416), APC anti-human CD25 antibody (302609), PE anti-mouse/rat/human FOXP3 antibody (320007), True-Nuclear Transcription Factor Buffer Set (424401), Permeabilization Wash Buffer (421002), and Cell Activation Cocktail (423303) (all from Biolegend, San Diego, USA).

2.6 | ELISA

A fully automated microplate reader (Multiskan Mk3 model; Thermo Scientific, Massachusetts, USA) was utilized to evaluate the contents of Th17 cytokines (serum IL-17 [ml058051] and IL-6 [ml058097]), Treg cytokines (serum IL-10 [ml064299] and TGF-β1 [ml022522]), and sIL-2R (ml038117). The above ELISA kits were available from mlbio (Shanghai, China) with high sensitivity and specificity, non-reactivity with other cytokines, and intra- and inter-plate coefficients of variation of < 10%.

2.7 | Determination of Disease Progression

The 3-year follow-up records of patients were harvested, and RA-ILD patients were assigned into the non-progression and progression groups. As previously described [21, 23], the criteria for determining the progression of lung disease were the following: (i) self-reported worsening of symptoms (dyspnea, cough, worsening of chest pain, and weight loss); (ii) a decrease in lung function (a decrease in FVC by more than 10% and/or a decrease in DLCO by more than 15%); and (iii) imaging changes: an increase in lesions by more than 20% compared with the baseline HRCT scans or the appearance of new signs of infiltration. If at least one of these criteria was met, the lung lesion was considered to progress, and vice versa, it was considered not to progress.

2.8 | Statistical Analysis

Sample size estimation was implemented using G*Power 3.1.9.7 (University of Düsseldorf, Germany) to ensure adequacy for the independent samples *t*-test, Mann–Whitney *U* test, and chi-square test. For data analysis and visualization, SPSS27.0 statistical software (available from IBM Corp., Armonk, NY, USA) and GraphPad Prism 9.5 software (available from GraphPad Software Inc., San Diego, CA, USA) were utilized. The normal distribution of data was tested with the Kolmogorov–Smirnov method; normally distributed measurement data were depicted as mean ± standard deviation, with independent samples *t*-test for two-group comparisons; non-normally distributed measurement data were depicted as median (minimum, maximum), with Mann–Whitney *U* test for two-group comparisons. Categorical data were expressed as the number of cases (percentages), with chi-squared test for two-group comparisons. Spearman's correlation coefficients were adopted for correlation analysis. ROC curves were plotted for analyzing the predictive value of serum sIL-2R levels and the Th17/Treg ratio for disease progression in RA-ILD patients, and the AUC values of multiple ROC curves were compared with the Delong test in MedCalc software (20.0.15, available from MedCalc Software Ltd., Ostend, Belgium). Independent dangerous factors for disease progression in RA-ILD patients were screened with the multivariate Cox regression analysis. Statistical significance was interpreted at $p < 0.05$.

3 | Results

3.1 | Baseline Data of Participants

No noticeable differences existed between the RA and RA-ILD groups with regard to age, gender, BMI, number of swollen joints and painful joints, and use of csDMARDs and bDMARDs (all $p > 0.05$). Obvious differences were detected in the duration of RA, smoking history, current treatment medication, DAS28, FEV1, FVC, FEV1/FVC, DLCO, and use of corticosteroids and other medications between the two groups (all $p < 0.01$) (Table 1).

3.2 | RA-ILD Patients Have Elevated Serum sIL-2R Levels and Th17/Treg Immune Imbalance

As unveiled by ELISA detection of related cytokines and flow cytometric results (Figure 1), RA-ILD patients had augmented serum levels of sIL-2R, Th17 cells, IL-17, and IL-6, an elevated ratio of Th17/Treg, and decreased Treg cell levels and IL-10 and TGF-β1 levels (all $p < 0.001$). In addition, serum sIL-2R levels, Th17/Treg, and related cytokines (IL-17, IL-6, IL-10, and TGF-β1) were further examined in RA-ILD patients with different patterns of injury (NSIP and UIP). The results revealed that serum sIL-2R levels and IL-17 levels in UIP patients were dramatically higher than those in NSIP patients (both $p < 0.05$), with no statistically significant differences in the ratio of Th17/Treg and the levels of related cytokines (IL-6, IL-10, and TGF-β1) (all $p > 0.05$; Figure S2).

3.3 | Serum sIL-2R Levels Are Positively Linked to the Th17/Treg Ratio in RA-ILD Patients

We further analyzed the correlation of serum sIL-2R levels with the Th17/Treg ratio and related cytokines (IL-17, IL-6, IL-10, and TGF-β1) in RA-ILD patients. The results of Spearman's

TABLE 1 | Comparison of baseline data between RA patients and RA-ILD patients.

Indicator	RA group ($n=194$)	RA-ILD group ($n=137$)	p
Gender (case, %)			
Male	95 (48.97)	58 (42.34)	0.233
Female	99 (51.03)	79 (57.66)	
Age (years)	54.86 ± 8.01	56.20 ± 7.11	0.115
BMI (kg/m^2)	23.42 ± 2.61	23.16 ± 2.32	0.351
RA onset (year)	8.52 ± 2.35	10.29 ± 2.04	< 0.001
ILD onset (year)	—	4.26 ± 1.35	—
Smoking history (case, %)	65 (33.51)	81 (59.12)	< 0.001
Number of swollen joints	4 (1, 8)	4 (1, 7)	0.707
Number of painful joints	6 (1, 12)	7 (1, 12)	0.071
DAS28	4.03 ± 0.85	4.68 ± 1.02	< 0.001
Therapeutic medicine			
csDMARDs (case, %)	182 (93.81)	124 (90.51)	0.263
bDMARDs (case, %)	108 (55.67)	86 (62.77)	0.196
Corticosteroids (case, %)	33 (17.01)	88 (64.23)	< 0.001
Other (case, %)	0 (0.00)	5 (3.65)	0.007
Lung function indicators			
FEV1 (L)	2.61 ± 0.23	2.24 ± 0.32	< 0.001
FVC (L)	3.54 ± 0.47	3.34 ± 0.49	< 0.001
FEV1/FVC (%)	74.21 ± 3.63	67.29 ± 5.97	< 0.001
DLCO (%)	27.78 ± 1.56	25.25 ± 1.74	< 0.001
Types of histopathology			
NSIP	—	31 (22.63)	—
UIP	—	106 (77.37)	—

Note: The normal distribution of data was tested with the Kolmogorov–Smirnov test; normally distributed measurement data were depicted as mean ± standard deviation, with independent samples *t*-test for two-group comparisons; non-normally distributed measurement data were expressed as median (minimum, maximum), with the Mann–Whitney *U* test for two-group comparisons. Categorical data were expressed as the number of cases and percentages, with the chi-squared test for two-group comparisons.
Abbreviations: bDMARDs, biologic disease-modifying antirheumatic drugs; BMI, body mass index; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; DLCO, diffusing capacity of the lung for carbon monoxide; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; NISP, nonspecific interstitial pneumonia; UIP, usual interstitial pneumonia.

analysis demonstrated a marked positive link of serum sIL-2R levels to IL-17 levels and the Th17/Treg ratio in RA-ILD patients ($r=0.432$, $r=0.576$, both $p<0.001$, Figure 2A,B), a notable negative link to IL-10 levels ($r=-0.466$, $p<0.001$, Figure 2D), and no remarkable link to IL-6 and TGF-β1 levels (both $p>0.05$, Figure 2C,E).

3.4 | Analysis of Serum sIL-2R Levels and the Th17/Treg Ratio in RA-ILD Patients With Varying Disease Progression

Subsequently, we harvested the 3-year follow-up records of patients and categorized them into the non-progression group ($n=105$) and the progression group ($n=32$). Between the two group, no remarkable differences were noted with regard to age, gender, BMI, duration of RA, smoking history, number of swollen joints and painful joints, treatment modality, FVC, and types of histopathology (all $p>0.05$), whereas there were notable differences in terms of duration of ILD, DAS28, FEV1, FEV1/FVC, and DLCO (all $p<0.05$) (Table S1). We further analyzed serum sIL-2R levels and the Th17/Treg ratio in RA-ILD patients with different disease progression. The findings exhibited that serum sIL-2R and IL-17 levels and the Th17/Treg ratio were higher ($p<0.001$, Figure 3A,D,E) and IL-10 levels were lower ($p<0.001$, Figure 3G) in the progression group than in the non-progression group.

3.5 | Serum sIL-2R Levels Can Assist in Predicting Disease Progression in RA-ILD Patients

Based on the aforesaid results, we plotted ROC curves for evaluating the predictive value of serum sIL-2R levels and the Th17/Treg ratio alone and in combination for disease progression in RA-ILD patients. It was observed that serum sIL-2R assisted in the prediction of disease progression in RA-ILD patients (AUC = 0.807 [95% CI: 0.730–0.869], cut off = 433.3, sensitivity = 84.37%, and specificity = 66.67%). The Th17/Treg ratio (%) assisted in predicting disease progression in RA-ILD patients (AUC = 0.746 [95% CI: 0.665–0.817], cut off = 70.25, sensitivity = 87.50%, and specificity = 63.81%); the combination of serum sIL-2R and the Th17/Treg ratio (%) assisted in the prediction of disease progression in RA-ILD patients (AUC = 0.826 [95% CI: 0.752–0.885], sensitivity = 84.37%, and specificity = 72.38%) (all $p<0.001$, Figure 4). Finally, the comparative analysis of AUC values by MedCalc indicated that the AUC of serum sIL-2R levels combined with the Th17/Treg ratio for predicting disease progression in RA-ILD patients was slightly higher than that of serum sIL-2R levels ($p=0.516$) or the Th17/Treg ratio ($p=0.066$) alone, with no prominent difference (Table S2).

3.6 | Elevated Serum sIL-2R Serves as an Independent Risk Factor for Disease Progression in RA-ILD Patients

Finally, for accurately assessing the influence of serum sIL-2R levels on the disease condition of RA-ILD patients, we used the disease status of RA-ILD patients 3 years after treatment (0 = no progression, 1 = progression) as the dependent variable and subsequently used the indicators with $p<0.05$ in Table S1; Figure 3 as the independent variables for the multivariate Cox regression analysis. The findings presented that the DAS28 score, serum

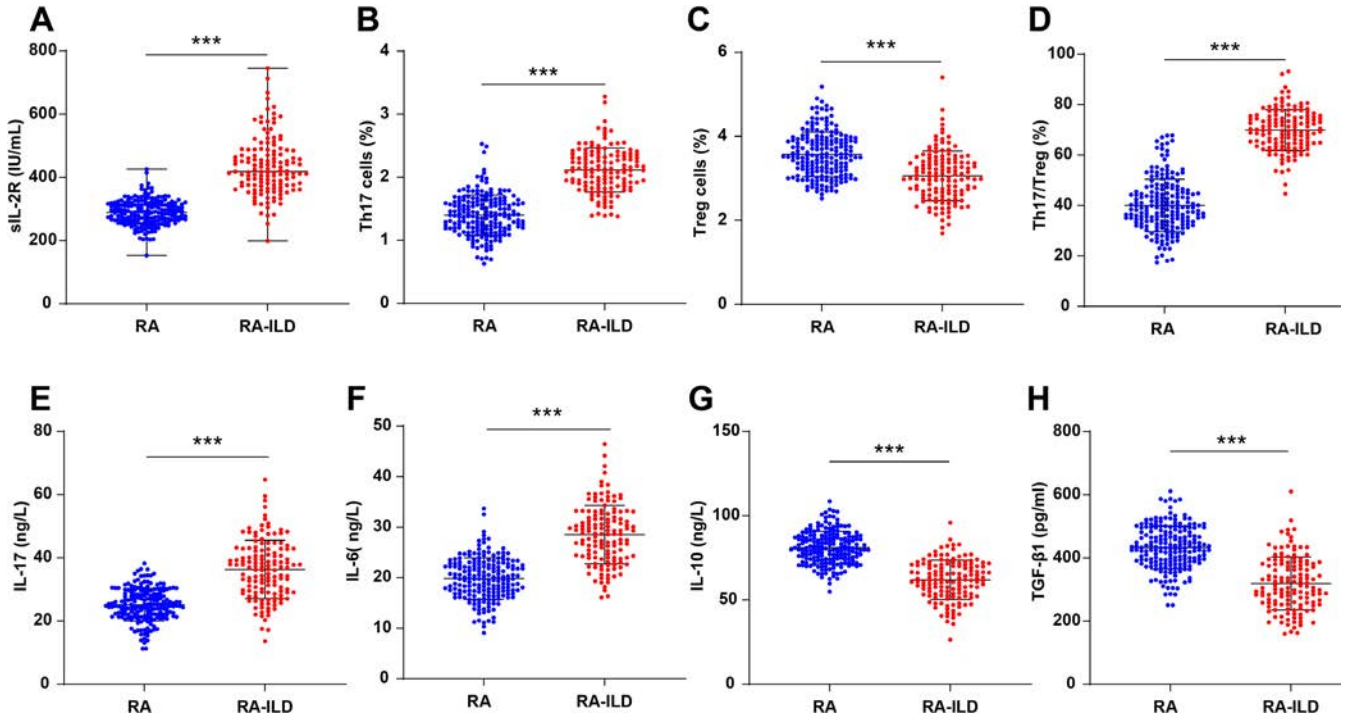

FIGURE 1 | Comparisons of serum sIL-2R levels, Th17/Treg ratio, and related cytokines between the two groups. Serum sIL-2R (A) and IL-17 (E), IL-6 (F), IL-10 (G), and TGF-β1 (H) expression levels were detected by ELISA, and Th17 (B) and Treg (C) cell levels were tested by flow cytometry, and Th17/Treg (D) was calculated. Normally distributed measurement data were depicted as mean ± standard deviation, with independent samples *t*-test for two-group comparisons; non-normally distributed measurement data were expressed as median (minimum, maximum), with Mann–Whitney *U* test for two-group comparisons. ***$p < 0.001$.

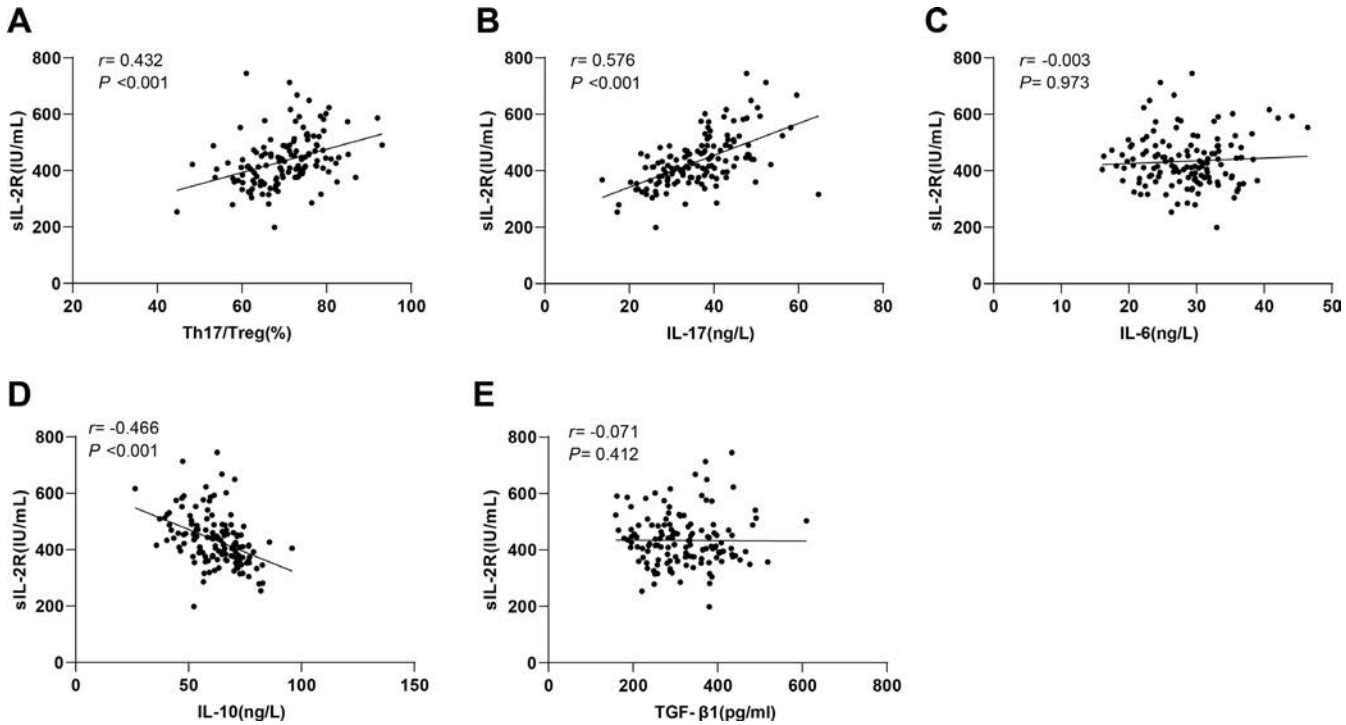

FIGURE 2 | Correlation of serum sIL-2R with the Th17/Treg ratio and related cytokines in RA-ILD patients. Spearman's analysis was used to analyze the correlations of serum sIL-2R with the Th17/Treg ratio and related cytokines [Th17/Treg (A), IL-17 (B), IL-6 (C), IL-10 (D), and TGF-β1 (E)]. r is the correlation coefficient.

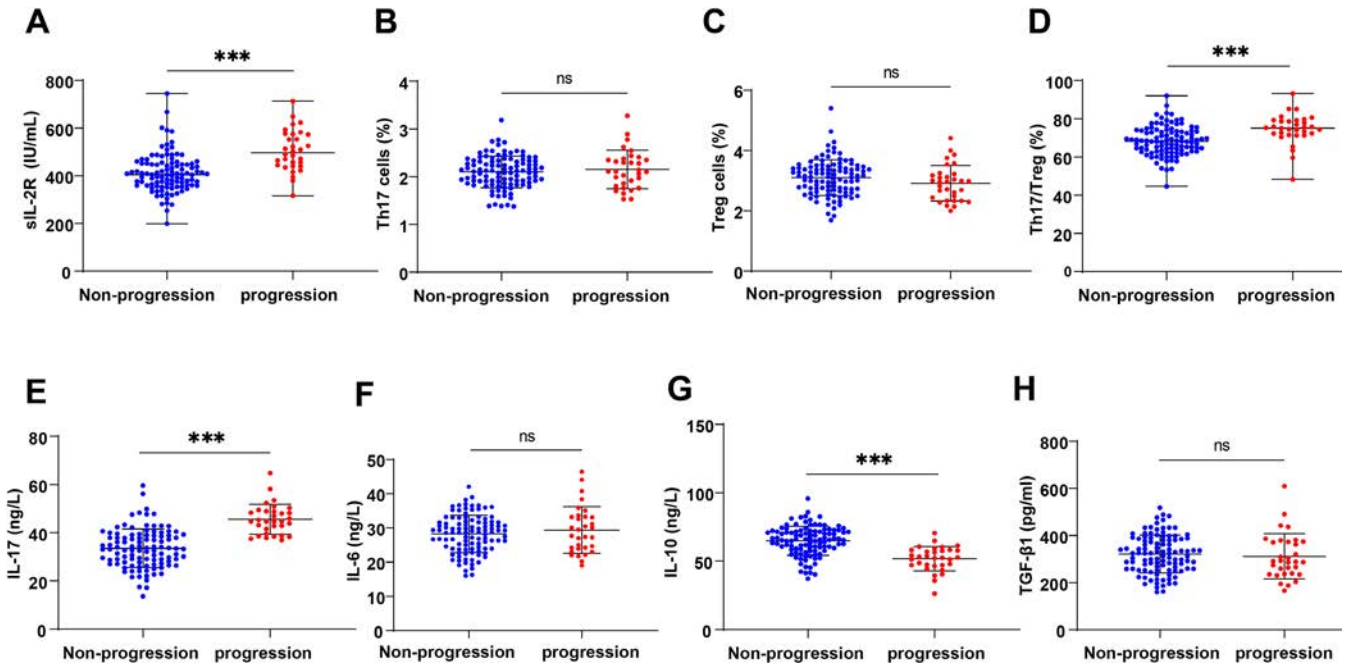

FIGURE 3 | Comparisons of serum sIL-2R levels, Th17/Treg ratio, and related cytokines between the two groups. Serum sIL-2R (A) and IL-17 (E), IL-6 (F), IL-10 (G), and TGF-β1 (H) expression levels were detected by ELISA, and Th17 (B) and Treg (C) cell levels were tested by flow cytometry, and Th17/Treg (D) was calculated. Normally distributed measurement data were depicted as mean ± standard deviation, with independent samples *t*-test for two-group comparisons; non-normally distributed measurement data were expressed as median (minimum, maximum), with Mann–Whitney *U* test for two-group comparisons. ***$p < 0.001$, ns $p > 0.05$.

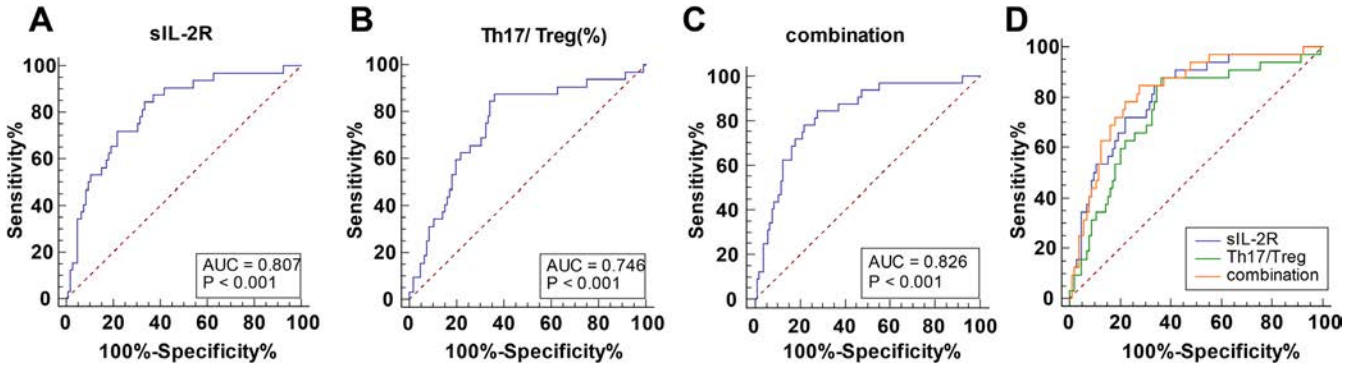

FIGURE 4 | Serum sIL-2R levels and Th17/Treg ratio for predicting disease progression in RA-ILD patients. Predictive value of serum sIL-2R levels (A) and the Th17/Treg ratio (B) alone and in combination (C–D) for disease progression in patients with RA-ILD by ROC curve analysis.

sIL-2R, and elevated Th17/Treg ratio were independent hazardous factors for disease progression in RA-ILD patients, and elevated FEV1 and FEV1/FVC were protective factors (all $p < 0.05$, Table 2).

4 | Discussion

This paper mainly explored the link between serum sIL-2R levels and Th17/Treg immune balance in RA-ILD patients and their prognostic value. Our results indicated an elevation in serum sIL-2R levels in RA-ILD patients, which was positively correlated with the Th17/Treg ratio. More importantly, these two factors could assist in predicting the disease progression of RA-ILD patients.

In this research, RA-ILD patients exhibited higher Th17 cell proportions, Th17 cytokines (IL-17 and IL-6), and Th17/Treg ratio but lower Th17 cell proportions and Treg cytokines (IL-10 and TGF-β1) than RA patients, illustrating the existence of Th17/Treg immune imbalance. Th17 and Treg cells are important for immune homeostasis, among which Th17 cells show a promoting role while Treg cells exhibit a suppressive role in autoimmunity and inflammatory responses [24]. Inflammation is caused by the broken balance between inflammatory responses and self-tolerance, namely Th17/Treg immune imbalance [25]. Immune dysregulation is a vital manifestation of RA-ILD, and targeting Th17/Treg immune imbalance may be a potent therapeutic approach for RA-ILD [26]. IL-17 cytokines, including IL-17A, IL-17F, IL-6, and IL-23, are implicated in the progression of multiple inflammatory and autoimmune diseases, like multiple

TABLE 2 | Multivariate Cox regression analysis of factors affecting disease progression in patients with RA-ILD.

Item	*p*	HR	95%CI
ILD onset (year)	0.283	1.148	0.892 ~ 1.477
DAS28	0.021	1.762	1.089 ~ 2.853
FEV1 (L)	0.029	0.143	0.025 ~ 0.816
FEV1/FVC (%)	0.014	0.897	0.823 ~ 0.978
DLCO (%)	0.475	0.900	0.675 ~ 1.201
Th17/Treg (%)	0.017	1.071	1.012 ~ 1.134
IL-10 (ng/L)	0.089	0.963	0.922 ~ 1.006
IL-17 (ng/L)	0.071	1.049	0.996 ~ 1.105
sIL-2R (IU/ml)	0.039	1.004	1.000 ~ 1.008

sclerosis, RA, psoriasis, ankylosing spondylitis, and osteoarthritis [27, 28]. Treg cells release inhibitory cytokines that limit the generation of pro-inflammatory cytokines and impair effector T cell activation and proliferation [29]. In this study, we further found that IL-17 levels and the Th17/Treg ratio increased while IL-10 levels decreased in RA-ILD patients with disease progression, indicating a possible correlation of Th17/Treg immune imbalance with disease progression. RA-ILD with different patterns of injury (UIP, NSIP, and OP) show different clinical responses to immune modulators, suggesting different cytokine profiles. Accordingly, we further classified the RA-ILD patients included in this study into two groups according to the types of histopathology: UIP ($n = 106$; 77.37%) and NSIP (31; 22.63%) groups. The results revealed that serum sIL-2R levels and IL-17 levels in UIP patients were dramatically higher than those in NSIP patients (both $p < 0.05$), with no statistically significant differences in the ratio of Th17/Treg and the levels of related cytokines (IL-6, IL-10, and TGF-β1) (all $p > 0.05$). This result may be attributed to the fact that the two subtypes, UIP and NSIP, are very similar in clinical and imaging manifestations and both are related to Th17/Treg immune balance; NSIP has milder clinical symptoms and better prognosis than UIP since milder inflammatory responses in NSIP lead to slight differences in the levels of pro-inflammatory factors between patients with the two subtypes.

The most pivotal finding in this paper was that serum sIL-2R levels were positively relevant to the Th17/Treg ratio and that elevated serum sIL-2R levels in combination of the Th17/Treg ratio could assist in predicting disease progression in RA-ILD. IL-2 constitutes one of the key cytokines in the immune system that participates in the modulation of protective immunity, as well as the maintenance of Treg-mediated immune tolerance [30, 31]. IL-2 can act on cells expressing either trimeric high-affinity IL-2R or dimeric low-affinity IL-2R; the activated T lymphocytes not only up-regulated cellular IL-2R levels but also secreted sIL-2R [16]. Serum sIL-2R, a soluble form of IL-2R, is helpful to distinguish disease progression from damage in inflammatory myopathies [32]. An elevation in serum sIL-2R levels are noted in sarcoidosis, one type of ILD, showing diagnostic and prognostic values for chronic sarcoidosis [33]. Although serum sIL-2R has been widely accepted as a diagnostic marker for RA with its potential of reflecting lymphocyte activation [34, 35], there is currently no study mentioning the relation between serum sIL-2R and RA-ILD. An existing study has linked serum sIL-2R concentrations to Th17 cell subsets and the Th17/Treg ratio in dermatomyositis [18]. Consistently, our paper suggested a positive link of serum sIL-2R concentrations to the Th17/Treg ratios in RA-ILD. Furthermore, based on ROC curves, this research demonstrated the superior performance of serum sIL-2R levels in conjunction with the Th17/Treg ratio in predicting disease progression in RA-ILD patients. Ultimately, this study highlighted increased DAS28 score, serum sIL-2R levels, and Th17/Treg ratio as hazardous markers while elevated FEV1 and FEV1/FVC as protective markers for RA-ILD, contributing to developing new diagnostic and prognostic markers for this disease.

Altogether, this research validates the link between serum sIL-2R and Th17/Treg immune balance in the course of RA-ILD. Based on this, the combined detection of serum sIL-2R levels and the Th17/Treg ratio shows a superior predictive value for disease progression in RA-ILD patients. Nevertheless, limitations still exist in this article. First, potential bias cannot be avoided since it is a single-center retrospective study with a relatively small sample size. Second, only short-term prognosis was assessed in this study. Regular surveillance of serum sIL-2R levels can be conducted in future studies to further explore the influence of serum sIL-2R levels on the long-term survival of RA-ILD patients. Third, this research only selected the most representative Th17 cytokines (IL-17 and IL-6) and Treg cytokines (IL-10 and TGF-β1) and preliminarily explored the levels of these cytokines in the serum of RA-ILD patients. Fourth, the mechanism of sIL-2R and Th17/Treg immune balance in regulating the occurrence and development of RA-ILD is still incompletely clarified. Fifth, some factors that contribute to disease progression in RA-ILD may be related to disease activity measured by DAS-28. Follow-up studies will focus on this aspect to exclude the influence of DAS-28 on the results, thus increasing the accuracy of our findings. All these issues warrant more extensive and in-depth research. Nevertheless, this study provided information for new prognostic markers of RA-ILD patients.

Author Contributions

Yaxiong Jin, Yixue Guo are the guarantors of the integrity of the entire study and contributed to the data acquisition. Yaxiong Jin, Chunying Zhang contributed to the study concepts, manuscript preparation, and review. Yong He, Bin Ding contributed to the statistical analysis and clinical studies. Qian Niu, Chunying Zhang, and Yang Fu contributed to the study design, literature research, and definition of intellectual content. Yong He, Qian Niu, and Ling Tang contributed to the data analysis and manuscript editing; All authors read and approved the final manuscript.

Acknowledgments

The authors have nothing to report.

Ethics Statement

The research was granted by the Research Ethics Committee of West China Hospital, and all participants were informed of the experimental purpose and signed informed consent forms.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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LETTER TO THE EDITOR

Nailfold Capillaroscopy as Predictor for Autoantibody Test Results

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Dear editor,
Nailfold capillaroscopy (NFC) is an established diagnostic method for several autoimmune diseases, as it allows drawing conclusions about the clinical characteristics of patients. Among these diseases are systemic sclerosis, Lupus erythematosus, and idiopathic inflammatory myopathies, such as dermatomyositis. All of these may present with associated or even specific autoantibodies. We investigated whether the morphologies and patterns in NFC also have a predictive value for sensitization to myositis-specific and myositis-associated autoantibodies.

We retrospectively collected data from 233 patients who were treated at the Department of Dermatology between November 2017 and December 2022. The patients were screened for myositis-specific (MSA) and myositis-associated autoantibodies (MAA) by EUROLINE immunoblot (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). There was no screening for antinuclear (ANA) or extractable nuclear antibodies (ENA) in our study. Of the total population, 51 patients underwent NFC within 90 days before or after immunoblot. NFC was performed on 8 fingers of both hands. Changes in capillary morphology and flow velocity were documented on the basis of the nomenclature of Sander et al. [1]. The immunoblot was able to detect 16 different antibodies. The results showed the following prevalences: Mi2α 13.7% (7), Mi2β 3.9% (2), TIF1γ 5.9% (3), MDA5 0.0% (0), NXP2 2.0% (1), SAE1 3.9% (2), Ku 2.0% (1), PM-Scl75 5.9% (3), PM-Scl100 5.9% (3), Jo-1 2.0% (1), SRP 3.9% (2), PL-72.0% (1), PL-12 2.0% (1), EJ 0.0% (0), OJ 0.0% (0) and Ro-52 3.9% (2). 36.8% (7) of antibody-positive patients were simultaneously sensitized to 2–4 antibodies. We compared the positive group (PG) of 37.3% (19) who had a positive immunoblot result with the negative group (NG) of 62.7% (32) who were tested negative. The individual files were reviewed in order to determine the patients' diagnoses. The diagnoses dermatomyositis, systemic sclerosis, inclusion body myositis, lupus disease, and Raynaud's phenomenon were included. The subtypes of dermatomyositis included dermatomyositis, clinically amyopathic dermatomyositis, paraneoplastic dermatomyositis, and overlap myositis. Lupus disease includes chilblain LE, LE tumidus, and subacute cutaneous LE. Cases without a confirmed diagnosis of an autoimmune disease were identified on the basis of the individual documentation and assigned to the "Other" category for the analysis. The descriptive analysis revealed no significant differences for the patient characteristics reported in Table 1.

NFC was well-assessed in 62.7% (32) of the patients. In 76.5% (39) of cases, the flow was normal, while in 3.9% (2) of cases, the flow was slowed.

The most common morphologies overall were ectasia with 78.4% (40), hemorrhage with 56.9% (29), branching with 54.9% (28), megacapillary with 52.9% (27) and edema with 43.1% (22).

There was a statistically significant difference between the positive and negative groups for the occurrence of branching ($p = 0.046$), which occurred more frequently in the negative group (65.6% vs. 36.8%) (Figure 1b). To determine the potential predictive value of NFC for the outcome of the immunoblot, we conducted a logistic regression analysis. The baseline model only contained the morphology branching. The result was not significant, but there was a tendency for the presence of branching to be associated with a 69.4% reduced chance of a positive immunoblot result compared to cases without branching (OR 0.306, $p = 0.050$, 95% CI 0.094–0.998). In further exploratory

TABLE 1 | Report of patient characteristics separately for immunoblot (IB) positive and negative group.

	Total ($N=51$)	IB positive group 37.3% ($N=19$)	IB negative group 62.7% ($N=32$)	p
Sex (♀/♂), frequency in % (N)				
	♀66.7 (34) ♂33.3 (17)	♀57.9 (11) ♂42.1 (8)	♀71.9 (23) ♂28.1 (9)	0.365
Age at the time of laboratory testing, mean (SD) [years]				
	51.18 (20.424)	50.2 (22.188)	51.8 (19.649)	0.798
Clinical diagnosis, frequency in % (N)				
Dermatomyositis disease	43.1 (22)	68.4 (13)	28.1 (9)	
Dermatomyositis	17.6 (9)	21.1 (4)	15.6 (5)	
Clinical amyopathic dermatomyositis	9.8 (5)	10.5 (2)	9.4 (3)	
Paraneoplastic dermatomyositis	3.9 (2)	10.5 (2)	0.0 (0)	
Overlap myositis	11.8 (6)	26.3 (5)	3.1 (1)	
Systemic sclerosis[a]	9.8 (5)	10.5 (2)	9.4 (3)	
Inclusion body myositis	2.0 (1)	0.0 (0)	3.1 (1)	
Lupus disease[b]	5.9 (3)	0.0 (0)	9.4 (3)	
Raynaud phenomenon	9.8 (5)	10.5 (2)	9.4 (3)	
Others	29.4 (15)	10.5 (2)	40.6 (13)	

[a]Systemic sclerosis includes diffuse and limited cutaneous forms.
[b]Lupus disease includes chilblain LE, LE tumidus, and subacute cutaneous LE. Cases without a confirmed diagnosis of an autoimmune disease are described in the "others" category.

analyses, additional morphologies were included in the models. A model including both branching and rarefication/avascular fields predicted immunoblot outcome better than branching alone. The presence of branching significantly decreased the likelihood of autoantibody detection ($p=0.035$, OR 0.259, 95% CI 0.074–0.908). Conversely, rarefication or avascular fields showed a tendency towards a positive result ($p=0.281$, OR 2.260, 95% CI 0.514–9.950). Factors such as gender, age at the time of the immunoblot, and disease duration did not significantly influence immunoblot outcome. However, there was a non-significant trend that the chance of a positive immunoblot increased slightly with each additional year of disease duration ($p=0.266$; OR 1.097; 95% CI 0.932–1.292).

Previous studies have investigated the relationship between NFC and antibodies in patients with various rheumatic diseases. Some reported significant differences in capillary changes for different antibody findings. In a study by Mugii et al., anti-TIF1γ-positive patients were significantly more likely to have rarefaction than anti-MDA5 or anti-ARS-positive patients with idiopathic inflammatory myositis ($N=71$) [2]. In Wakura et al., hemorrhage was more pronounced in MDA5-positive patients than in anti-ARS-positive patients with dermatomyositis ($N=27$) [3]. Santos et al. reported associations of the morphologies rarefaction and branching with the occurrence of anti-MDA5 antibodies in idiopathic inflammatory myositis (IIM) patients ($N=95$) [4]. Mercer et al. found a lower capillary density in anti-Jo-1-positive patients compared to anti-Jo-1-negative patients with idiopathic inflammatory myositis ($N=24$) [5]. In contrast, we analyzed the patient collective according to positive or negative autoantibody status, so that both mono- and poly-sensitized, as well as seronegative, patients were included. The different study designs make it difficult to compare the results.

The logistic regression analysis showed a lower chance of antibody sensitization when branching was detected in the NFC. There are some studies that also created logistic regression models. In this way, Chebbi et al. were able to establish rarefication as an indicator for the presence of anti-U1RNP in SLE patients ($N=54$) [6]. In a study by Müller et al. on adults with Raynaud's phenomenon, the occurrence of certain morphologies such as reduced capillary density, avascular fields, or branching was related to ANA-titres ≥320 ($N=671$) [7]. The logistic regression analysis tested whether certain morphologies can be used as an indicator for the presence of antibodies. We showed that the chance of autoantibody positivity for MSA/MAA was reduced when branching was detected in NFC. Other morphologies were not significant. We therefore conclude that NFC findings cannot be used to decide whether antibody testing is appropriate. To date, NFC has been used as a complementary test in the diagnosis of autoimmune diseases. Our data show that NFC is a suitable diagnostic tool to support the clinical diagnosis of autoimmune diseases in the absence of detectable autoantibodies (Figure 1c). In clinical practice, clinical diagnosis and synthesis of multiple diagnostic procedures remain crucial to diagnose autoimmune diseases.

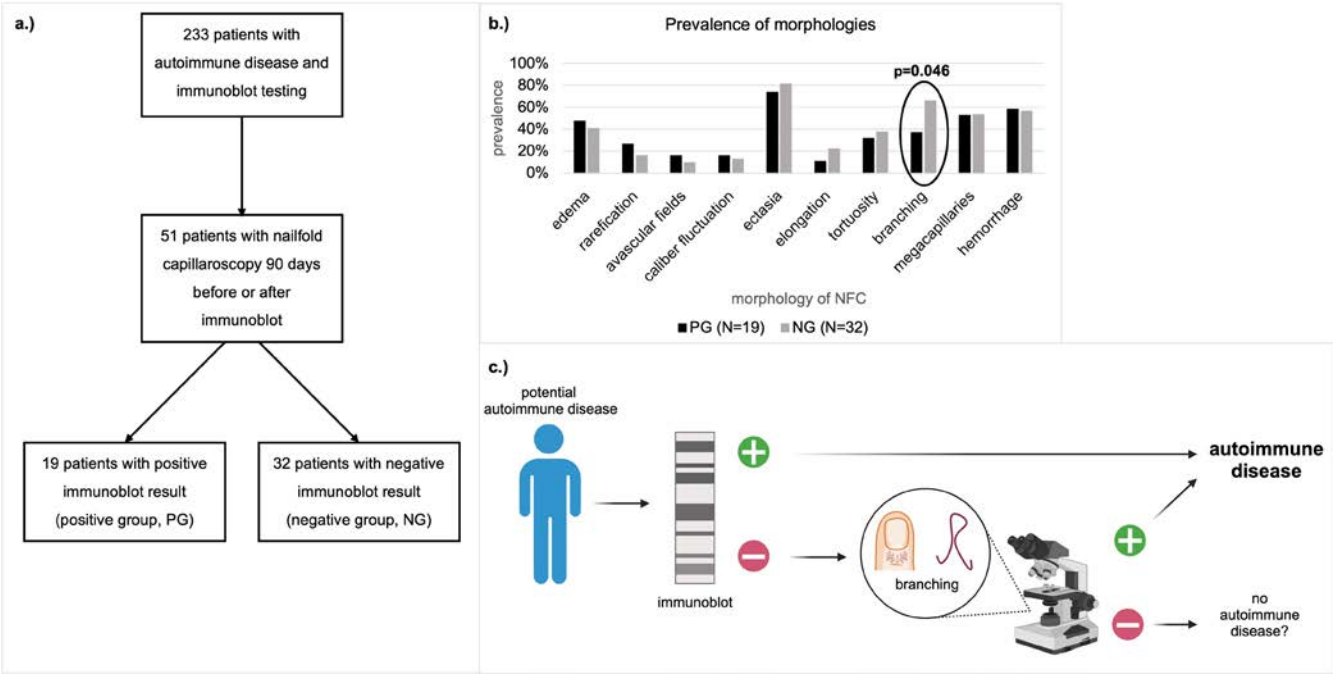

FIGURE 1 | (a) Inclusion criteria of the study population and formation of the subgroups shown in a flow chart. (b) Prevalence of the individual morphologies of nailfold capillaroscopy separately for positive and negative groups. The *x*-axis shows the individual morphologies of nailfold capillaroscopy. The *y*-axis shows the prevalence of the morphology separately for PG and NG in %. The morphology branching was observed statistically significantly more frequently in the NG ($p = 0.046$). p, *p* value; *N*, number. (c) Schematic illustration of the conclusions. Branching morphology in NFC has a predictive value for the negative autoantibody testing using a dermatomyositis immunoblot. A negative immunoblot result does not refute an autoimmune diagnosis, and NFC is a suitable diagnostic tool to support the clinical diagnosis of autoimmune diseases in the absence of detectable autoantibodies. The image was created with BioRender (Science Suite Inc., Toronto, Canada).

The model strategy of logistic regression analysis can serve as a basis for future prospective studies and could help to alleviate the inconsistencies between study results independent of study designs. During planning, the calculation of the sample size, the matching by age and gender, and a comparison group of healthy subjects should be considered. In addition, it would be prudent to expand the antibody profile to include ANA/ENA.

Author Contributions

Mara Karcher performed formal analysis, investigation, data curation, visualization, and contributed to conceptualization, methodology, validation, and writing, reviewing, and editing of the original draft. Anke Strölin contributed to conceptualization, methodology, supervision, reviewing, and editing of the original draft. Sebastian Volc contributed to conceptualization, methodology, validation, supervision, writing, reviewing, and editing of the original draft. All authors read and approved the final manuscript.

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Ethics Statement

This project was approved by the ethics committee of the Medical Faculty of Eberhard Karls University Tübingen.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Mara Karcher
Anke Strölin
Sebastian Volc

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ORIGINAL ARTICLE

Clinical Significance of Hematological Indices as Disease Activity Markers in Patients With Ankylosing Spondylitis Following Treatment With Tumor Necrosis Factor Inhibitors

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ABSTRACT

Background: The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Ankylosing Spondylitis Disease Activity Score with C-reactive protein (ASDAS-CRP) are commonly used to measure disease activity in patients with AS.

Aim: This study was conducted to determine the power of hematological indices to serve as disease activity markers in AS patients treated with tumor necrosis factor (TNF) inhibitors.

Methods: A total of 222 patients with AS were recruited and classified into active disease (BASDAI ≥ 4, $n = 158$) and remission (BASDAI < 4, $n = 64$) groups. The active group was treated with TNF inhibitors for 3 months. Composite indices such as BASDAI and ASDAS-CRP were measured to assess disease activity. Hematological indices as alternative disease activity markers including neutrophil-to-lymphocyte ratio (NLR), neutrophil-to-hemoglobin and lymphocyte (NHL) score, systemic immune-inflammation index (SII), and platelet-to-lymphocyte ratio (PLR) were assessed.

Results: Patients with active AS showed higher NLR, NHL score, SII, and PLR than those in remission. ASDAS-CRP and BASDAI values at baseline were significantly associated with all hematological indices, including NLR, NHL score, SII, and PLR. Similar to the improvement of BASDAI and ASDAS-CRP scores following TNF inhibitors treatment, NLR, NHL score, SII, and PLR markedly decreased after treatment with TNF inhibitors for 3 months ($p < 0.001$ for all, respectively). All hematological indices closely predicted major improvement ($\Delta \geq 2.0$ of ASDAS-CRP) following treatment with TNF inhibitors.

Conclusion: This study indicated that four hematological indices may be useful markers of disease activity and predictors of treatment response to TNF inhibitors in patients with AS. Further studies in larger populations are needed to validate the usefulness of hematological indices as measures of disease activity in patients with AS.

Abbreviations: AS, ankylosing spondylitis; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C-reactive protein; DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; NHL, neutrophil-to-hemoglobin and lymphocyte; NLR, neutrophil-to-lymphocyte ratio; NSAIDs, nonsteroidal anti-inflammatory drugs; PLR, platelet-to-lymphocyte ratio; RA, rheumatoid arthritis; SII, systemic immune–inflammation index; SLE, systemic lupus erythematosus; SpA, spondyloarthritis; TNF, tumor necrosis factor.

1 | Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease characterized by predominant involvement of the axial skeleton, resulting in bone ankylosis and sometimes also affecting peripheral joints, enthesis, or extra-articular organs [1, 2]. Although the pathogenic mechanisms of AS have not been clearly determined, some genetic factors such as HLA-27, non-HLA-B27 MHC alleles, and non-MHC genes including *ERAP1* may contribute to inflammation and new bone formation in spondyloarthritis (SpA) [3, 4]. Significant advances have been made in diverse pharmacological options including nonsteroidal anti-inflammatory drugs (NSAIDs), biologic disease-modifying antirheumatic drugs (bDMARDs), or targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs) and multidisciplinary strategies for the treatment of axial SpA (axSpA) [4, 5].

Numerous measures for assessing disease activity and severity and predicting prognosis are currently available in clinical practice and research in AS. In the assessment of disease activity, the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), which is a self-administered patient questionnaire, has been widely used in AS [6]. The Ankylosing Spondylitis Disease Activity Score (ASDAS), which is a composite index generated by both patient-oriented ratings and laboratory measures such as C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR), is now available for radiographic and nonradiographic axSpA [7, 8].

The mechanisms and postulated functions for acute phase proteins such as CRP, ESR, and fibrinogen in the inflammatory response are well understood [9]. In addition, changes in the differential components of white blood cells (WBCs) and platelets are associated with systemic or subclinical inflammation in acute or chronic inflammatory disorders. Recently, numerous studies have found that the neutrophil/lymphocyte ratio (NLR) or platelet/lymphocyte ratio (PLR) could be closely associated with disease activity measures, such as the BASDAI and ASDAS-CRP and other acute phase reactants, and also with disease severity in AS [10–18]. Hematological indices such as NLR or PLR have been found to reflect disease activity well in patients with AS receiving tumor necrosis factor (TNF) inhibitors [10, 12, 15]. In addition to NLR and PLR, novel hematological indices such as the systemic immune-inflammation index (SII) were associated with disease activity indices in patients with AS [19–22]. However, no data about neutrophil/(hemoglobin × lymphocyte) (NHL) score are available in AS.

Although BASDAI and ASDAS-CRP are commonly used to assess axSpA, more reasonable markers for the assessment of disease activity and severity, treatment response, and prognosis are needed in clinical practice. This study investigated whether hematological indices such as NLR, PLR, SII, and NHL scores were associated with activity indices including BASDAI and ASDAS-CRP and closely reflected changes in treatment response in AS patients treated with TNF inhibitors.

2 | Subjects and Methods

2.1 | Study Population

A total of 222 patients were consecutively enrolled in the outpatient clinic of the Division of Rheumatology at Daegu Catholic University Medical Center from January 2020 to December 2023. All patients met the modified New York criteria as diagnostic criteria for AS proposed by the American College of Rheumatology [23]. Exclusion criteria included patients with psoriatic SpA, reactive arthritis, inflammatory bowel disease-related SpA, and undifferentiated SpA. bDMARDs, including TNF inhibitors or interleukin-17 (IL-17) inhibitors, can be initiated when patients have high disease activity (ASDAS ≥ 2.1) according to the Assessment of SpondyloArthritis International Society (ASAS)-EULAR recommendations for axSpA [5]. However, the National Health Insurance Act in Korea recommends the initiation of bDMARDs when disease activity is BASDAI of 4 or higher. We therefore classified patients into two groups: active disease (BASDAI ≥ 4) and remission (BASDAI < 4) (Figure 1). Patients with active disease ($n = 158$) received TNF inhibitors if at least two nonsteroidal anti-inflammatory drugs (NSAIDs) were ineffective and their BASDAI was 4 or higher [5]. Patients with BASDAI < 4 were assigned to the remission group ($n = 64$). Because this study involves retrospective analysis, the need for informed consent from study participants was waived. The study was approved by the Institutional Review Board of Daegu Catholic University Hospital (CR-24-057-L).

2.2 | Collection of Clinical Data

Demographic data including age (year), sex, disease duration (year), body mass index (kg/m^2), and HLA-B27 positivity at

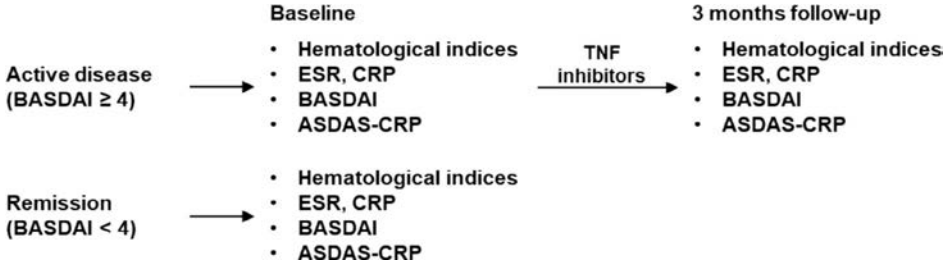

FIGURE 1 | Study population flowchart. ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score-CRP; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate. Hematological indices include NLR, PLR, SII, and NHL scores.

baseline were identified. Disease-related clinical features such as peripheral arthritis, uveitis, and enthesitis were identified after reviews of medical records. Acute phase reactants, including CRP and ESR, were also assessed. We identified current medications including NSAIDs, methotrexate, sulfasalazine, and corticosteroids being used by patients with AS and TNF inhibitors such as infliximab, etanercept, adalimumab, and golimumab at study enrollment.

2.3 | Assessment of Disease Activity Indices in AS

This study evaluated disease-related disease activity markers such as BASDAI and ASDAS-CRP [6, 7]. Among patients with active disease with a BASDAI ≥ 4, we arbitrarily classified them into two groups: $2.1 \leq$ ASDAS-CRP < 3.5 for high activity and ASDAS-CRP ≥ 3.5 for very high activity based on ASDAS-CRP [7]. A change of ≥ 2.0 units of ASDAS-CRP between pretreatment and treatment with TNF inhibitors was defined as a "major improvement."

2.4 | Assessment of Hematological Indices

Hematological data such as total WBC ($10^3/\mu L$) and differential WBC components including neutrophils ($10^3/\mu L$) and lymphocytes ($10^3/\mu L$), hemoglobin (g/dL), and platelets ($10^3/\mu L$) were collected to construct hematological indices before and 3 months after the administration of TNF inhibitors in the active disease group and at baseline in the remission group. Hematological indices such as NLR, PLR, NHL score, and SII were calculated by these formulas using neutrophil, lymphocyte, hemoglobin, and platelet as follows: NLR = neutrophil/lymphocyte ratio, PLR = platelet/lymphocyte ratio, NHL score (g/dL) = neutrophil/(hemoglobin × lymphocyte) ratio, and SII = platelet × neutrophil/lymphocyte ratio [24].

2.5 | Statistical Analysis

Data are presented as median (interquartile ranges) and numbers (%) of cases. The normality distribution of data was assessed by the Kolmogorov–Smirnov test. Comparisons of categorical variables between the active disease and remission groups were performed using the Chi-square test. The Mann–Whitney test was used to compare parametric variables between two groups (active disease vs. remission and $2.1 \leq$ ASDAS-CRP < 3.5 vs. ASDAS-CRP ≥ 3.5, respectively). A comparison of changes in hematological indices between baseline and a 3-month follow-up in the active disease group was performed using the Wilcoxon signed-rank test. Spearman's correlation was used to estimate the correlation between hematological indices and AS-related disease activity markers. In addition, the performance of hematological indices in predicting major improvement in patients treated with TNF inhibitors was evaluated using receiver operating characteristic (ROC) curve analysis with estimation of the area under the curve (AUC) and 95% confidence intervals (CIs). Statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). The results were considered statistically significant if the p-value was < 0.05.

3 | Results

3.1 | Baseline Characteristics

A total of 222 patients, including 158 active disease patients and 64 remission patients, were analyzed in this study (Table 1 and Figure 1). Patients with active disease who received TNF inhibitors for at least 3 months had BASDAI of 4 or higher at baseline. Active disease patients had more frequent peripheral arthritis than remission patients ($p = 0.004$). Traditional disease activity indices, including ESR, CRP, BASDAI, and ASDAS-CRP, were significantly higher in active disease patients than those in remission patients ($p < 0.001$ for all). The use of methotrexate, sulfasalazine, and corticosteroid was higher in active disease patients than in remission patients. The median doses of corticosteroids used in active patients were significantly higher than those in remission patients ($p < 0.001$). The median doses of corticosteroids in active patients before the administration of TNF inhibitors were 2.5 mg (0.0, 5.0), and the doses of corticosteroids administered after the administration of TNF inhibitors were 0.0 mg (0.0, 2.5).

There are significant differences in complete blood counts (CBCs) and differential counts, including WBC, hemoglobin, platelets, and neutrophils, between active disease and remission groups ($p < 0.001$ for all), but not in lymphocytes ($p > 0.05$). In the assessment of differences in hematological indices based on CBCs between the two groups, active disease patients had significantly higher NLR, NHL score, SII, and PLR compared with patients in remission.

3.2 | Correlation Between Hematological Indices and Disease Activity Indices

We assessed whether hematological indices were associated with disease activity indices such as BASDAI, ASDAS-CRP, ESR, and CRP (Table 2). All hematological indices including NLR, NHL score, SII, and PLR were significantly associated with ESR ($p < 0.001$ for all), CRP ($p < 0.001$ for all), BASDAI ($p < 0.001$ for all), and ASDAS-CRP ($p < 0.001$ for all) in total AS patients, respectively. In active AS patients ($n = 158$), ASDAS-CRP, ESR, and CRP were significantly associated with all hematological indices, including NLR, NHL score, SII, and PLR, but not BASDAI. In contrast, neither BASDAI nor ASDAS-CRP was associated with all hematological indices in remission patients ($n = 64$). However, associations were found between NHL score and ESR as well as between SII and CRP. In addition, only ASDAS-CRP in remission patients was associated with ESR ($p = 0.002$) and CRP ($p < 0.001$), respectively, but not BASDAI.

3.3 | Changes in Hematological Indices Between Before and After Use of Anti-TNF Inhibitors

We compared disease activity indices and hematological indices before and after the use of TNF inhibitors in active disease patients (Figure 2). BASDAI and ASDAS-CRP after the use of TNF inhibitors significantly decreased, compared with before the use of TNF inhibitors (7.2 [6.6, 7.8] vs. 2.8 [2.6, 3.3], $p < 0.001$ and 4.1 [3.7, 4.6]

TABLE 1 | Baseline characteristics of study population.

Variables	Active disease ($n=158$)	Remission ($n=64$)	p
Age (year)	40.0 (32.0, 48.0)	38.0 (25.3, 49.8)	0.438
Gender (male)	138 (87.3)	53 (82.8)	0.378
Disease duration (year)	8.0 (2.7, 14.3)	9.3 (3.7, 20.2)	0.242
Body mass index (kg/m^2)	24.2 (21.6, 27.4)	23.7 (22.2, 25.3)	0.240
HLA-B27 positivity (n, %)	150 (94.9)	59 (92.2)	0.429
Clinical features (n, %)			
Peripheral arthritis	73 (46.2)	16 (25.0)	0.004
Uveitis	37 (23.4)	16 (25.0)	0.802
Enthesitis	25 (15.8)	10 (15.6)	0.971
Disease activity indices			
ESR (mm/h)	29.0 (16.0, 53.0)	12.5 (7.3, 25.0)	<0.001
CRP (mg/L)	12.6 (6.0, 23.9)	2.8 (0.8, 4.4)	<0.001
BASDAI	7.2 (6.6, 7.8)	2.3 (1.2, 3.4)	<0.001
ASDAS-CRP	4.1 (3.7, 4.6)	1.6 (1.0, 2.1)	<0.001
Medications (n, %)			
NSAIDs	157 (99.4)	62 (96.9)	0.145
Methotrexate	54 (34.2)	4 (6.3)	<0.001
Sulfasalazine	144 (91.1)	48 (75.0)	0.001
Corticosteroid	113 (71.5)	15 (23.4)	<0.001
Corticosteroid (mg/day)	2.5 (0.0, 5.0)	0.0 (0.0, 0.0)	<0.001
TNF inhibitors (n, %)			
Infliximab	39 (24.7)		
Etanercept	34 (21.5)		
Adalimumab	72 (45.6)		
Golimumab	13 (8.2)		
Hematological indices			
White blood count ($\times10^3/\mu$L)	8.4 (6.9, 9.4)	7.1 (6.3, 8.3)	<0.001
Hemoglobin (g/dL)	13.9 (12.8, 14.8)	14.6 (14.0, 15.4)	<0.001
Platelet ($\times10^3/\mu$L)	281.5 (240.0, 344.0)	228.0 (196.3, 265.5)	<0.001
Neutrophil ($\times10^3/\mu$L)	5.2 (4.2, 6.2)	4.2 (3.3, 5.0)	<0.001
Lymphocytes ($\times10^3/\mu$L)	2.1 (1.7, 2.6)	2.2 (1.8, 2.5)	0.853
NLR	2.4 (1.8, 3.2)	1.9 (1.6, 2.6)	0.001
NHL score (g/dL)	0.17 (0.13, 0.24)	0.12 (0.11, 0.17)	<0.001
SII	698.3 (471.4, 1007.6)	444.6 (331.4, 589.0)	<0.001
PLR	138.2 (106.7, 178.7)	113.5 (88.9, 132.8)	<0.001

Note: Data were described as median (interquartile range) for quantitative variables and number of cases (%) for qualitative variables. p-values were calculated by Chi-square test or Fisher's exact test for qualitative variables and Mann–Whitney U test for quantitative variables.
Abbreviations: ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score-CRP; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NHL, neutrophil-to-hemoglobin and lymphocyte; NLR, neutrophil-to-lymphocyte ratio; NSAIDs, non-steroidal anti-inflammatory drugs; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index; TNF, tumor necrosis factor.

TABLE 2 | Correlation between hematological indices and disease activity indices in ankylosing spondylitis at baseline.

Disease activity group	Disease activity indices	Hematological indices							
		NLR		NHL score		SII		PLR	
		r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Total ($n=222$)	BASDAI	0.241	<0.001	0.286	<0.001	0.364	<0.001	0.262	<0.001
	ASDAS-CRP	0.393	<0.001	0.443	<0.001	0.568	<0.001	0.454	<0.001
	ESR	0.359	<0.001	0.446	<0.001	0.543	<0.001	0.436	<0.001
	CRP	0.416	<0.001	0.452	<0.001	0.595	<0.001	0.484	<0.001
Active disease ($n=158$)	BASDAI	0.110	0.170	0.121	0.131	0.135	0.090	0.066	0.410
	ASDAS-CRP	0.413	<0.001	0.439	<0.001	0.532	<0.001	0.433	<0.001
	ESR	0.342	<0.001	0.436	<0.001	0.538	<0.001	0.464	<0.001
	CRP	0.442	<0.001	0.460	<0.001	0.571	<0.001	0.484	<0.001
Remission ($n=64$)	BASDAI	0.077	0.543	0.096	0.453	0.012	0.926	−0.040	0.752
	ASDAS-CRP	0.069	0.587	0.095	0.456	0.143	0.260	0.011	0.930
	ESR	0.199	0.114	0.247	0.049	0.219	0.082	0.068	0.591
	CRP	0.176	0.165	0.214	0.089	0.339	0.006	0.136	0.284

Note: *p*-values were calculated by Spearman's rank correlation analysis. Data were described as correlation coefficient (*r*).
Abbreviations: ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score-CRP; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NHL, neutrophil-to-hemoglobin and lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index.

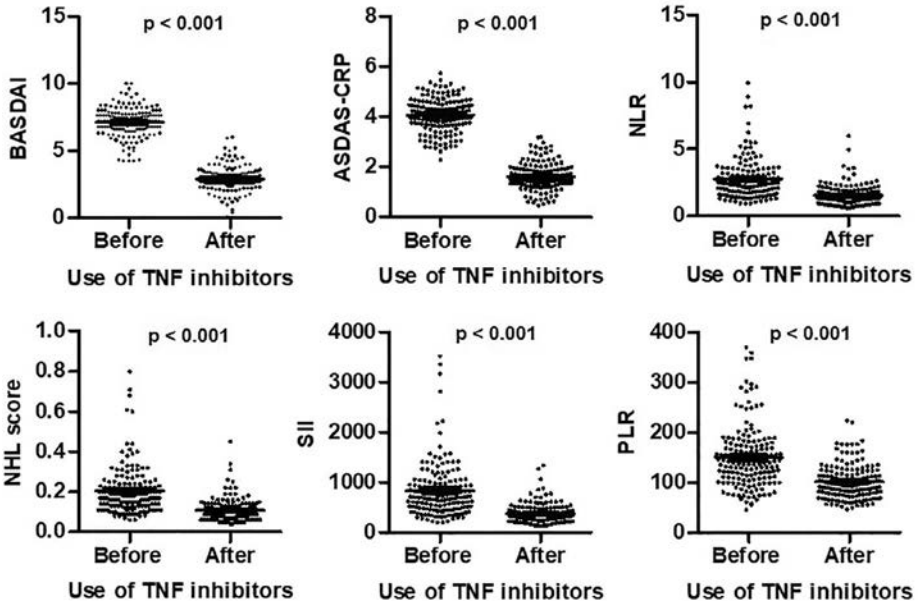

FIGURE 2 | Comparison of disease activity indexes and hematological indexes before and after the use of TNF inhibitors. *p*-values were calculated by the Wilcoxon signed-rank test. ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score-CRP; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; NHL, neutrophil-to-hemoglobin ratio and lymphocyte; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index; TNF, tumor necrosis factor.

vs. 1.5 [1.3, 1.9], $p<0.001$, respectively). NLR, NHL score, SII, and PLR significantly decreased after the use of TNF inhibitors, compared to before the use of TNF inhibitors (2.4 [1.9, 0.32] vs. 1.3 [1.1, 1.8], $p<0.001$; 0.18 [0.13, 0.24] vs. 0.10 [0.08, 0.12] $p<0.001$; 698.3 [471.4, 1007.6] vs. 322.4 [246.0, 430.7], $p<0.001$; and 138.2 [106.7, 178.7] vs. 95.3 [76.9, 118.1], $p<0.001$, respectively).

3.4 | Differences in Hematological Indices Based on ASDAS-CRP in Active AS Patients

Among active disease patients receiving TNF inhibitors, 30 patients had $2.1 \leq$ ASDAS-CRP < 3.5 and 128 patients had ASDAS-CRP ≥ 3.5 at baseline. We compared the differences

TABLE 3 | Comparison of the differences in hematological indices at baseline according to disease activity based on ASDAS-CRP in active disease patients.

Hematological indices	Disease activity based on ASDAS-CRP		
	2.1 ≤ ASDAS-CRP < 3.5	ASDAS-CRP ≥ 3.5	*p*
NLR	1.7 (1.4, 2.4)	2.5 (2.0, 3.3)	< 0.001
NHL score	0.12 (0.10, 0.17)	0.19 (0.14, 0.25)	< 0.001
SII	434.9 (312.2, 641.7)	758.4 (546.7, 1069.9)	< 0.001
PLR	106.2 (80.1, 135.2)	147.0 (114.9, 183.0)	< 0.001

Note: Data were described as median (interquartile range) for quantitative variables and number of case (%) for qualitative variables. *p*-values were calculated by Mann–Whitney *U* test.
Abbreviations: ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score-CRP; NHL, neutrophil-to-hemoglobin and lymphocyte; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index.

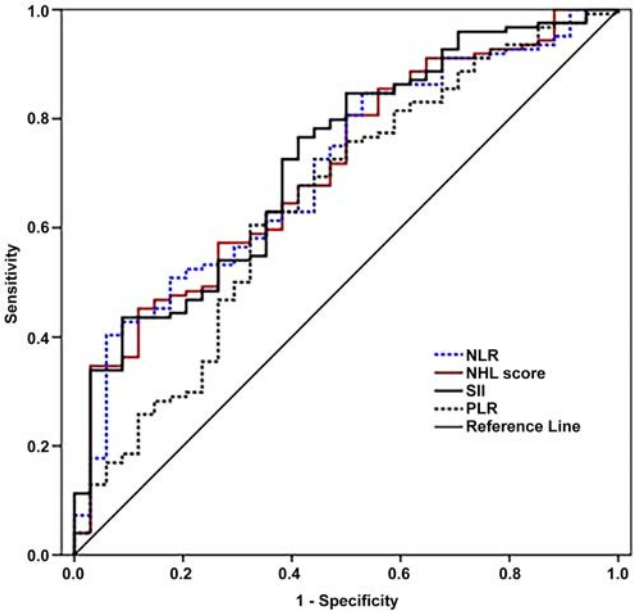

FIGURE 3 | Receiver operating characteristic curve analysis of hematological indices in the major improvement after receiving TNF inhibitors. AUC, area under the curve; NHL, neutrophil-to-hemoglobin and lymphocyte; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index.

in hematological indices between 2.1 ≤ ASDAS-CRP < 3.5 and ASDAS-CRP ≥ 3.5 in patients with active disease (Table 3). Patients with very high disease activity (ASDAS-CRP ≥ 3.5) showed significant increases in NLR, NHL score, SII, and PLR, compared with those with high disease activity (2.1 ≤ ASDAS-CRP < 3.5) ($p < 0.001$ for all).

3.5 | Determination of Predictive Value of Hematological Indices for Major Improvement Following Treatment With TNF Inhibitors Using ROC Curves

Among 158 active disease patients receiving TNF inhibitors, 124 patients (78.5%) achieved major improvement, and 151 patients (95.6%) achieved clinically important improvement after treatment with TNF inhibitors. A ROC analysis was used to determine the value of hematological indices as predictors of major improvement after the use of TNF inhibitors in active AS patients (Figure 3). The AUCs for hematological indices were illustrated as follows: 0.707 (0.613–0.801), $p < 0.001$ for NLR; 0.709 (0.615–0.804), $p < 0.001$ for NHL score; 0.725 (0.631–0.819), $p < 0.001$ for SII; and 0.649 (0.541–0.757), $p = 0.008$ for PLR.

4 | Discussion

The NLR and PLR have emerged as simple indicators of disease activity and severity in variable inflammatory or immune-mediated diseases using ratios between neutrophils or platelets and lymphocytes released to the peripheral bloodstream [25, 26]. Neutrophils are immune cells with effective antimicrobial abilities against exogenous or endogenous pathological stimuli [27]. Abundance of neutrophils in the blood may cause harmful damage in compromised and even healthy host tissues. Accumulating evidence suggests that the pathological properties of neutrophils contribute to the development or progression of numerous inflammatory and autoimmune diseases [25]. Neutrophils can modulate the innate and adaptive immunity of immune responses by cytokine production by macrophages and activation and recruitment of dendritic cells, together with the promotion of T-cell antigen presentation and B-cell activation and antibody production. In addition, lymphocytes are immune cells that are responsible for the immunomodulatory response to invading microbes or foreign molecules in a host [28]. Abnormal variations in lymphocyte counts may indicate diverse physiological and pathological conditions such as bacterial or fungal infections, autoimmune rheumatic diseases, or intense exercise.

Meta-analyses have demonstrated that NLR could be a reliable biomarker in the diagnosis and monitoring of disease activity in some autoimmune rheumatic diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [29, 30]. Considering the clinical relevance of NLR in AS, numerous studies have explored whether NLR is associated with susceptibility, disease activity, and disease severity in patients with AS. Most of these studies found that NLR in patients with AS was significantly higher than that in controls [11–14, 16]. However, some studies found no difference in NLR levels between AS patients and controls [10, 17]. In this study, we found higher NLR values in the active disease group (BASDAI ≥ 4) compared with those in the remission group (BASDAI < 4), which was consistent with the findings of other case–control studies [16, 17]. However, there was no difference in NLR values between the active (BASDAI ≥ 4) and inactive (BASDAI < 4) groups in Chinese AS patients [18]. The present study found that AS patients with very high disease activity (ASDAS-CRP ≥ 3.5) showed much higher NLR than did patients with high activity (2.1 ≤ ASDAS-CRP < 3.5) in the active disease group (BASDAI ≥ 4). Given the association between NLR and AS-specific disease activity indices, NLR was

markedly associated with BASDAI [11], but other studies did not find similar associations [14, 17]. In this study, there was a close association between NLR and AS-specific activity indices such as BASDAI and ASDAS-CRP in total or active disease patients, but no association was noted for patients in remission. Given the association between NLR and two traditional markers of acute phase reactants, previous studies also reported that NLR was closely related to inflammatory responses based on ESR and CRP [12, 14, 16]. This study also found evidence for a significant association between NLR and ESR and CRP in active disease patients, but not in those in remission. This difference may be due to the fact that only about 60% of patients had increased ESR and CRP despite high disease activity [31]. We observed that some hematological indices are only partially correlated with other serologic inflammation markers such as ESR or CRP in patients in remission. Furthermore, nonserological components such as back pain, morning stiffness, or global patient assessment in patients with low disease activity or remission may have only a minimal impact on hematological indices [21]. Therefore, hematological indices are thought to have a poor correlation with BASDAI and ASDAS-CRP in patients with low disease activity.

Platelets are important immune cells in peripheral blood and are known to be involved in thrombosis and hemostasis [32]. They are closely related to diverse autoimmune rheumatic diseases, prothrombotic disorders, and metabolic diseases [26]. Changes in platelet counts reflect disease activity through excessive shifting to inflammatory or damaged tissues in autoimmune rheumatic diseases. Along with NLR, numerous studies have been conducted on PLR in relation to disease activity in AS. The validity of PLR as a marker of disease activity in AS or in differences from the control group is somewhat inconsistent depending on the study designs. Some studies found that active patients (BASDAI ≥ 4) had higher PLR values compared with inactive patients (BASDAI < 4) [17, 18]. In addition, Al-Osami et al. [17] reported evidence of a close association between PLR and BASDAI. Not only the disease activity of PLR but PLR values also increased with the progression of the max sacroiliitis grade of AS ($p = 0.0246$) and were higher in max Bath Ankylosing Spondylitis Radiology Index (BASRI) ≥ 2 of the hip joint compared to BASRI < 2 ($p = 0.0284$), reflecting the severity of AS [18]. In addition, a positive correlation was seen between PLR and ESR. In contrast, PLR values in AS patients were lower or no different from those in the control group [10, 13, 17]. In the present study, PLR values were in the higher active disease group compared with the remission group and were significantly associated with other disease activity indices, including BASDAI, ASDAS-CRP, ESR, and CRP, particularly in patients with active disease.

Besides neutrophils, lymphocytes, and platelets among the components of hematological indices, the NHL score is another hematological index that additionally considers hemoglobin. Inflammation induces changes in the bone marrow hematopoiesis environment, triggering increased production of innate immune effector cells, while inhibiting the differentiation and maturation of lymphoid cells and erythrocytes [33]. Anemia related to inflammation can develop due to impaired erythropoiesis and iron sequestration in patients with chronic illnesses, such as autoimmune rheumatic diseases, chronic infection, neoplasms, and renal failure [34]. The present study consistently found significantly lower hemoglobin levels in active disease patients compared with those in remission. The NHL score is an independent marker associated with disease activity in RA and is a predictor of major adverse cerebrocardiovascular events (MACCEs) at 2 years in acute myocardial infarction [24, 35]. However, no data on the association between the NHL score and AS-related disease activity are available. We found that active disease patients had a higher NHL score than did patients in remission. In addition, the NHL score was positively correlated with BASDAI, ASDAS-CRP, ESR, and CRP, suggesting that the NHL score may serve as a useful indicator of disease activity in patients with AS.

A recent meta-analysis study evaluated the diagnostic role of SII, another novel hematological index, in diverse immune-mediated rheumatic diseases such as RA, SLE, and AS [36]. The meta-analysis using four different studies demonstrated that SII was significantly higher in AS patients ($n = 365$) than in controls ($n = 288$) (standardized mean differences = 0.88, 95% CI 0.71–1.05, $p < 0.001$) [19–22]. Considering the diagnostic accuracy of the SII for AS, the AUC values of the ROC for SII were 0.832 (0.622 in sensitivity and 0.96 in specificity) [22]. In addition, the predictive value of SII for new-onset AS was still significant (OR 0.006, 95% CI 1.002–1.010, $p = 0.003$). In a comparison of SII between active disease and remission patients, three different clinical studies found that SII was higher in the active group (BASDAI ≥ 4) than in the remission group (BASDAI < 4) [19–21]. In the assessment of the diagnostic performance of the SII for active disease, Wu et al. [19] showed that the AUC values for SII were 0.877 (0.868 in sensitivity and 0.833 in specificity). In the assessment of the relationship of SII to other disease activity indicators for AS, SII was markedly associated with ESR, CRP, ASDAS, or BASDAI [19–21]. The present study also confirmed that SII was higher in the active disease group compared with the remission group, and there was a close association between SII and ESR, CRP, ASDAS, or BASDAI. This suggests that SII is a promising indicator not only to assess disease activity but also to predict the development of AS.

Treatment strategies for axSpA have advanced significantly in recent years and now include nonpharmacologic and pharmacologic interventions [5]. Treatment options to manage disease activity and symptoms have expanded with the use of TNF inhibitors, IL-17 inhibitors, and JAK inhibitors. In recent decades, TNF inhibitors have been the first option for AS in patients who do not respond to NSAIDs. Several factors related to a favorable response to TNF inhibitors have been identified, including younger age, increased CRP levels, and HLA-B27 positivity [37]. Some clinical studies evaluated whether the use of TNF inhibitors affects hematological indices including NLR and PLR in AS patients [10, 12, 15]. Boyraz et al. [10] demonstrated that PLR in patients treated with TNF inhibitors was significantly lower than in controls, whereas NLR did not differ between the two groups. In a comparison of NLR between TNF inhibitors and NSAIDs, patients receiving TNF inhibitors had significantly lower NLR than those with NSAIDs [12]. In an analysis of changes in NLR and PLR at 3 and 6 months after initiation of TNF inhibitors in Turkish AS patients, both NLR and PLR were markedly reduced compared with pretreatment [15]. In the present study, we also found significantly reduced NLR, PLR, SII, and NHL scores at 3 months after TNF inhibitors treatment

compared with pretreatment. Few clinical studies have examined the ability of hematological indices to predict treatment outcomes after the administration of TNF inhibitors in patients with AS. This study confirmed the potential of hematological indices to serve as predictors of major improvement after treatment with TNF inhibitors. It suggests that hematological indices such as NLR or PLR may be good indicators that reflect the response to treatment.

Glucocorticoids, which have powerful anti-inflammatory and immunosuppressive properties, have been widely used as a therapeutic agent for various inflammatory and immune-mediated diseases [38]. One of their main actions is to increase the number of WBCs, especially neutrophils, whereas counts of eosinophils and lymphocytes decrease in the peripheral blood through various mechanisms. This suggests that glucocorticoids administered to patients with AS to control inflammation may affect the number of neutrophils and lymphocytes in peripheral blood, resulting in higher hematological indices compared to patients who received fewer corticosteroids. In this study, we found that there was a correlation between doses of corticosteroid and hematological indices (data not shown). However, hematological indices showed a higher correlation with ESR and CRP (Table 2). Therefore, it is difficult to conclude that hematological indices are dependent on corticosteroids.

There are several limitations to findings of this study. First, the study population was retrospectively recruited from patients at a single university-based medical center. Because the clinical and laboratory data were extracted from a single center, there may be problems validating data from multiple centers. Second, the subject composition of previous studies was as follows: AS patients versus controls or active group versus inactive group. No healthy control group participated in this study because the main purpose of this study was to verify the availability of hematological indices according to AS activity or TNF inhibitor treatment. Third, hematological indices are inflammation markers based on the components of blood cells. There are accumulated data showing that these indices are closely related to disease activity in patients with AS (Table 4). However, it does not seem to have the potential to replace composite disease activity measures such as BASDAI, ASDAS-CRP, and BASFI, which include patient-reported queries and objective inflammatory markers, to assess inflammation, function status, and general health. To overcome these limitations in clinical practice, it is thought that assessment of the normal ranges or cut-off values of each hematological index is necessary.

5 | Conclusion

The relevance of novel biomarkers using counts of neutrophils, platelets, and lymphocytes measured in peripheral blood has been closely examined in studies of numerous inflammatory, neoplastic, infectious, and cardiovascular diseases [25, 26]. It has been attempted to determine whether hematological indices are related to disease activity and severity in patients with AS (Table 4). In addition to hematological indices introduced in previous studies such as NLR and PLR, novel indices are being introduced [20–22]. In this study, in addition to the widely used NLR, PLR, and SII, the clinical usefulness of the novel index NHL, which has not been analyzed in patients with AS, was evaluated. This study found that significant differences in NLR, PLR, NHL score, and SII were evident between active disease and remission in AS patients. All four hematological indices were significantly associated with BASDAI, ASDAS-CPR, ESR, and CRP, particularly in the active disease group. NLR, PLR, NHL score, and SII were potential predictors of major

TABLE 4 | Summary of the role of hematological indices as disease activity markers in ankylosing spondylitis.

Previous studies	Study populations	Results[a]
Boyraz I et al. [10]	105 AS/50 controls	NLR: AS = control, PLR: AS < control
Kucuk A et al. [11]	102 AS/60 controls	NLR: AS > control
Gökmen F et al. [12]	96 AS/81 controls	NLR: AS > control, TNFi user < NSAIDs user
Bozan N et al. [13]	30 AS/35 controls	NLR: AS > control, PLR: AS = control
Enginar AU et al. [15]	203 AS	NLR and PLR: before TNFi use > after TNFi use
Mercan R et al. [14]	140 AS/117 controls	NLR: AS > control
Xu S et al. [16]	765 AS/701 controls	NLR: inactive AS < active AS
Al-Osami MH et al. [17]	132 AS/181 controls	NLR and PLR: AS = control and inactive AS < active AS
Liang T et al. [18]	180 AS/95 non-AS	NLR: inactive AS = active AS, PLR: inactive AS < active AS
Wu J et al. [19]	136 AS/63 controls	SII: AS > control and inactive AS < active AS
Taha SI et al. [20]	50 AS/100 controls	NLR: AS = control, PLR and SII: AS > control
Sariyildiz A et al. [21]	100 AS/50 controls	NLR, PLR, SII: AS > control and inactive AS = active AS
Luo Q et al. [22]	79 AS/75 controls	NLR, PLR, SII: AS > control

Abbreviations: AS, ankylosing spondylitis; NLR, neutrophil-to-lymphocyte ratio; NSAIDs, non-steroidal anti-inflammatory drugs; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index; TNFi, tumor necrosis factor inhibitor.
[a]The statistical differences of each hematological index between two groups are indicated by > (greater than), < (less than), = (equal to), or ≥ (greater than or equal to). Patients with BASDAI < 4 are considered to be inactive (or remission) AS, and BASDAI ≥ 4 are considered to be active AS.

improvement 3 months after treatment with TNF inhibitors. Further studies with larger sample sizes and prospective designs may be needed to validate the usefulness of hematological indices in the assessment of disease activity and clinical outcomes in patients with AS.

Author Contributions

Conceptualization: Seong-Kyu Kim. Data curation: Jung-Yoon Choe and Seong-Kyu Kim. Formal analysis: Seong-Kyu Kim. Investigation: Jung-Yoon Choe and Seong-Kyu Kim. Methodology: Seong-Kyu Kim. Supervision: Seong-Kyu Kim. Writing – original draft: Seong-Kyu Kim. Writing – review and editing: Seong-Kyu Kim.

Acknowledgments

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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ORIGINAL ARTICLE

Evaluation of Inflammatory Scores as Diagnostic Markers for Polymyalgia Rheumatica

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ABSTRACT

Objective: Polymyalgia rheumatica (PMR) is a chronic inflammatory rheumatic disease affecting older adults, with symptoms often overlapping with those of other conditions, thus making its diagnosis challenging. The present study evaluated the utility of laboratory-based inflammatory scores, including the systemic immune-inflammation index (SII), C-reactive protein-to-albumin ratio (CAR), albumin-to-globulin ratio (AGR), and prognostic nutritional index (PNI), as diagnostic tools for PMR and explored their association with clinical manifestations.

Methods: This retrospective study analyzed the medical records of 156 patients diagnosed with PMR and 408 diagnosed with rheumatoid arthritis (RA) between 2001 and 2021 to evaluate the diagnostic performance of inflammatory scores in distinguishing between the two conditions. Statistical analyses, including receiver operating characteristic (ROC) curve analysis and logistic regression, were used to assess the accuracy and effectiveness of the scores.

Results: Patients with PMR exhibited higher SII and CAR scores and lower AGR and PNI values than those with RA. CAR demonstrated the best diagnostic performance, with an area under the ROC curve (AUC) of 0.823 (95% confidence interval [CI], 0.784–0.861), followed by SII (AUC, 0.797 [95% CI, 0.757–0.837]), AGR (AUC, 0.696 [95% CI, 0.648–0.743]), and PNI (AUC, 0.691 [95% CI, 0.641–0.741]). Combining these scores improved diagnostic ability, with an AUC of approximately 0.835. Furthermore, elevated SII or CAR values and decreased AGR or PNI values were associated with leucocytosis, anemia, elevated erythrocyte sedimentation rate/C-reactive protein, hypalbuminaemia, fever, weight loss, and headache.

Conclusions: Serological inflammatory scores, such as SII, CAR, AGR, and PNI, were useful as diagnostic markers for PMR, with combined scores enhancing overall diagnostic accuracy.

1 | Introduction

Polymyalgia rheumatica (PMR) is a chronic inflammatory disorder that predominantly affects older adults, typically presenting in individuals > 50 years of age. Characterized by muscle pain and stiffness, particularly in the shoulders, hips, and neck, PMR significantly impairs daily functioning and quality of life [1]. These clinical symptoms are usually bilateral, most pronounced in the morning, gradually improving during the day, and worsening after rest or during periods of prolonged inactivity [2]. Adult females are affected 2–3 times more frequently than males, and 40%–50% of patients may develop systemic symptoms, including fever, fatigue, malaise, weight loss, and depression [3]. Although PMR is relatively common, its diagnosis remains challenging due to its nonspecific symptoms, clinical features overlapping with other conditions, and the absence of definitive diagnostic tests [4].

The diagnosis of PMR is primarily based on clinical presentation, patient history, and physical examination and is supported by laboratory findings of elevated levels of inflammatory markers [5]. Current classification criteria from the American College

Summary

- This study evaluated the utility of inflammatory scores, including systemic immune-inflammation index (SII), C-reactive protein-to-albumin ratio (CAR), albumin-to-globulin ratio (AGR), and prognostic nutritional index (PNI), as diagnostic tools for polymyalgia rheumatica (PMR).
- Combination of these inflammatory scores enhances diagnostic accuracy and may be especially valuable in clinical settings where advanced imaging tools may be limited.
- Findings suggest that inflammatory scores derived from routine laboratory investigations offer a cost-effective and accessible means of assisting in the diagnosis of PMR.

of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) recommend that PMR diagnosis includes age > 50 years, bilateral shoulder pain, elevated erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP) level, and prompt response to glucocorticoid treatment [6]. However, these criteria are not definitive; they lack specific pathological findings to confirm the diagnosis, and elevated ESR and CRP levels are nonspecific indicators of inflammation that may also be present in various other inflammatory, infectious, and malignant conditions.

Differentiating PMR from other diseases is a crucial—yet challenging—task. Conditions, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), giant cell arteritis (GCA), and various malignancies, can present with symptoms similar to those of PMR, particularly in the early stages of the disease [7, 8]. This difficulty is compounded by the risk for misdiagnosis and subsequent inappropriate treatment. Misdiagnosing PMR can result in unnecessary glucocorticoid exposure, and failure to identify more serious underlying conditions, such as malignancy, can have severe consequences [9]. Given these challenges, reliable and noninvasive diagnostic tools are urgently needed [10].

Emerging evidence suggests that laboratory-based inflammatory scores are valuable tools for evaluating the inflammatory status of patients with systemic autoimmune disorders [11–13]. These indices include the systemic immune inflammation index (SII), C-reactive protein-to-albumin ratio (CAR), albumin-to-globulin ratio (AGR), and prognostic nutritional index (PNI) [13]. Given their potential utility, we aimed to assess the effectiveness of these inflammation scores in the diagnosis of PMR. Additionally, we evaluated their diagnostic performance and explored their association with clinical manifestations and disease activity.

2 | Methods

2.1 | Study Design and Population

This single-centre, retrospective study was conducted at Ajou University Hospital (Gyeonggi-do, Korea) using data collected from electronic medical records between January 2001 and August 2021. Patients with PMR were included if they were diagnosed according to the 2012 Provisional Classification Criteria from the EULAR/ACR. The exclusion criteria included systemic inflammatory conditions, malignancies, or infections. The control group consisted of patients diagnosed with RA who were ≥ 50 years of age and fulfilled either the 1987 ACR or 2010 ACR/EULAR classification criteria [14, 15]. A relapse was defined as the aggravation or reappearance of PMR symptoms and an elevation in inflammatory markers, typically occurring during glucocorticoid tapering or discontinuation, and requiring re-escalation of therapy. In cases of suspected relapses, careful clinical evaluation was conducted to rule out other conditions, such as osteoarthritis or comorbidities, that might be masked by glucocorticoids [16]. The study protocol was reviewed and approved by the Institutional Review Board of Ajou University Hospital (AJIRB-MDB-2021-447). The requirement for informed consent was waived.

2.2 | Data Collection

Data collected for this study included age at diagnosis, sex, symptom duration, follow-up period, clinical symptoms and signs, laboratory results, treatments, and outcomes for both the PMR and RA groups. The inflammatory scores analyzed included SII, CAR, AGR, and PNI. These scores were calculated as follows: SII = platelet count × (neutrophil count/lymphocyte count); CAR = CRP level/albumin level; AGR = albumin level/(total protein—albumin level); and PNI = albumin level + 0.005 × lymphocyte count.

Inflammatory marker measurements, including SII, CAR, AGR, and PNI, were calculated based on laboratory results obtained at the time of diagnosis before initiating any treatment. This ensured that measurements reflected baseline inflammatory activity without treatment influence, maintaining consistency across PMR and RA groups for valid comparisons.

2.3 | Statistical Analysis

Statistical analyses were performed using SPSS version 25.0 (IBM Corporation, Armonk, NY, USA). Continuous variables are expressed as mean ± standard deviation (SD); differences with $p < 0.05$ were considered to be statistically significant. Comparisons between the PMR and RA groups for categorical variables were performed using the chi-squared or Fisher's exact tests, whereas continuous variables were compared using an independent *t*-test or Mann–Whitney *U* test. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic performance of the inflammatory scores, with the area under the ROC curve (AUC) indicating overall diagnostic accuracy. A logistic regression model was also developed to assess the diagnostic effectiveness of combined biomarkers. Pearson's correlation analysis was used to explore the relationships between inflammatory scores and disease activity indices. Differences in scores based on clinical symptoms among patients with PMR were evaluated using an independent *t*-test.

3 | Results

3.1 | Demographic and Baseline Characteristics

The present study included 156 patients diagnosed with PMR and 408 with RA; demographic and baseline characteristics are summarized in Table 1. Patients with PMR had a mean (±SD) age of 65.6 ± 9.63 years, were slightly older than those with RA (64.1 ± 10.3 years), with no significant difference ($p = 0.106$). The proportion of females in the PMR group was 70.5% and 77.9% in the RA group, although the difference was not statistically significant. The mean duration from symptom onset to diagnosis was significantly shorter in patients with PMR (approximately 5 months) than in those with RA (approximately 9 months) ($p < 0.001$). Rheumatoid factor positivity (17.9% versus [vs.] 81.9%; $p < 0.001$), anti-cyclic citrullinated peptide antibody positivity (0% vs. 53.2%; $p < 0.001$), tender joint count (3.4 ± 4.9 vs. 9.8 ± 8.1; $p < 0.001$), and swollen joint count (1.5 ± 3.6 vs. 4.5 ± 5.6; $p < 0.001$) were notably higher in those with RA compared to those with PMR. Regarding laboratory investigation results, patients with PMR exhibited significantly higher mean levels of leukocytes (9472.9 ± 3264.5/μL vs. 7592.2 ± 2318.7/μL; $p < 0.001$), neutrophils (6917.9 ± 2934.7/μL vs. 4775.5 ± 2030.9/μL; $p < 0.001$), monocytes (691.3 ± 480.5/μL vs. 555.9 ± 404.6/μL; $p = 0.002$), platelets (354.4 ± 106.2 × 103/μL vs. 287.1 ± 90.3 × 103/μL; $p < 0.001$), ESR (69.5 ± 24.7 mm/h vs. 41.7 ± 28.0 mm/h; $p < 0.001$), CRP (6.9 ± 6.3 mg/dL vs. 1.7 ± 2.7 mg/

TABLE 1 | Demographic and baseline characteristics of patients with polymyalgia rheumatica (PMR) and rheumatoid arthritis (RA).

	PMR patients ($n = 156$)	RA patients ($n = 408$)	p
Age, years	65.6 ± 9.63	64.1 ± 10.3	0.106
Female, *n* (%)	110 (70.5)	318 (77.9)	0.065
Duration of illness to hospital, month	5.06 ± 6.73	9.07 ± 10.7	**< 0.001**
Smoking, *n* (%)	16 (10.3)	41 (10.0)	0.942
Alcohol, *n* (%)	20 (12.8)	55 (13.5)	0.837
RF positivity, *n* (%)	28 (17.9)	334 (81.9)	**< 0.001**
Anti-CCP antibody positivity, *n* (%)	0 (0)	217 (53.2)	**< 0.001**
ANA positivity, *n* (%)	42 (26.9)	116 (28.4)	0.669
Tender joint count	3.4 ± 4.9	9.8 ± 8.1	**< 0.001**
Swollen joint count	1.5 ± 3.6	4.5 ± 5.6	**< 0.001**
Visual analogue scale for pain	6.2 ± 2.2	5.0 ± 2.1	**< 0.001**
PMR activity score	38.6 ± 33.6	NA	NA
DAS28-ESR	NA	5.17 ± 1.42	NA
CDAI	NA	15.2 ± 12.3	NA
Leukocyte/μL	9472.9 ± 3264.5	7592.2 ± 2318.7	**< 0.001**
Neutrophils/μL	6917.9 ± 2934.7	4775.5 ± 2030.9	**< 0.001**
Lymphocytes/μL	1678.2 ± 641.3	2081.1 ± 687.2	**< 0.001**
Monocytes/μL	691.3 ± 480.5	555.9 ± 404.6	**0.002**
Hemoglobin, g/dL	11.5 ± 1.6	12.6 ± 1.4	**< 0.001**
Platelets × 10^3/μL	354.4 ± 106.2	287.1 ± 90.3	**< 0.001**
ESR, mm/h	69.5 ± 24.7	41.7 ± 28.0	**< 0.001**
CRP, mg/dL	6.9 ± 6.3	1.7 ± 2.7	**< 0.001**
Total protein, g/dL	7.2 ± 0.6	7.3 ± 0.5	**0.008**
Albumin, g/dL	3.9 ± 0.5	4.2 ± 0.4	**< 0.001**
NLR	4.9 ± 3.3	2.6 ± 1.5	**< 0.001**
PLR	0.2 ± 0.1	0.1 ± 0.1	**< 0.001**
MLR	0.5 ± 0.4	0.3 ± 0.2	**< 0.001**

Note: Values are mean ± SD or *n* (%). Bold values indicate significant *p*-value.
Abbreviations: ALPL, alkaline phosphatase; ALT, alanine transaminase; ANA, antinuclear antibody; AST, aspartate aminotransferase; CCP, cyclic citrullinated peptide; CDAI, clinical disease activity index; CRP, C-reactive protein; DAS28, disease activity score in 28 joints; ESR, erythrocyte sedimentation rate; MLR, monocyte-to lymphocyte ratio; NA, not applicable; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; RF, rheumatoid factor.

dL; $p < 0.001$), neutrophil-to-lymphocyte ratio (NLR, 4.9 ± 3.3 vs. 2.6 ± 1.5; $p < 0.001$), platelet-to-lymphocyte ratio (PLR, 0.2 ± 0.1 vs. 0.1 ± 0.1; $p < 0.001$), and monocyte-to-lymphocyte ratio (MLR, 0.5 ± 0.4 vs. 0.3 ± 0.2; $p < 0.001$) compared to patients with RA. In contrast, hemoglobin (11.5 ± 1.6 g/dL vs. 12.6 ± 1.4 g/dL; $p < 0.001$), lymphocyte count (1678.2 ± 641.3/μL vs. 2081.1 ± 687.2; $p < 0.001$), and albumin level (3.88 ± 0.49 g/dL vs. 4.21 ± 0.39 g/dL; $p < 0.001$) were significantly higher in the RA group. In terms of disease activity, the PMR-activity score in patients with PMR had a mean value of 38.6, while the patients with RA had a mean disease activity score 28-ESR of 5.17 and mean clinical disease activity index of 15.2.

3.2 | Management and Treatment Outcomes in Patients With PMR

An overview of the treatment strategies and outcomes for 156 patients with PMR during follow-up, which had a mean duration of 36.8 ± 18.5 months, is presented in Table 2. Among these patients, 71 (45.5%) experienced a relapse, whereas 85 (54.5%) did not. All patients underwent glucocorticoid therapy (100%) at a cumulative dose of 3.8 g (prednisone equivalent). Additionally, 121 (77.6%) patients were treated with non-steroidal anti-inflammatory drugs (NSAIDs) and 139 (89.1%) were prescribed glucocorticoid-sparing therapies. Glucocorticoid-sparing treatments includedhydroxychloroquine, $n = 52$ (33.3%); methotrexate, $n = 92$ (59.0%); sulfasalazine, $n = 20$ (12.8%); and leflunomide, $n = 5$ (3.2%).

TABLE 2 | Treatment and prognosis of patients with polymyalgia rheumatica during follow-up.

Variables	Data ($n = 156$)
Follow-up period, months	36.8 ± 18.5
Disease pattern	
Relapsing course, *n* (%)	71 (45.5)
Without relapsing, *n* (%)	85 (54.5)
Medications administered	
Glucocorticoid, *n* (%)	156 (100)
Cumulative dose, g (prednisone-equivalent)	3.8 ± 3.0
NSAIDs, *n* (%)	121 (77.6)
Glucocorticoid-sparing therapies, *n* (%)	139 (89.1)
Hydroxychloroquine, *n* (%)	52 (33.3)
Methotrexate, *n* (%)	92 (59.0)
Sulfasalazine, *n* (%)	20 (12.8)
Leflunomide, *n* (%)	5 (3.2)

Note: Values are mean ± SD or *n* (%).
Abbreviation: NSAIDs, nonsteroidal anti-inflammatory drugs.

3.3 | Diagnostic Utility of Inflammatory Scores Between PMR and RA

A comparison of various laboratory inflammatory markers in patients with PMR and RA, highlighting their diagnostic utility, is presented in Table 3. The SII was significantly higher in the PMR group (1758.4 ± 1279.8) compared with the RA group (772.5 ± 622.6), with an AUC of 0.797, a cut-off value of 877.8, a sensitivity of 72.4%, and a specificity of 72.5% ($p < 0.001$). The CAR was also elevated in those with PMR (1.9 ± 1.9) versus RA (0.5 ± 0.8), demonstrating the highest diagnostic performance, with an AUC of 0.823, a cut-off value of 0.75, a sensitivity of 71.1%, and a specificity of 80.8% ($p < 0.001$). The AGR was lower in patients with PMR (1.2 ± 0.3) than in those with RA (1.4 ± 0.3), with an AUC of 0.696, a cut-off of 1.33, a sensitivity of 60.5%, and a specificity of 69.2% ($p < 0.001$). The PNI was also significantly lower in the PMR group (12.3 ± 3.43) compared with the RA group (14.6 ± 3.53), with an AUC of 0.691, a cut-off of 12.5, a sensitivity of 72.5%, and a specificity of 60.9% ($p < 0.001$). ROC curve analyses (Figure 1A,B) indicated that CAR exhibited the highest AUC (0.823), followed by SII (0.797), AGR (0.696), and PNI (0.691). Combining these inflammatory scores improved diagnostic accuracy, resulting in an AUC of approximately 0.835 (Figure 1C). In a subgroup analysis of seronegative RA (SNRA) cases, we evaluated the diagnostic performance of SII, CAR, AGR, and PNI in distinguishing PMR from SNRA. Interestingly, these inflammatory scores demonstrated even better diagnostic ability compared to the overall RA group. CAR showed the highest AUC value of 0.930, followed by SII at 0.911, AGR at 0.810, and PNI at 0.765 (Table S1).

3.4 | Association Between Inflammatory Scores and Disease Activity Markers

The association between inflammatory scores (i.e., SII, CAR, AGR, and PNI) and disease activity markers in patients with PMR was

TABLE 3 | Comparison of laboratory inflammatory markers between patients with PMR and RA.

	PMR patients ($n = 156$)	RA patients ($n = 408$)	*p*	AUC	Cut-off	Sensitivity	Specificity
SII	1758.4 ± 1279.8	772.5 ± 622.6	**< 0.001**	0.797	877.8	72.4	72.5
CAR	1.9 ± 1.9	0.5 ± 0.8	**< 0.001**	0.823	0.75	71.1	80.8
AGR	1.2 ± 0.3	1.4 ± 0.3	**< 0.001**	0.696	1.33	60.5	69.2
PNI	12.3 ± 3.4	14.6 ± 3.5	**< 0.001**	0.691	12.5	72.5	60.9

Note: Values are mean ± SD or *n* (%). Bold values indicate significant *p*-value.
Abbreviations: AGR, albumin/globulin ratio; CAR, C-reactive protein/albumin ratio; PNI, prognostic nutritional index (serum albumin +0.005 × peripheral lymphocyte count); SII, systemic immune-inflammation index (platelet count × neutrophil/lymphocyte count at diagnosis).

analyzed (Table 4). Leukocyte count was significantly and positively correlated with SII ($r=0.558$; $p<0.001$) and CAR ($r=0.378$; $p<0.001$), and significantly and negatively correlated with AGR ($r=-0.219$; $p<0.001$). Hemoglobin levels were negatively correlated with SII ($r=-0.335$; $p<0.001$) and CAR ($r=-0.447$; $p<0.001$) but positively correlated with AGR ($r=0.440$; $p<0.001$). Platelet counts were positively correlated with SII ($r=0.584$; $p<0.001$) and CAR ($r=0.384$; $p<0.001$) and negatively correlated with AGR ($r=-0.385$; $p<0.001$). ESR was positively correlated with SII ($r=0.410$; $p<0.001$) and CAR ($r=0.510$; $p<0.001$) and negatively correlated with AGR ($r=-0.630$; $p<0.001$). CRP levels exhibited strong positive correlations with SII ($r=0.636$; $p<0.001$) and CAR ($r=0.987$; $p<0.001$) and negatively correlated with AGR ($r=-0.442$; $p<0.001$). Finally, albumin levels were significantly and negatively correlated with SII ($r=-0.470$; $p<0.001$) and CAR ($r=-0.647$; $p<0.001$) and exhibited a strong positive correlation with AGR ($r=0.689$; $p<0.001$).

Furthermore, NLR demonstrated a strong positive correlation with SII ($r=0.894$; $p<0.001$) and CAR ($r=0.540$; $p<0.001$) and a negative correlation with AGR ($r=-0.284$; $p<0.001$). PLR was also positively correlated with SII ($r=0.810$; $p<0.001$) and CAR ($r=0.403$; $p<0.001$) and negatively correlated with AGR ($r=-0.395$; $p<0.001$). MLR was positively correlated with both SII ($r=0.456$; $p<0.001$) and CAR ($r=0.404$; $p<0.001$) and negatively correlated with AGR ($r=-0.169$; $p<0.001$).

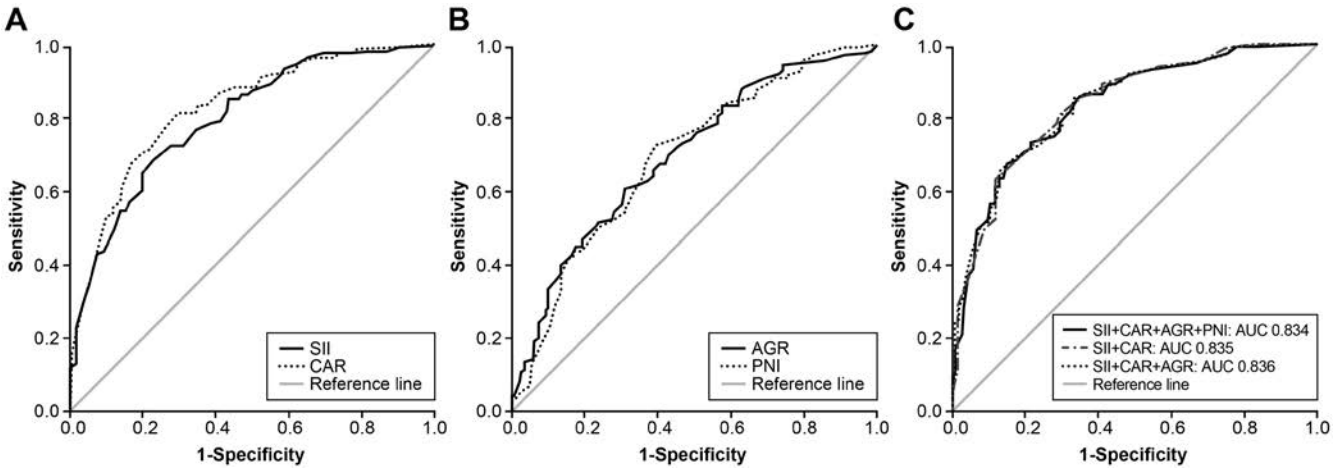

FIGURE 1 | Receiver operating characteristic (ROC) curve analysis of the diagnostic utility of inflammatory scores in patients with polymyalgia rheumatica (PMR) (A) ROC curve values for PMR diagnosis were 0.823 for the CAR (95% confidence interval [CI], 0.784–0.861) and 0.797 for the SII (95% CI, 0.757–0.837). (B) ROC curve values for PMR diagnosis were 0.696 for the AGR (95% CI: 0.648–0.743) and 0.691 for the PNI (95% CI: 0.641–0.741). (C) ROC Curve analysis of combined inflammatory scores for the diagnosis of PMR. CAR, C-reactive protein-to-albumin ratio; CI, confidence interval; SII, systemic immune-inflammation index (platelet count × neutrophil/lymphocyte count at diagnosis); AGR, albumin-to-globulin ratio; PNI, Prognostic Nutritional Index (serum albumin +0.005 × peripheral lymphocyte count).

TABLE 4 | Correlation between disease activity markers and inflammatory scores in patients with PMR.

	Correlation coefficient, *r* (*p*)			
Disease activity markers	**SII**	**CAR**	**AGR**	**PNI**
Leukocyte	0.558 (**<0.001**)	0.378 (**<0.001**)	−0.219 (**<0.001**)	0.167 (**<0.001**)
Hemoglobin	−0.335 (**<0.001**)	−0.447 (**<0.001**)	0.440 (**<0.001**)	0.057 (**<0.001**)
Platelet	0.584 (**<0.001**)	0.384 (**<0.001**)	−0.385 (**<0.001**)	−0.019 (0.644)
ESR	0.410 (**<0.001**)	0.510 (**<0.001**)	−0.630 (**<0.001**)	−0.260 (**<0.001**)
CRP	0.636 (**<0.001**)	0.987 (**<0.001**)	−0.442 (**<0.001**)	−0.394 (**<0.001**)
Albumin	−0.470 (**<0.001**)	−0.647 (**<0.001**)	0.689 (**<0.001**)	0.418 (**<0.001**)
NLR	0.894 (**<0.001**)	0.540 (**<0.001**)	−0.284 (**<0.001**)	−0.638 (**<0.001**)
PLR	0.810 (**<0.001**)	0.403 (**<0.001**)	−0.395 (**<0.001**)	−0.705 (**<0.001**)
MLR	0.456 (**<0.001**)	0.404 (**<0.001**)	−0.169 (**<0.001**)	−0.397 (**<0.001**)

Note: Bold values indicate significant *p*-value.
Abbreviations: AGR, albumin/globulin ratio; CAR, C-reactive protein/albumin ratio; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MLR, monocyte-to lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PMR, polymyalgia rheumatica; PNI, prognostic nutritional index (serum albumin +0.005 × peripheral lymphocyte count); SII, systemic immune-inflammation index (platelet count × neutrophil/lymphocyte count at diagnosis).

3.5 | Relationships Between Inflammatory Scores and Clinical Manifestations

A comparison of inflammatory scores, based on clinical manifestations in patients with PMR, is presented in Table 5. The SII was significantly higher in patients experiencing fever (2484.4 ± 1654.5 vs. 1499.5 ± 1004.6; $p < 0.001$), weight loss (2151.4 ± 1403.6 vs. 1627.4 ± 1214.0; $p = 0.041$), and headache (2450.9 ± 1698.8 vs. 1657.9 ± 1190.2; $p = 0.011$). Similarly, the CAR was significantly elevated in patients with fever (3.4 ± 2.4 vs. 1.4 ± 1.3; $p < 0.001$), weight loss (2.9 ± 2.1 vs. 1.6 ± 1.7; $p = 0.001$), and headache (2.8 ± 2.3 vs. 1.8 ± 1.8; $p = 0.036$). In contrast, AGR was significantly lower in patients with weight loss (1.1 ± 0.2 vs. 1.3 ± 0.3; $p < 0.001$), and PNI was notably lower in patients with fever (10.9 ± 3.5 vs. 12.8 ± 3.3; $p = 0.003$) and headache (10.6 ± 2.7 vs. 12.5 ± 3.5; $p = 0.018$).

4 | Discussion

The 2012 classification criteria for diagnosing PMR have a sensitivity of 68% and a specificity of 78%; however, their sensitivity in distinguishing RA from PMR is only 65% [6]. Due to the difficulty in differentiating PMR from other diseases using these criteria alone, recent recommendations have increasingly emphasized imaging techniques, such as ultrasonography, magnetic resonance imaging (MRI), and fluorodeoxyglucose positron emission tomography (FDG-PET), to aid in the diagnosis of PMR [17]. However, these techniques are not standardized, and their utility is limited by variations in interpretation depending on the expertise of the radiologist in PMR-related imaging. Therefore, our findings, which suggest that specific inflammatory scores from routine laboratory investigations can be used to diagnose PMR, may be highly beneficial in clinical practice.

The SII, CAR, AGR, and PNI used in this study were initially developed as prognostic markers for solid tumors but have recently been applied to autoimmune diseases [18–20]. Research suggests that SII is linked to predicting the risk for RA, AGR and PNI are associated with disease activity in SLE, and CAR is related to disease activity in axial spondyloarthritis [21–24]. Although these indices are not specific to any single disease, their elevation or alteration in specific clinical contexts makes them valuable for assessing disease severity, prognosis, and treatment response across various conditions. These findings

TABLE 5 | Comparison of inflammatory scores according to manifestations in patients with PMR.

Manifestations	SII	*p*	CAR	*p*	AGR	*p*	PNI	*p*
Fever								
(+) = 41	2484.4 ± 1654.5	**< 0.001**	3.4 ± 2.4	**< 0.001**	1.2 ± 0.3	0.595	10.9 ± 3.5	**0.003**
(−) = 115	1499.5 ± 1004.6		1.4 ± 1.3		1.2 ± 0.2		12.8 ± 3.3	
Weight loss								
(+) = 39	2151.4 ± 1403.6	**0.041**	2.9 ± 2.1	**0.001**	1.1 ± 0.2	**< 0.001**	11.5 ± 2.9	0.092
(−) = 117	1627.4 ± 1214.0		1.6 ± 1.7		1.3 ± 0.3		12.5 ± 3.6	
Depression								
(+) = 28	1949.5 ± 1126.4	0.342	1.9 ± 1.7	0.815	1.2 ± 0.2	0.801	12.5 ± 2.4	0.686
(−) = 128	1716.5 ± 1611.2		1.9 ± 1.9		1.2 ± 0.3		12.2 ± 3.6	
Headache								
(+) = 19	2450.9 ± 1698.8	**0.011**	2.8 ± 2.3	**0.036**	1.2 ± 0.3	0.809	10.6 ± 2.7	**0.018**
(−) = 135	1657.9 ± 1190.2		1.8 ± 1.8		1.2 ± 0.3		22.5 ± 3.5	
Morning stiffness								
(+) = 94	1739.8 ± 1289.4	0.824	1.8 ± 1.8	0.389	1.2 ± 0.2	0.934	12.3 ± 3.2	0.907
(−) = 62	1786.5 ± 1275.0		2.1 ± 1.9		1.2 ± 0.3		12.2 ± 3.8	
Shoulder involvement								
(+) = 143	1741.3 ± 1269.7	0.625	1.9 ± 1.9	0.096	1.2 ± 0.3	0.111	12.3 ± 3.4	0.480
(−) = 13	1946.2 ± 1426.8		2.8 ± 1.9		1.1 ± 0.3		11.6 ± 4.5	
Hip involvement								
(+) = 106	1750.3 ± 1212.8	0.915	2.0 ± 1.9	0.710	1.2 ± 0.3	0.126	12.2 ± 3.4	0.565
(−) = 50	1775.4 ± 1424.3		1.9 ± 1.8		1.3 ± 0.3		12.5 ± 0.5	

Note: Bold values indicate significant *p*-value.
Abbreviations: AGR, albumin/globulin ratio; CAR, C-reactive protein/albumin ratio; PNI, prognostic nutritional index (serum albumin +0.005 × peripheral lymphocyte count); SII, systemic immune-inflammation index (platelet count × neutrophil/lymphocyte count at diagnosis).

support the broader utility of these markers in autoimmune diseases. However, to our knowledge, studies specifically focusing on PMR have not yet been published. As such, the present study investigated and documented the role of these indices in the diagnosis of PMR.

Given the diagnostic challenges of differentiating PMR from RA, especially using traditional classification criteria, our study focused on these two groups using specific inflammatory indices. We found statistically significant differences across all four indices between patients with PMR and those with RA, with the SII and CAR demonstrating particularly high diagnostic performance, supported by AUCs of 0.797 and 0.823, respectively, highlighting their potential use as reliable diagnostic markers. Similar to our findings, other studies investigating diagnostic biomarkers using routine laboratory investigations have reported AUC values ranging from 0.65 to 0.85 for various autoimmune and autoinflammatory diseases [25, 26]. Our study further demonstrated that the SII and CAR not only outperformed the traditional classification criteria in terms of sensitivity and specificity, but also demonstrated diagnostic performance comparable with that of advanced imaging techniques. Recent studies have indicated that FDG-PET and MRI have sensitivities of approximately 90% and specificities between 75% and 80% for diagnosing PMR [27, 28]. Considering the high cost and time demands associated with these imaging modalities, the inflammatory scores investigated in our study represent a valuable and cost-effective alternative.

Although AGR and PNI alone did not exhibit strong diagnostic capabilities, combining these inflammatory scores with SII and CAR significantly improved diagnostic accuracy, achieving an AUC of approximately 0.85. The combination of these four markers (SII, CAR, AGR, and PNI) enhanced diagnostic accuracy by leveraging the complementary strengths of each. This multifaceted approach enables a more comprehensive assessment of disease characteristics and improves both sensitivity and specificity. Additionally, the use of a combination of biomarkers can help minimize false positives and false negatives, leading to more reliable and precise diagnostic outcomes [29].

The incidence of PMR is steadily increasing, with approximately 25% of patients requiring long-term treatment exceeding 4 years, underscoring a growing disease burden [30]. Given this trend, the early and accurate identification of PMR, along with the development of more effective treatment strategies, is essential to mitigate the risks and challenges associated with prolonged therapy. However, despite the prevalence of PMR, there is a paucity of studies utilizing easily accessible inflammatory scores as diagnostic or prognostic markers for PMR in routine clinical practice [31]. Although ESR and CRP are commonly used, there are few widely recommended hematological markers specifically for PMR, and those available often have limited diagnostic value. Research investigating routine blood tests has suggested that markers such as NLR and PLR may be associated with disease activity in PMR [32]. Additionally, NLR has been proposed as a potential predictor of steroid resistance, providing further insight into the clinical course of the disease [33].

The use of ferritin autoantibodies to diagnose PMR has been explored; however, this test is not commonly performed in routine clinical practice due to its limited accessibility [34]. Other biomarkers, such as cytokines (e.g., BAFF, CXCL, and interleukin-6) and enzyme markers (e.g., MMP3) exhibit promising diagnostic performance, with AUC values ranging from 0.8 to 0.97 [35, 36]. However, these studies often compared biomarkers with healthy controls rather than those of other autoimmune diseases, leaving their differential diagnostic abilities uncertain. In contrast, our study demonstrated that the inflammatory scores we used—SII, CAR, AGR, and PNI—exhibited robust differential diagnostic capabilities against RA and are easily accessible in routine clinical practice. These scores showed particular promise in challenging cases, such as distinguishing PMR from SNRA, where traditional serological markers like RF and anti-CCP are absent. While not intended to replace established PMR diagnostic criteria, they may serve as valuable adjunctive tools, particularly in complex cases such as SNRA or overlapping syndromes. By providing additional data on systemic inflammation, these scores may enhance diagnostic accuracy when used alongside established criteria like ESR and CRP. Further validation studies are needed to confirm their role within existing diagnostic workflows.

In addition to evaluating the diagnostic utility of the inflammatory scores, we explored their relationship with clinical symptoms and disease activity. We found notable correlations between these scores and standard inflammatory markers, such as ESR and CRP, indicating their alignment with established measures of inflammation. Furthermore, our inflammatory scores demonstrated significant associations with PMR disease activity indicators, such as NLR and PLR, and were linked to common PMR symptoms, including fever, weight loss, and headache, reinforcing their clinical relevance [32]. The markers we examined—SII, CAR, AGR, and PNI—have been shown in broader clinical studies to offer additional associations with disease prognosis and mortality, as demonstrated in previous studies, further supporting their potential prognostic value in PMR [11–13]. Although these findings are significant, limitations of the present study include the incomplete assessment of vasculitis. In patients with PMR and concurrent GCA, the risk for severe complications, such as blindness and aneurysm(s), is a major concern, emphasizing the need for diagnostic markers that can distinguish between the two conditions [37]. However, not all patients with PMR in this study underwent vasculitis evaluation; as such, this aspect was not sufficiently addressed. Further research is required to determine whether the inflammatory scores used in our study can reliably differentiate between PMR and PMR-associated with GCA.

The present study had several limitations, the first of which was its retrospective design, which may have introduced selection bias; as such, prospective studies are needed to validate these findings in larger and more diverse patient populations. Second, although the inflammatory scores demonstrated a robust diagnostic performance, they may not fully capture the complexity of PMR, especially in cases involving symptoms overlapping with other autoimmune diseases. Furthermore, the study did not account for potential confounding factors, such as variations in treatment regimens or comorbid conditions, which may influence inflammatory markers.

5 | Conclusions

Our study highlights the value of inflammatory scores, such as the SII, CAR, AGR, and PNI, in diagnosing PMR, which is often challenging due to its nonspecific symptoms. These scores, which are derived from routine and inexpensive blood tests, provide a cost-effective and accessible diagnostic tool. They offer a quick and objective measure of systemic inflammation, distinguishing PMR from other conditions such as RA. Moreover, they correlate significantly with clinical manifestations and disease severity, thus enhancing their utility in the comprehensive assessment of PMR. Notably, combining these scores improved diagnostic accuracy, making them especially valuable in clinical settings where access to advanced diagnostic tools may be limited.

Author Contributions

J.-W.K., J.-Y.J., C.-H.S., and H.-A.K. contributed to the study design and data collection, analysis, and interpretation. J.-W.K. and H.-A.K. contributed to the data collection and/or data interpretation. All authors revised the manuscript and gave final approval for submission.

Ethics Statement

This single-center, retrospective study was approved by the Institutional Review Board of Ajou University Hospital (AJIRB-MDB-2021-447).

Consent

The requirement for informed consent was waived due to the retrospective nature of this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All available data are reported in the manuscript and Supporting Information.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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ORIGINAL ARTICLE

Risk of Gout Among Patients With Tuberculosis: A Nationwide Cohort Study in South Korea

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ABSTRACT

Aim: Medications used for tuberculosis (TB) treatment are thought to increase uric acid levels and influence the occurrence of gout. The objective of this study was to evaluate the risk of gout in patients with TB.

Methods: We searched the South Korean National Health Claims database for incident cases of TB. After identifying patients diagnosed with gout within 6 months of TB diagnosis, the risk compared to the general population was estimated by calculating the standardized incidence ratios (SIRs). A nested case–control analysis among patients with TB was performed by matching subjects diagnosed with and without gout in a 1:5 ratio to identify the risk factors for gout.

Results: Of the 3848 patients with gout, the proportions of males, patients aged ≥ 70 years, and those with a diagnosis within the first 2 months were 70.2%, 33.0%, and 52.8%, respectively. The incidence of gout in patients with TB was significantly higher than in the general population (overall SIR: 1.42, sex-adjusted SIR: 1.32, age-adjusted SIR: 1.04). Conditional logistic regression analysis indicated that hypertension (odd ratio [OR] 1.43, 95% confidence interval (CI) 1.31–1.58), heart failure (OR 1.19, 95% CI 1.01–1.39), chronic kidney disease (OR 2.47, 95% CI 1.99–3.06), and use of pyrazinamide (OR 1.02, 95% CI 1.02–1.02) and ethambutol (OR 1.00, 95% CI 1.00–1.01) were associated with gout.

Conclusion: The increased risk of gout in patients with TB and the association between comorbidities and TB medications underscore the need for higher clinical awareness in this population.

1 | Introduction

Gout is one of the most common forms of inflammatory arthritis in adults and is characterized by the deposition of monosodium urate crystals in the joints—usually in the foot, ankle, and knee—or the periarticular tissues, causing intense pain, swelling, redness, and warmth [1]. Although acute attacks of gout are mostly self-limiting and resolve within 2 weeks [2], if left untreated, recurrent episodes of pain can progress to the chronic phase of the disease. Progression of the disease to chronic gout, represented by the formation of tophi, can destroy the involved joints and lead to substantial functional impairment [3]. Gout frequently affects men and older patients, with its incidence and prevalence growing continuously in recent decades [4]. Although gout was previously considered more common in Europe and the Americas than in Asia, epidemiological studies have demonstrated that the number of patients with gout is rapidly increasing, even in Eastern populations [5]. Thus, the increasing global burden of gout underscores the importance of identifying high-risk populations prone to developing this potentially debilitating disease.

Tuberculosis (TB) is a highly prevalent infection worldwide and a significant public health concern [6]. It is the leading cause of death attributed to an infectious agent [7], and while there has been substantial progress in decreasing the global burden of TB, eliminating it is still challenging. Transmitted primarily through the respiratory tract, TB affected 10.6 million individuals in 2022, with 1.3 million succumbing to the disease [8]. These data underscore the need for increased clinical attention to understand the complications associated with TB. Patients with TB are typically prescribed a combined regimen of isoniazid (INH), ethambutol (ETB), rifampin (RFP), and pyrazinamide (PZA). Since PZA and ETB impact uric acid levels in the circulation by decreasing the clearance of uric acid in the kidneys [9], it could be hypothesized that patients with TB are at an increased risk of developing gout. However, to our knowledge, the incidence of gout in patients with TB has not been described in the literature. Therefore, the main objective of this study was to identify the risk of gout in patients with TB using the Health Insurance Review & Assessment (HIRA) database, focusing on the drugs used for TB treatment and comorbid diseases.

2 | Methods

2.1 | Data Acquisition

We performed a retrospective analysis by searching the HIRA database for the calendar years 2009–2021 (data acquisition approval number: M20230414001). The HIRA database, an organization run by the South Korean government, collects records of hospital care usage covered by the National Health Insurance (NHI). Since NHI is the sole insurer approved in South Korea, and hospitals requesting financial reimbursement from the government must record the treatment provided to the patient, the healthcare usage patterns of most residents (> 50 million individuals) are included in the HIRA database, making it an optimal source for epidemiologic studies [10]. This study was approved by the Institutional Review Board of Severance Hospital (IRB approval no: 4–2023-0288). As the HIRA database only provides deidentified data to prevent the extrusion of personal information, the requirement for obtaining informed consent from the patients was waived.

2.2 | Study Design and Definition of TB, Gout, and Comorbidities

First, we extracted patients with TB in the database who were diagnosed as TB between 2009 and 2021 using the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10) code A15-19 [11]. After identifying those prescribed ≥ 2 first-line TB drugs (INH, ETB, RFP, and PZA) upon initial diagnosis and applying a two-year washout, those prescribed with TB drugs for > 6 months were selected. Finally, when patients diagnosed with gout prior to the TB diagnosis were excluded, the total number of patients was 228 744. The index date for the TB group was set as the initial date of TB diagnosis and the prescription of two or more first-line TB drugs.

We defined patients as having gout when the ICD-10 code of M10 (either in a principal or first–fifth additional diagnosis) was assigned and the patient was admitted to the hospital or visited the outpatient department on more than two occasions within 1 month. Considering that the standard regimen for TB treatment is prescribed for 6 months, only cases of gout occurring within 6 months of TB diagnosis were evaluated, and a total of 3848 patients with gout were identified (Figure 1). The patients' comorbidities included hypertension (I10–15), diabetes mellitus (E10–14), dyslipidemia (E78), ischemic heart disease (I20–25), heart failure (I50), chronic kidney disease (N18), and moderate/severe liver disease (I85.0, I85.9, I86.4, I98.2, K70.4, K71.1, K72.1, K72.9, and K76.5–76.7) within 1 year of the TB index date.

2.3 | Nested Case–Control (NCC) Analysis

To identify the effect of comorbidities and TB medications on the onset of gout, we performed an NCC analysis by matching age, sex, TB index date, TB type (extrapulmonary [A17 and A18] or pulmonary [A15, A16, and A19]), and follow-up duration after TB diagnosis in a 1:5 ratio in patients diagnosed with and without gout (Figure 1). The use of TB medications was categorized into ETB and PZA users and nonusers to evaluate the effects of these drugs on the incidence of gout. As INH and RFP are core drugs used for treating TB [12], they were not included as covariates in the NCC analysis.

2.4 | Statistical Analysis

Data of continuous variables are presented as means ± standard deviations, and the differences were compared using the student's t-test; categorical variables are presented as frequencies and proportions and compared using the chi-square test. The relative risk of gout in patients with TB compared to that in the general population was estimated by calculating the incidence rate (IR) and crude and adjusted standardized incidence ratios (SIRs). The incidence of gout in the general population was adopted from the numerical estimates in 2015, as previously described [13]. Moreover, factors associated with the occurrence of gout in the NCC-matched population were estimated using conditional logistic regression. Statistical analyses were performed using either the SAS 9.4 Enterprise Guide (SAS Institute Inc., Cary, NC, USA) or R 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria), with a two-tailed p value < 0.05 considered significant.

3 | Results

3.1 | Age and Sex Distribution of Patients With Gout and TB and the Risk of Gout Compared to the General Population

Among patients with TB affected by gout, the proportions of male and female individuals were 70.2% and 29.8%. The incidence of gout in patients > 70 years old was the highest (33.0%), followed by 21.0% in the 50–59-year age group and 17.7% in the 60–69-year age group. The proportions of patients who developed gout

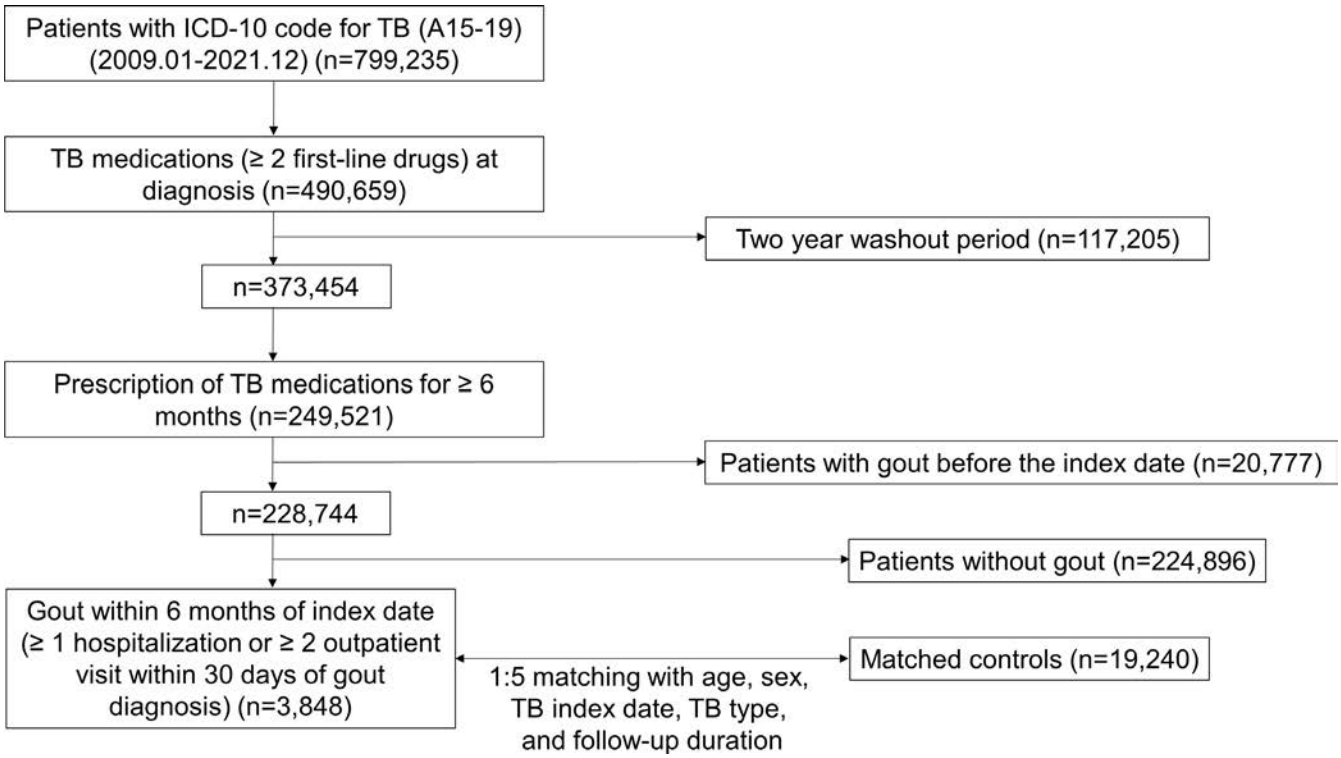

FIGURE 1 | A flowchart illustrating the selection of eligible patients for this study. TB, tuberculosis.

at < 2 months, between 2–4 months, and > 4 months were 52.8%, 29.4%, and 17.8%, respectively (Figure 2).

The overall IR of gout in patients with TB was 2.74/1000 person-years, and the IR of gout in patients with TB was higher in male than in female patients and was the highest in the ≥ 70-years age group. The risk of gout in the study population was significantly higher than that in the general population (overall and sex- and age-adjusted SIRs: 1.42, 95% confidence interval [CI] 1.37–1.46; 1.32, 95% CI 1.28–1.37; and 1.04, 95% CI 1.01–1.08; respectively). This heightened risk of gout remained consistent in male and female patients and the 0–29-year and 50–59-year age groups when the patients were categorized according to sex and age (Table 1).

3.2 | Comparison of Baseline Characteristics Among Patients With TB With and Without Gout After Matching

Upon comparing the characteristics of patients diagnosed with and without gout after matching, we found that the insurance type was comparable between the groups ($p = 0.120$). Underlying diseases such as hypertension, dyslipidemia, ischemic heart disease, heart failure, and chronic kidney disease were more prevalent in patients with gout. Regarding TB medication, INH, RFP, PZA, and ETB were more frequently prescribed to patients with gout than to those without gout during the follow-up (all $p < 0.001$) (Table 2).

3.3 | Factors Associated With Gout

Unadjusted conditional logistic regression analysis indicated that underlying diseases of hypertension, dyslipidemia, ischemic heart disease, heart failure, and chronic kidney disease and PZA and ETB use were associated with gout in patients with TB. When the factors were adjusted, hypertension (odd ratio [OR] 1.43, 95% CI 1.31–1.58), heart failure (OR 1.19, 95% CI 1.01–1.39), chronic kidney disease (OR 2.47, 95% CI 1.99–3.06), and PZA (OR 1.02, 95% CI 1.02–1.02) and ETB (OR 1.00, 95% CI 1.00–1.01) use were revealed to influence the occurrence of gout (Table 3).

4 | Discussion

The rapid increase in the number of patients with gout in Eastern countries could be ascribed to the introduction of Western lifestyle measures and dietary patterns, the rise of obesity within the general population, an aging society, and the widespread use of medications influencing uric acid levels. The increase in gout in the public community has important societal implications, as it could influence patients' quality of life, decrease work productivity, and result in greater healthcare expenditures [14]. Patients with TB experience elevated serum uric acid levels due to the effects of therapeutic agents; however, the specific risk of developing gout in this population is unknown. Furthermore, although a previous investigation identified putative factors for gout during TB, the incidence of gout in patients with TB was not elucidated [15]. In the present study, analysis of the HIRA database revealed that patients with TB had a significantly higher risk of developing gout than the general population. Intriguingly, in patients with TB, underlying diseases such as hypertension, heart failure, and chronic kidney disease and PZA and ETB contributed to the incidence of gout.

Hyperuricemia, defined as a serum uric acid level > 7 mg/dL in men and > 6 mg/dL in women, is a typical laboratory finding

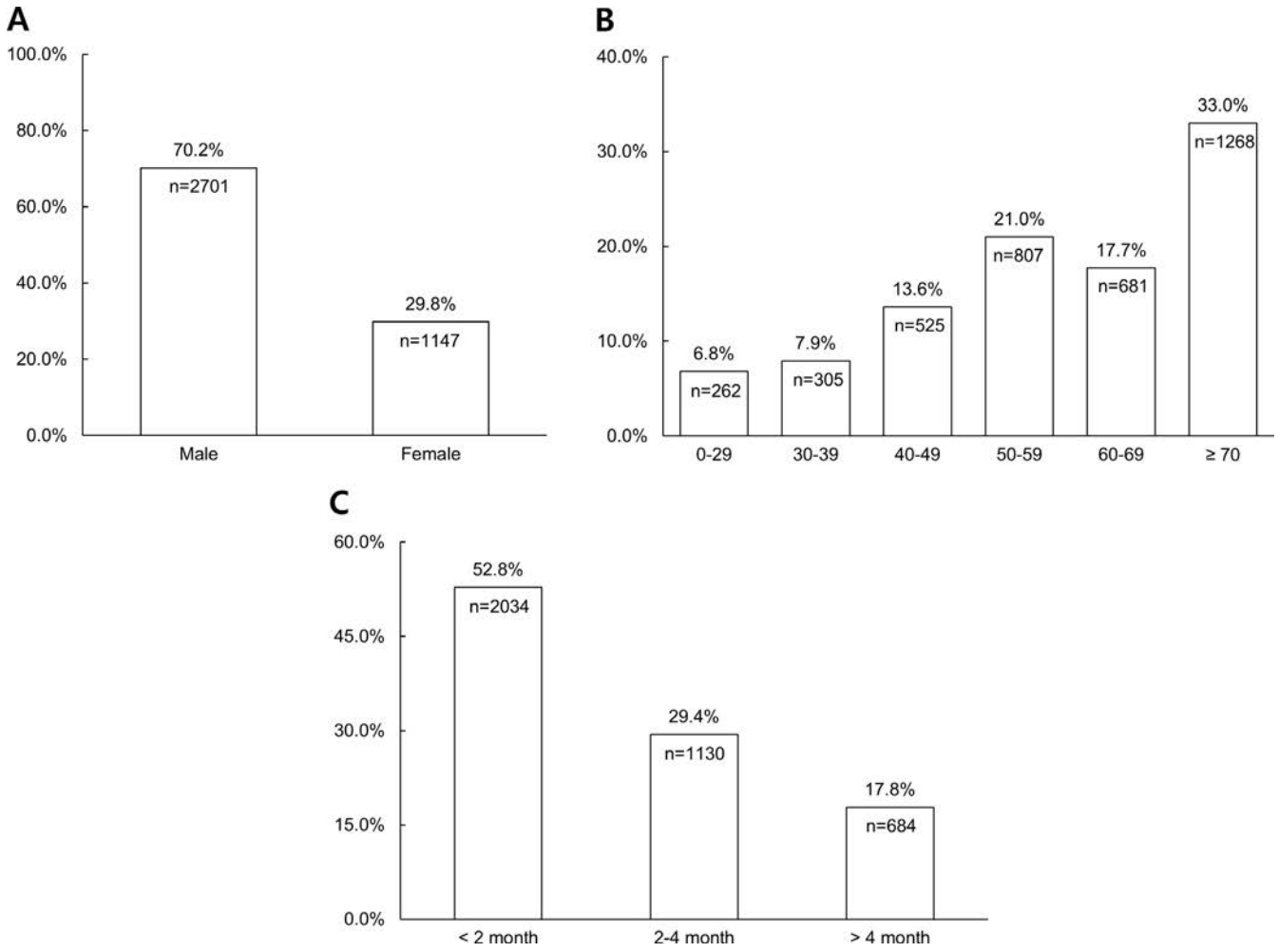

FIGURE 2 | Demographic characteristics of TB patients with gout. The proportion of patients is presented according to (A) sex, (B) age distribution, and (C) the period of gout diagnosis after TB. TB, tuberculosis.

TABLE 1 | Incidence rate of gout in the TB group compared to the general population.

	IR/1000 PY (95% CI)	SIR (95% CI)	Expected	Observed
Overall population	2.74 (2.65–2.82)	1.42 (1.37–1.46)	2711.58	3848
Sex		1.32 (1.28–1.37)[a]	2907.83	3848
Male	3.46 (3.33–3.59)	1.08 (1.04–1.13)	2490.39	2701
Female	1.83 (1.73–1.94)	2.75 (2.59–2.91)	417.44	1147
Age		1.04 (1.01–1.08)[b]	3688.83	3848
0-29	1.18 (1.04–1.32)	1.44 (1.27–1.62)	182.51	262
30–39	1.65 (1.47–1.84)	0.80 (0.72–1.00)	379.09	305
40–49	2.41 (2.21–2.62)	1.09 (0.99–1.19)	481.95	525
50–59	3.16 (2.95–3.39)	1.20 (1.12–1.28)	673.14	807
60–69	3.49 (3.23–3.76)	1.05 (0.97–1.13)	647.80	681
≥ 70	3.84 (3.63–4.05)	0.96 (0.91–1.01)	1324.34	1268

Abbreviation: TB, tuberculosis.
[a]The value indicates the sex-adjusted standardized incidence ratio.
[b]The value indicates the age-adjusted standardized incidence ratio.

TABLE 2 | Baseline characteristics of TB patients with and without gout after matching.

Variables	Total ($n = 23\,088$)	Patients with gout ($n = 3848$)	Patients without gout ($n = 19\,240$)	p
Age, years	59.14 ± 17.45	59.14 ± 17.45	59.14 ± 17.45	—
Distribution of patient age				
0–29	1572 (6.8)	262 (6.8)	1310 (6.8)	—
30–39	1830 (7.9)	305 (7.9)	1525 (7.9)	
40–49	3150 (13.6)	525 (13.6)	2625 (13.6)	
50–59	4842 (21.0)	807 (21.0)	4035 (21.0)	
60–69	4086 (17.7)	681 (17.7)	3405 (17.7)	
≥ 70	7608 (33.0)	1268 (33.0)	6340 (33.0)	
Sex, *n* (%)				
Male	16 206 (70.2)	2701 (70.2)	13 505 (70.2)	—
Female	6882 (29.8)	1147 (29.8)	5735 (29.8)	
Type of TB, *n* (%)				
Extrapulmonary TB	2982 (12.9)	497 (12.9)	2485 (12.9)	—
Pulmonary TB	20 106 (87.1)	3351 (87.1)	16 755 (87.1)	
Insurance type, *n* (%)				
National Health Insurance	21 277 (92.2)	3522 (91.5)	17 755 (92.3)	0.120
Medical Aid	1811 (7.8)	326 (8.5)	1485 (7.7)	
Underlying disease, *n* (%)				
Hypertension	8607 (37.3)	1654 (43.0)	6953 (36.1)	< 0.001
Diabetes mellitus	6324 (27.4)	1038 (27.0)	5286 (27.5)	0.539
Dyslipidemia	8481 (36.7)	1505 (39.1)	6976 (36.3)	< 0.001
Ischemic heart disease	2450 (10.6)	451 (11.7)	1999 (10.4)	0.016
Heart failure	1270 (5.5)	259 (6.7)	1011 (5.3)	< 0.001
Chronic kidney disease	528 (2.3)	150 (3.9)	378 (2.0)	< 0.001
Moderate/severe liver disease	160 (0.7)	29 (0.8)	131 (0.7)	0.696
TB medication, *n* (%)				
Isoniazid	21 061 (91.2)	3825 (99.4)	17 236 (89.6)	< 0.001
Rifampicin	21 225 (91.9)	3812 (99.1)	17 413 (90.5)	< 0.001
Pyrazinamide	20 079 (87.0)	3787 (98.4)	16 292 (84.7)	< 0.001
Ethambutol	19 161 (83.0)	3372 (87.6)	15 789 (82.1)	< 0.001

Abbreviation: TB, tuberculosis.

observed in patients with gout [16]. Since excess uric acid is responsible for the formation of urate crystals and their evolution into symptomatic disease [1], the development of hyperuricemia is indispensable in gout, although hyperuricemia may not occur in some patients [17]. Uric acid is endogenously produced in the liver as the final product of purine metabolism, in which purines derived from dietary sources or endogenous synthesis are broken down into xanthine and further converted into uric acid by the enzyme xanthine oxidase. Under normal circumstances, uric acid is transported in the bloodstream and filtered through the kidneys, with approximately 70%–80% excreted in the urine and the remainder eliminated through the gastrointestinal tract [18]. The elevation of uric acid levels is largely understood to be a consequence of two main factors. First, increased uric acid production can result from the consumption of purine-rich diets, excessive alcohol consumption, or the presence of metabolic disorders. Second, decreased renal excretion of uric acid may be attributed to renal insufficiency, medications, or genetic abnormalities that affect uric acid transporters in the kidneys [19]. Since PZA and ETB increase uric acid levels in the

TABLE 3 | Conditional logistic regression analysis associated with the incidence of gout.

Variables	Crude OR (95% CI)	*p*	Adjusted OR (95% CI)	*p*
Insurance type				
National Health Insurance	1.00 (ref)		1.00 (ref)	
Medical Aid	1.11 (0.98–1.26)	0.111	1.07 (0.94–1.22)	0.319
Underlying disease				
Hypertension	1.47 (1.35–1.59)	< 0.001	1.43 (1.31–1.58)	< 0.001
Diabetes mellitus	0.97 (0.90–1.06)	0.508	0.84 (0.76–1.00)	0.051
Dyslipidemia	1.15 (1.07–1.24)	< 0.001	1.08 (0.99–1.18)	0.081
Ischemic heart disease	1.16 (1.03–1.30)	0.011	0.96 (0.84–1.09)	0.502
Heart failure	1.32 (1.14–1.53)	< 0.001	1.19 (1.01–1.39)	0.038
Chronic kidney disease	2.05 (1.69–2.49)	< 0.001	2.47 (1.99–3.06)	< 0.001
Moderate/severe liver disease	1.11 (0.74–1.66)	0.621	1.34 (0.87–2.05)	0.186
TB medication usage				
Pyrazinamide/day	1.02 (1.02–1.03)	< 0.001	1.02 (1.02–1.02)	< 0.001
Ethambutol/day	1.01 (1.01–1.01)	< 0.001	1.00 (1.00–1.01)	< 0.001

Abbreviation: OR, odds ratio.

circulation, the elevated incidence of gout in patients with TB in the present study is principally explained by the effect of anti-TB medications.

Our NCC analysis indicated that PZA and ETB use increased the odds of developing gout, supporting the fact that drugs are crucial factors contributing to the occurrence of gout in patients with TB. Nonetheless, TB infection itself could be proposed as a risk factor for gout because it can increase the production of uric acid and may affect urate solubility by altering acid–base homeostasis following infection [20]. Given that these processes facilitate the nucleation and growth of monosodium urate crystals in the human body [21], it can be inferred that TB is associated with a greater onset of gout. However, an increase in uric acid level alone is not sufficient to diagnose gout, as a suggestive clinical episode is required. Notably, previous studies have demonstrated that toll-like receptor 2/4 signaling, nucleotide-binding oligomerization domain-like receptor protein 3 inflammasome activation, and the subsequent release of interleukin-1, a crucial cytokine responsible for inducing inflammation in gout [22, 23], are enhanced in TB. Therefore, the simultaneous activation of pertinent pathways for gout attack during TB infection suggests a potential association between TB and an elevated risk of gout.

Analysis of the South Korean National Health Claims database indicated that the incidence of gout per 1000 person-years was 1.52 in 2009 and 1.94 in 2015 [13]. In the present study, the number of patients who developed gout was 2.74/1000 person-years among patients with TB, and the overall, sex-, and age-adjusted SIR for gout was significantly higher in patients with TB than in the general population. In addition to the use of PZA and ETB, the comorbid risk factors hypertension, heart failure, and chronic kidney disease were significantly associated with gout incidence in patients with TB, suggesting that greater caution and active evaluation are required among these patients when clinical signs and symptoms that raise the possibility of gout occur. In particular, as most gout events occur within the first 2 months of TB treatment, it is advisable for attending physicians to pay more attention during this period.

This study had some limitations. First, although the number of included patients was high, this study was performed retrospectively using the HIRA database. Furthermore, an analysis stratifying according to uric acid levels and glomerular filtration rate could not be performed because the database does not include laboratory data on uric acid level and renal function. Second, although the duration of usage and selection of TB medication could vary according to drug susceptibility, the site involved, and side effects, such data could not be investigated. Third, data on lifestyle patterns, including alcohol ingestion, smoking, and the frequency and intensity of exercise, which may potentially influence serum uric acid levels, were unavailable in the HIRA database, and adjustment for these factors was not possible [24]. Fourth, given that the incidence of gout was defined according to the ICD-10 code alone, cases of gout may have been overestimated, although further specification was performed to increase diagnostic accuracy. Fifth, it should be taken into account that hypertension and chronic kidney disease—which were identified as risk factors for gout in TB—are also related to TB occurrence in the general population and might have affected data interpretation. Thus, additional studies are required to overcome the limitations of the present study and elucidate the risk of gout in patients with TB.

In conclusion, the present study's results indicate that the risk of gout is higher in patients with TB than in the general population. Underlying diseases such as hypertension, heart failure, chronic kidney disease, and PZA and ETB use were associated with the occurrence of gout in patients with TB, underscoring the need for higher vigilance for gout in this patient group.

Author Contributions

Conceptualization: Ha J.W., Kim C.Y., and Ahn S.S.; data curation: Han M.; formal analysis: Han M. and Jung I.; funding acquisition: none; Investigation: Han M., Ha J.W., Kim C.Y., and Ahn S.S.; methodology: Han M., Ha J.W., Kim C.Y., and Ahn S.S.; project administration: Han M., Ha J.W., Kim C.Y., and Ahn S.S.; resources: Han M., Ha J.W., and Ahn S.S.; Software: Han M. and Jung I.; supervision: Jung I.; validation: Han M. and Ahn S.S.; Visualization: Han M., Ha J.W., Kim C.Y., and Ahn S.S.; writing – original draft: Han M., Ha J.W., Kim C.Y., and Ahn S.S.; writing – review and editing: Han M., Ha J.W., Jung I., Kim C.Y., and Ahn S.S.

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The authors have nothing to report.

Ethics Statement

This study was approved by the Institutional Review Board of Severance Hospital, and the requirement for informed consent from the patients was waived, as the HIRA only provides de-identified data to prevent the extrusion of personal information (IRB approval no: 4–2023-0288).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The dataset generated and analyzed for this study cannot be publicly shared according to the Personal Information Protection Act of South Korea. The Korea National Health Insurance Sharing Service (contact via https://nhiss.nhis.or.kr; contact: +82-33-736-2432, 2433) is responsible for distributing HIRA data for scientific research to researchers after obtaining formal approval.

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ORIGINAL ARTICLE

Exploring the Interaction Between HLA-B27 and Other Risk Factors of Valvular Heart Disease in Axial Spondyloarthritis

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Keywords: axial spondyloarthritis | HLA-B27 | valvular heart disease

ABSTRACT

Objectives: HLA-B27 plays a critical role in axial spondyloarthritis (axSpA). Valvular heart disease (VHD) is a life-threatening extra-articular manifestation of axSpA. The evidence for the association between HLA-B27 and VHD in axSpA is still scarce and controversial. In this study, we aim to mainly explore the association of HLA-B27 and VHD in axSpA, and the interaction between HLA-B27 and other risk factors of VHD in axSpA.

Methods: We analyze cross-sectional data of axSpA patients from 2016 to 2022 in Shenzhen Second People's Hospital. Multivariable logistic regression models were fitted to evaluate the association between HLA-B27 and VHD in axSpA patients. When discovering the interaction between HLA-B27 and sex and disease duration, we made stratified analyses.

Results: Included were 444 axSpA patients with echocardiography during inpatient admission. Males were 299, and females were 145. In the adjusted model of multivariable logistic analysis, only age increased the risk of VHD (OR 1.054; 95% CI 1.021–1.087). To detect interactions between HLA-B27 and other variables that affected the outcome of VHD, sex and categorical disease duration were found to interact with HLA-B27 after being adjusted by age ($p < 0.05$). In sex-stratified analysis, male patients with HLA-B27 increased the risk of VHD (OR 11.2; 95% CI 1.40–89.36) after being adjusted by age. In stratified analysis of disease duration, over 24 months of duration increased the risk of VHD (OR 5.86; 95% CI 1.27–27.07).

Conclusions: Our study provided evidence that age was the only independent risk factor for VHD in axSpA. HLA-B27 interacted with sex and disease duration to increase the risk of VHD in axSpA patients.

1 | Introduction

The remarkably strong association of ankylosing spondylitis (AS) with HLA-B27 was first discovered independently by groups in London and California in 1972 [1, 2]. Epidemiologic studies find that AS is very rare in the indigenous populations of southern parts of Africa that lack HLA-B27, indicating that the prevalence of AS roughly directly correlates with the prevalence of HLA-B27 in the population [3]. Classification criteria for axSpA, revised by the Assessment of SpondyloArthritis International Society (ASAS)

Yupeng Lai and Yanpeng Zhang contributed equally to this work.

in 2009, include patients with chronic back pain (> 3 months) and age at onset less than 45 years, in the presence of sacroiliitis plus at least one typical SpA feature, or in the presence of HLA-B27 plus at least two other SpA features [4, 5]. These criteria have further confirmed the important role of HLA-B27 in axSpA.

Valvular heart disease (VHD), as one of the extra-articular manifestations of axSpA, which could threaten the life of patients with axSpA, has aroused researchers' curiosity and enthusiasm for many years. Studies have described the association between VHD and axSpA. Studies including axSpA or AS patients were more likely to report the prevalence of aortic valve diseases. Studies noted that the prevalence of aortic regurgitation was significantly higher in AS patients than expected from general population data [6, 7]. A report using cardiac magnetic resonance imaging detected that valvulitis and aortitis were associated with ankylosing spondylitis [8]. Studies have also found that the prevalence of aortic incompetence increases with age, disease duration, and the presence of peripheral arthritis [9]. Although aortic valve disease was the common topic in AS, the prevalence of mitral valve disease appeared to be more than that of aortic disease. In a recent cohort study including 3780 AS patients, 199 patients developed mitral valve disease while 53 patients developed aortic valve disease [10]. Despite the prevalence reports on aortic valve disease and mitral valve disease, only a few studies have researched the relationship between HLA-B27 and VHD in axSpA. A study found that HLA-B27 was an important genetic risk factor for the development of lone aortic regurgitation [11]. A small sample study found that HLA-B27 was present in similar proportions in the presence vs. absence of aortic regurgitation [6]. Another similar research about heart diseases was HLA-B27-associated cardiac diseases concerning conduction disturbance [12, 13]. Some other studies even reported paradoxical results about the association between HLA-B27 and VHD [14]. The reason for the lack of definite recognition of the association between HLA-B27 and VHD in axSpA is probably that positively associated results are concealed by other risk factors, such as age, disease duration, and the presence of peripheral arthritis, which are described in previous studies.

The aim of our study is to uncover the association between HLA-B27 and VHD in axSpA and to explore the interaction between HLA-B27 and other risk factors of VHD in axSpA.

2 | Methods

2.1 | Study Design

This is a cross-sectional study, with data extracted from hospitals information systems.

2.2 | Study Population

For this cross-sectional study, original data of axSpA inpatients for the first time of hospitalization from 2016 to 2022 in Shenzhen Second People's Hospital were collected. Firstly, 548 patients were included according to the ASAS 2009 classification criteria for axSpA or modified New York's criteria [15]. The 1984 modified New York's criteria for ankylosing spondylitis (AS) are consistent with the ASAS 2009 classification criteria for axSpA in patients with sacroiliitis [16]. After excluding patients without echocardiography and HLA-B27, 444 patients were ultimately included in the study.

2.3 | HLA-B27 Assessment

HLA-B27 examination data were collected from the information systems of Shenzhen Second Hospital. HLA-B27 data were recorded as two groups (positive and negative).

2.4 | VHD Ascertainment

VHD was defined as valvular heart disease ascertained by echocardiography, including mitral stenosis, mitral regurgitation, tricuspid stenosis, tricuspid regurgitation, aortic valve stenosis, aortic valve regurgitation, pulmonary valve stenosis, and pulmonary valve regurgitation.

2.5 | Variables Measurement

Demographic and clinical variables included sociodemographic characteristics (age, sex, marital status), lifestyle factors and medical conditions (ever smoke, ever alcohol intake, exercise, body mass index [BMI], diabetes, cardiovascular diseases [17], gout, and hypertension), axSpA-related clinical and laboratory characteristics (systolic pressure [SBP], diastolic blood pressure [DBP], disease duration, enthesitis, dactylitis, iritis, psoriasis, inflammatory bowel disease [IBD], erythrocyte sedimentation rate [ESR], C-reactive protein [CRP], creatine [Cr], alanine aminotransferase [ALT], aspartate aminotransferase[AST], low-density lipoprotein [LDL], Bath Ankylosing Spondylitis Disease Activity Index [BASDAI] [18], Ankylosing Spondylitis Disease Activity Score calculated with CRP [ASDAS] [19], Bath Ankylosing Spondylitis Functional Index [BASFI] [20], non-steroidal anti-inflammatory drugs [NSAIDs] intake, methotrexate [MTX] intake, sulfasalazine [SSZ] intake, thalidomide intake, tumor necrosis factor inhibitor [TNFi]) intake.

3 | Ethics Statement

The study was approved by the Ethics Committee of Shenzhen Second People's Hospital, with approval ID 20200601011-FS01, and a waiver of patient consent was provided by the Ethics Committee for this study. What is more, written consent was not required if individual privacy and commercial interests were not involved, according to the medical ethical law of China.

3.1 | Statistical Analysis

Data are presented as valid percentages and mean values with a standard deviation in 2 groups (non-VHD and VHD). Standardized difference was used to evaluate the heterogeneity of the baseline characteristics, and a standardized difference $p \geq 0.1$ indicated a negligible difference in potential confounders between the two study groups. The associations of VHD with

nominal, ordinal, and continuous variables were analyzed in a univariate binary regression model. To investigate whether HLA-B27 were independently associated with VHD, a multivariate logistic regression model was constructed from the differences of univariate regression baseline between non-VHD patients and VHD patients ($p < 0.10$ in univariate analyses). Univariate and multivariate logistic regression analyses were presented as estimated odds ratios (ORs) and 95% confidence intervals (CIs). For all other analyses, statistical significance was set at $p < 0.05$. Interaction analyses were performed to find out the other factors that influenced the association between HLA-B27 and VHD. Stratified analyses based on the interacting factors were performed to confirm the association between HLA-B27 and VHD in axSpA. We used multiple multivariate imputations to handle the missing data of covariates (exposure and outcome excluded) [21]. We made sensitivity analyses to compare imputed complete data and pre-imputation data. Data were analyzed using the statistical packages R (The R Foundation; http://www.r-project.org; version 3.4.3) and EmpowerStats (www.empowerstats.com; X&Y Solutions Inc).

4 | Results

4.1 | Characteristics of the Study

A total of 548 patients diagnosed as axSpA were enrolled in the original data set. After excluding 103 subjects without echocardiography examination and one subject without HLA-B27 examination, 444 patients were included in the data set for analysis. The 444 patients were divided into the non-VHD group and the VHD group according to the echocardiographic results. The non-VHD group included 347 patients, while the VHD group included 97 patients. In the VHD group, 53 patients suffered from mitral valve (MV) diseases, 65 patients suffered from tricuspid valve (TV) diseases, 34 patients suffered from aortic valve (AV) diseases, and 5 patients suffered from pulmonary valve (PV) diseases. The flowchart for including patients is presented in Figure 1. The differences in demographic and clinical characteristics between patients with VHD and those without are displayed in Table 1. Compared with the non-VHD group, patients with VHD were older, more often male, more often married, had a longer disease duration, higher ESR, and more frequently comorbid diabetes, cardiovascular diseases, and hypertension.

4.2 | Univariable and Multivariable Predictors of VHD

In the univariate logistic regression model, all the variables of characteristics in Table 1 were included to find the risk predictors of VHD. Age, sex, marital status, diabetes, cardiovascular disease, hypertension, disease duration, dactylitis, ALT, AST, and thalidomide were associated with VHD ($p < 0.05$). To further confirm the predictors of VHD, a multivariate logistic regression model was constructed, based on the differences of univariate regression baseline between non-VHD patients and VHD patients ($p < 0.10$ in univariate analyses). Thus, in addition to the above-mentioned variables, HLA-B27, ESR, and MTX were included in the multivariable logistic regression model. All the variables included in the multivariate logistic regression were detected for collinearity, and the variance inflation factors were low (VIF < 5). In the multivariate logistic regression model, age remained an independent predictor of VHD in axSpA (OR 1.054; 95% CI 1.021–1.087).

4.3 | Detecting Interactions Between HLA-B27 and Other Variables

Considering that HLA-B27 plays an important role in the pathogenesis of axSpA, it is possible that HLA-B27 also plays a part in the extra-articular manifestation of axSpA, such as VHD. However, few previous studies mentioned the relationship between HLA-B27 and VHD in axSpA patients. It is probable that the interaction between HLA-B27 and other variables impedes the real impact of HLA-B27 on VHD. Thus, we made a statistical detection to find the interaction of HLA-B27 and all the variables that were verified as risk factors in Table 2. Before exploring the interaction, we transformed the disease duration variable from a continuous variable to a categorical variable, with 24 months as the cutting point. The interaction detections between HLA-B27 and other variables affecting the outcome of VHD are displayed in Table 3. Sex, hypertension, and categorical disease duration were found to interact with HLA-B27. To further confirm these results, interaction detections were performed again between HLA-B27 and those three variables by adjusting for age, as age was ascertained in the previous univariate and multivariate analyses to be an independent predictor of VHD in axSpA. In the adjusted model, only sex and categorical disease duration interacted with HLA-B27 to impact VHD ($p < 0.05$). The crude analyses, as well as the adjusted models, were displayed

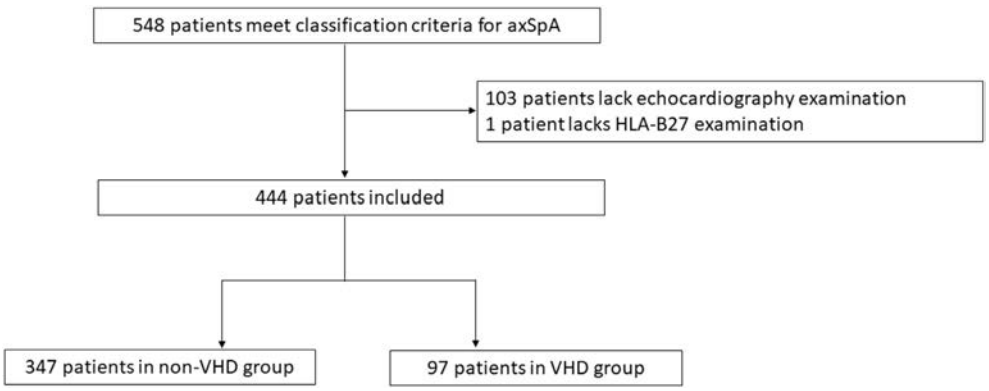

FIGURE 1 | The flowchart for including patients.

TABLE 1 | Demographic and disease characteristics of the 444 patients with or without VHD.

	Total ($n=444$)[a]	Non-VHD ($n=347$)[a]	VHD ($n=97$)[a]	p
Age (years)	(444) 36.82 ± 12.08	(347) 34.69 ± 10.60	(97) 44.44 ± 13.90	< 0.001
BMI (kg/m^2)	(419) 23.16 ± 3.80	(329) 23.18 ± 3.98	(90) 23.12 ± 3.08	1.000
SBP (mmHg)	(438) 118.75 ± 16.71	(346) 118.69 ± 14.07	(92) 118.97 ± 24.31	0.957
DBP (mmHg)	(438) 79.26 ± 10.40	(346) 78.95 ± 10.38	(92) 80.42 ± 10.46	0.440
Disease duration (months)	(443) 72.00 (24.00–120.00)	(347) 60.00 (24.00–120.00)	(96) 108.00 (24.00–168.50)	0.020
ESR (mm/h)	(433) 12.00 (6.00–31.00)	(337) 11.00 (5.00–30.00)	(96) 18.50 (9.00–35.25)	0.002
CRP (mg/L)	(443) 5.54 (1.96–17.80)	(346) 5.35 (1.91–17.80)	(97) 5.71 (2.00–17.73)	0.835
Creatine (umol/L)	(443) 65.98 ± 15.06	(347) 65.97 ± 14.54	(96) 66.01 ± 16.91	0.512
ALT (U/L)	(439) 14.40 (9.00–22.65)	(344) 15.15 (9.00–24.23)	(95) 12.00 (9.05–17.65)	0.032
AST (U/L)	(400) 15.00 (13.00–19.00)	(318) 16.00 (13.00–19.15)	(82) 15.00 (12.00–17.00)	0.009
LDL (mmol/L)	(405) 2.61 (2.06–3.14)	(318) 2.67 (2.10–3.17)	(87) 2.39 (1.90–3.00)	0.034
BASDAI	(282) 3.00 (1.72–4.60)	(230) 3.00 (1.70–4.60)	(52) 3.25 (1.80–4.53)	0.820
ASDAS	(279) 2.50 (1.60–3.20)	(227) 2.40 (1.63–3.20)	(52) 2.60 (1.55–3.30)	0.888
BASFI	(282) 1.30 (0.20–3.00)	(230) 1.20 (0.20–3.00)	(52) 1.50 (0.27–2.90)	0.952
Sex				0.011
Female	145 (32.66%)	103 (29.68%)	42 (43.30%)	
Male	299 (67.34%)	244 (70.32%)	55 (56.70%)	
Marital status				0.002
Unmarried	131 (29.98%)	117 (33.91%)	14 (15.22%)	
Married	290 (66.36%)	215 (62.32%)	75 (81.52%)	
Divorced	16 (3.66%)	13 (3.77%)	3 (3.26%)	
Ever smoke				0.116
No	367 (82.66%)	292 (84.15%)	75 (77.32%)	
Yes	77 (17.34%)	55 (15.85%)	22 (22.68%)	
Ever alcohol intake				0.720
No	398 (89.64%)	312 (89.91%)	86 (88.66%)	
Yes	46 (10.36%)	35 (10.09%)	11 (11.34%)	
Exercise				0.691
No	405 (97.83%)	313 (97.51%)	92 (98.92%)	
Yes	9 (2.17%)	8 (2.49%)	1 (1.08%)	
Diabetes				< 0.001
No	431 (97.07%)	343 (98.85%)	88 (90.72%)	
Yes	13 (2.93%)	4 (1.15%)	9 (9.28%)	
Cardiovascular diseases				0.026
No	434 (97.97%)	343 (98.85%)	91 (94.79%)	
Yes	9 (2.03%)	4 (1.15%)	5 (5.21%)	
Gout				0.918
No	422 (95.05%)	330 (95.10%)	92 (94.85%)	
Yes	22 (4.95%)	17 (4.90%)	5 (5.15%)	

(Continues)

TABLE 1 | (Continued)

	Total ($n = 444$)[a]	Non-VHD ($n = 347$)[a]	VHD ($n = 97$)[a]	*p*
Hypertension				0.008
No	412 (92.79%)	328 (94.52%)	84 (86.60%)	
Yes	32 (7.21%)	19 (5.48%)	13 (13.40%)	
Enthesitis				0.651
No	388 (88.79%)	304 (89.15%)	84 (87.50%)	
Yes	49 (11.21%)	37 (10.85%)	12 (12.50%)	
Dactylitis				0.004
No	404 (90.99%)	323 (93.08%)	81 (83.51%)	
Yes	40 (9.01%)	24 (6.92%)	16 (16.49%)	
Iritis				0.400
No	395 (88.96%)	311 (89.63%)	84 (86.60%)	
Yes	49 (11.04%)	36 (10.37%)	13 (13.40%)	
Psoriasis				0.886
No	434 (97.75%)	339 (97.69%)	95 (97.94%)	
Yes	10 (2.25%)	8 (2.31%)	2 (2.06%)	
IBD				1.000
No	439 (98.87%)	343 (98.85%)	96 (98.97%)	
Yes	5 (1.13%)	4 (1.15%)	1 (1.03%)	
HLA-B27				0.092
No	54 (12.16%)	47 (13.54%)	7 (7.22%)	
Yes	390 (87.84%)	300 (86.46%)	90 (92.78%)	
NSAIDs				0.890
No	153 (34.46%)	119 (34.29%)	34 (35.05%)	
Yes	291 (65.54%)	228 (65.71%)	63 (64.95%)	
MTX				0.087
No	404 (90.99%)	320 (92.22%)	84 (86.60%)	
Yes	40 (9.01%)	27 (7.78%)	13 (13.40%)	
SSZ				0.585
No	321 (72.30%)	253 (72.91%)	68 (70.10%)	
Yes	123 (27.70%)	94 (27.09%)	29 (29.90%)	
Thalidomide				0.030
No	415 (93.47%)	329 (94.81%)	86 (88.66%)	
Yes	29 (6.53%)	18 (5.19%)	11 (11.34%)	
TNFi				0.320
No	321 (72.30%)	247 (71.18%)	74 (76.29%)	
Yes	123 (27.70%)	100 (28.82%)	23 (23.71%)	

Note: All results are presented as (*N*) Mean + SD for continuous variables with normal distribution, (*N*) Median (Q1–Q3) for continuous variables without normal distribution, and *N* (%) for categorical variables.

Abbreviations: ALT, alanine aminotransferase; ASDAS, Ankylosing Spondylitis Disease Activity Score-CRP; AST, aspartate aminotransferase; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BMI, body mass index, diabetes, cardiovascular diseases, gout, and hypertension; Cr, creatine; CRP, C-reactive protein; DBP, diastolic blood pressure; ESR, erythrocyte sedimentation rate; HLA-B27, human leucocyte antigen B27; IBD, inflammatory bowel disease; LDL, low-density lipoprotein; MTX, methotrexate; NSAIDs, non-steroidal anti-inflammatory drugs; SBP, systolic pressure; SSZ, sulfasalazine; TNFi, tumor necrosis factor inhibitor.

[a]All % calculated for 444, 347, or 97 patients, respectively, unless stated otherwise. In case % were calculated for less than the maximal number of patients, data for some patients were missing, and the actual denominator is displayed.

TABLE 2 | Univariable and multivariable logistic regression models for risk factors of VHD.

Variable	Univariable odds ratios	95% CI	*p*
Age (years)	1.07	(1.05, 1.09)	< 0.0001
Sex			
Female	1.0	Ref.	
Male	0.55	(0.35, 0.88)	0.0121
Marital status			
Unmarried	1.0	Ref.	
Married	2.92	(1.58, 5.38)	0.0006
Divorced	1.93	(0.49, 7.61)	0.3482
Ever smoke			
No	1.0	Ref.	
Yes	1.56	(0.89, 2.71)	0.1182
Ever alcohol intake			
No	1.0	Ref.	
Yes	1.14	(0.56, 2.34)	0.7203
Exercise			
No	1.0	Ref.	
Yes	0.43	(0.05, 3.44)	0.4230
BMI (kg/m^2)	1.00	(0.94, 1.06)	0.9062
Diabetes			
No	1.0	Ref.	
Yes	8.77	(2.64, 29.14)	0.0004
Cardiovascular diseases			
No	1.0	Ref.	
Yes	4.71	(1.24, 17.90)	0.0229
Gout			
No	1.0	Ref.	
Yes	1.05	(0.38, 2.94)	0.9184
Hypertension			
No	1.0	Ref.	
Yes	2.67	(1.27, 5.63)	0.0097
SBP (mmHg)	1.00	(0.99, 1.01)	0.8877
DBP (mmHg)	1.01	(0.99, 1.04)	0.2258
Disease duration (months)	1.00	(1.00, 1.01)	0.0002
Enthesitis			
No	1.0	Ref.	
Yes	1.17	(0.59, 2.35)	0.6512
Dactylitis			
No	1.0	Ref.	
Yes	2.66	(1.35, 5.24)	0.0047

(Continues)

TABLE 2 | (Continued)

Variable	Univariable odds ratios	95% CI	*p*
Iritis			
No	1.0	Ref.	
Yes	1.34	(0.68, 2.63)	0.4015
Psoriasis			
No	1.0	Ref.	
Yes	0.89	(0.19, 4.27)	0.8864
IBD			
No	1.0	Ref.	
Yes	0.89	(0.10, 8.09)	0.9200
HLA-B27			
No	1.0	Ref.	
Yes	2.01	(0.88, 4.61)	0.0975
ESR (mm/h)	1.01	(1.00, 1.02)	0.0649
CRP (mg/L)	1.00	(0.99, 1.01)	0.8004
Creatine (umol/L)	1.00	(0.99, 1.02)	0.9810
ALT (U/L)	0.98	(0.96, 1.00)	0.0293
AST (U/L)	0.96	(0.92, 1.00)	0.0321
LDL (mmol/L)	1.04	(0.91, 1.18)	0.5628
BASDAI	0.98	(0.87, 1.10)	0.7083
ASDAS	1.08	(0.86, 1.36)	0.5054
BASFI	1.00	(0.89, 1.13)	0.9952
NSAIDs			
No	1.0	Ref.	
Yes	0.97	(0.60, 1.55)	0.8896
MTX			
No	1.0	Ref.	
Yes	1.83	(0.91, 3.71)	0.0912
SSZ			
No	1.0	Ref.	
Yes	1.15	(0.70, 1.88)	0.5851
Thalidomide			
No	1.0	Ref.	
Yes	2.34	(1.06, 5.13)	0.0344
TNFi			
No	1.0	Ref.	
Yes	0.77	(0.46, 1.29)	0.3213
Variable	**Multivariable odds ratios**	**95% CI**	***p***
Age (years)	1.054	(1.021, 1.087)	0.001

Note: All results are presented as β (95% CI) *p*-value for continuous variables, and OR (95% CI) *p*-value for categorical variables.

in Table 3 and also presented as Figure 2. We concluded that HLA-B27 interacted with sex and categorical disease duration to impact VHD.

4.4 | Stratified Analyses to Detect the Association Between HLA-B27 and VHD

Based on the previous interaction result, multivariate logistic analyses stratified by Sex and categorical disease duration were performed between HLA-B27 and VHD. According to the pathogenesis and disease progression of axSpA, assessment for axSpA is usually performed every 2 years [22]. Thus, we transform the disease duration variable from a continuous variable to a categorical variable, with 24 months as the cutting point, for further stratified analysis. Stratified analyses results are presented in Table 4. In the analyses stratified by sex, HLA-B27 was a risk factor for VHD in male patients (OR 8.74; 95% CI 1.71–65.31), in accordance with the result adjusted by age (OR 11.2; 95% CI 1.40–89.36). In the analyses stratified by categorical disease duration, HLA-B27 was a risk factor for VHD in patients with disease duration longer than 24 months (OR 4.67; 95% CI 1.09–20.10), in accordance with the result adjusted by age (OR 5.86; 95% CI 1.27–27.07). Results of stratified analyses are presented in Table 4.

4.5 | Sensitive Analyses With Multivariate Imputations

Although very few variables comprised missing data, to make results more robust, sensitive analyses were performed to compare original data with multivariate imputed data, in univariate logistic analyses, multivariate logistic analysis, and stratified analyses. Thus, we imputed missing data to acquire five replications of multivariate imputation for repeating the statistic program. The univariate logistic analyses based on multivariate imputed data achieved the same results as original data and found the same 14 risk predictors (such as Age, sex, marital status, diabetes, cardiovascular disease, hypertension, disease duration, dactylitis, ALT, AST, thalidomide, HLA-B27, ESR, MTX), according to the same criterion as previously performed ($p < 0.1$). These 14 variables were included in the multivariate logistic analyses and further confirmed that age is the independent risk factor of VHD (OR 1.057; 95% CI 1.027–1.088). Results were similar among five replications of imputed data. Results of univariate analyses based on one of the replications of multivariate imputed data were displayed Table S1. Results of multivariate analyses based on pool results of five replications of multivariate imputed data were displayed in Table S2. We also performed interaction detection between HLA-B27 and sex, hypertension, categorical disease

TABLE 3 | Interaction between HLA-B27 and other variables to affect the risk of VHD.

			VHD					
			Crude model			Adjusted model		
Exposure	**Interactive factor**	***N***	**OR**	**95% CI**	***p***	**OR**	**95% CI**	***p***
HLA-B27	Sex							
No	Female	19	Ref.			Ref.		
Yes	Female	126	0.87	(0.31, 2.46)	0.7877	0.73	(0.24, 2.23)	0.5858
No	Male	35	0.06	(0.01, 0.58)	0.0147	0.05	(0.01, 0.53)	0.0123
Yes	Male	264	0.56	(0.20, 1.53)	0.2575	0.58	(0.20, 1.69)	0.3174
p for interaction					0.0185			0.0086
HLA-B27	Categorical disease duration							
No	≤24	23	Ref.			Ref.		
Yes	≤24	110	0.85	(0.28, 2.55)	0.7710	0.84	(0.27, 2.64)	0.7672
No	>24	31	0.25	(0.04, 1.42)	0.1170	0.15	(0.02, 0.89)	0.0375
Yes	>24	279	1.16	(0.42, 3.24)	0.7769	0.82	(0.28, 2.40)	0.7198
p for interaction					0.0539			0.0384
HLA_B27	Hypertension							
No	No	48	Ref.			Ref.		
Yes	No	364	1.57	(0.68, 3.64)	0.2917	1.70	(0.71, 4.08)	0.2354
No	Yes	6	0.00	(0.00, Inf)	0.9815	0.00	(0.00, Inf)	0.9861
Yes	Yes	26	5.86	(1.93, 17.78)	0.0018	1.92	(0.57, 6.49)	0.2929
p interaction					0.0317			0.1046

Note: Adjusted Model is adjusted for age. The cutting point for categorical disease duration is 24 months. Results in this table is displayed as OR (95% CI) *p*-value.
Abbreviations: HLA-B27, exposure; VHD, dependent variable.

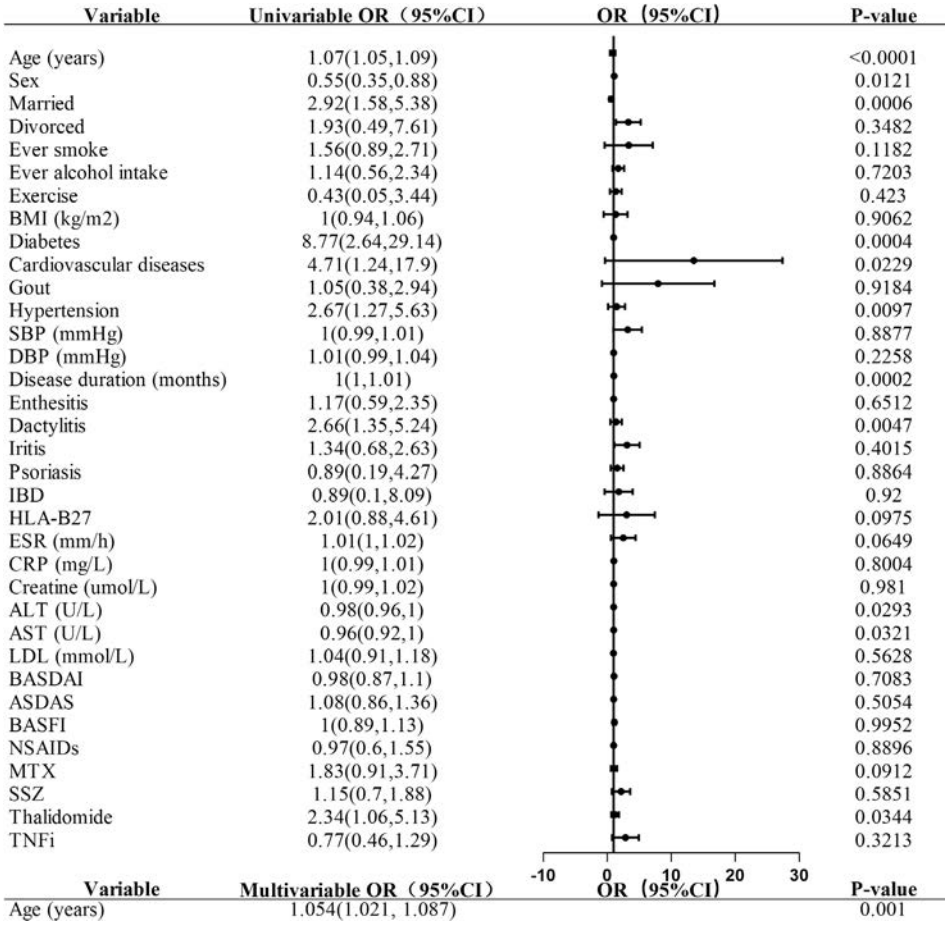

FIGURE 2 | Univariable and multivariable logistic regression models for risk factors of VHD.

duration based on imputed data and achieved the same results as original data. Results among five replications of imputed data were similar. Table S3 displayed one of the replications of multivariate imputed data for interaction detection. Stratified analyses were also performed with five replications of imputed data, and the pool results from five replications were the same as original data (Table S4). In the analyses stratified by sex, HLA-B27 was a risk factor for VHD in the male patients (OR 8.74; 95% CI 1.71–65.31). In the analyses stratified by categorical disease duration, HLA-B27 was a risk factor for VHD in patients with disease duration longer than 24 months (OR 4.73; 95% CI 1.10–20.34). The adjusted model displayed similar results to the crude model.

5 | Discussion

In the past decades, studies have found that AS patients, diagnosed with 1984 modified New York criteria, have a higher risk of VHD compared with the general population without AS [23]. In the recent years, patients diagnosed with axSpA according to the new ASAS 2009 classification criteria were also found to have a higher risk of cardiac rhythm disturbances and aortic regurgitation [7]. Only a few studies found that severe conduction system abnormalities and aortic regurgitation were associated with HLA-B27 [12]. Our study further explored the mechanism for the higher risk of VHD in axSpA, disclosed the association of HLA-B27 and VHD in axSpA, and uncovered the impact of the interaction between HLA-B27 and other factors on VHD in axSpA.

We primarily performed univariate analyses to determine the risk factors of VHD in axSpA. Age, sex, marital status, diabetes, cardiovascular disease, hypertension, disease duration, dactylitis, ALT, AST, and thalidomide were associated with VHD ($p < 0.05$), and HLA-B27, ESR, and MTX were possibly associated with VHD ($p < 0.1$). Our results were similar to some studies on the risk factors for VHD in axSpA, such as age, sex, disease duration, and dactylitis [9]. However, in the performance of further confirmation with multivariate analyses, only age turned out to be an independent risk factor of VHD in axSpA. In a general population-based study, VHD increased with age, from 0.3% of the 18–44-years-old to 11.7% of those aged 75 years and older [24]. The result of the previous study was similar to our result. Thus, age played an important role in VHD. All the other risk factors that increased with age, such as diabetes, cardiovascular disease, hypertension, and disease duration, displayed no significant impact on VHD with adjustment for age. When we considered the interaction of HLA-B27 with other risk factors, age needed to be adjusted.

When we come to the fact that HLA-B27 plays a prominent role in axSpA, we are curious about the association between HLA-B27 and VHD in axSpA. In previous years, studies have shown paradoxical results about the association between HLA-B27 and one of the VHDs, aortic valve disease [14]. Some studies

TABLE 4 | Stratified analyses for the association between HLA-B27 and VHD.

HLA-B27		VHD					
		Crude model			Adjusted model		
Subgroup	*N*	OR	95% CI	*p*	OR	95% CI	*p*
Male							
0	145	0.87	(0.31, 2.46)	0.7877	0.76	(0.25, 2.25)	0.6144
1	299	8.74	(1.17, 65.31)	0.0346	11.20	(1.40, 89.36)	0.0226
Diseaseduration categorical							
≤24	133	0.85	(0.28, 2.55)	0.7710	0.85	(0.27, 2.62)	0.7730
>24	310	4.67	(1.09, 20.10)	0.0383	5.86	(1.27, 27.07)	0.0236

Note: Adjusted model was adjusted for age.
Abbreviations: HLA-B27, variable; Subgroup, interactive factor; VHD, dependent variable.

found that patients with lone aortic incompetence were none with HLA-B27 [25]. Some studies found that the situation that patients with both lone aortic incompetence and excess HLA-B27 occurred only in patients with spondyloarthritis [26]. Some studies displayed that the prevalence of spondylitic heart disease increases steeply with the duration of the arthritis, and HLA-B27 was not an independent manifestation of VHD [27]. Some studies displayed that HLA-B27 was an important genetic risk factor for lone aortic regurgitation and severe conduction system abnormalities [11]. Our results have presented a more definite association between HLA-B27 and VHD in axSpA. Based on our multivariate analyses results, HLA-B27 was a possible risk factor for VHD in axSpA. The reason that it did not achieve significance was probably that the impact of HLA-B27 on VHD was concealed by other risk factors, such as age, disease duration, and presence of peripheral arthritis. Exploring the interaction between HLA-B27 and risk factors detected from univariate analyses assisted us in finding the interactive factors, such as sex and categorical disease duration. Then we stratified the data by sex and categorical disease duration, and we found in male sex HLA-B27 turned out to be a risk factor for VHD and in patients with over 24 months of disease duration HLA-B27 turned out to be a risk factor for VHD.

It has been a controversial question whether the male or female population is more inclined to suffer from VHD in the general population. In the 11,911 randomly selected adult population, VHD was diagnosed less often in women than in men (odds ratio 0.90, 0.81–1.01; $p = 0.07$) [24]. Studies concerning sex-related VHD displayed that women were predisposed to suffer from aortic stenosis, mitral regurgitation, non-rheumatic calcific mitral stenosis, tricuspid valve disease, and a higher risk of death in aortic regurgitation [28]. Other studies found that women more frequently suffered from mitral valve (MV) diseases such as mitral valve prolapse or rheumatic MV disease, while men more often develop aortic valve (AV) diseases, including aortic regurgitation or aortic stenosis associated with bicuspid AVs [29]. It is possible that patients with axSpA are different from the general population in VHD. A study shows that AS patients have a statistically higher risk of mitral valve, aortic valve, and tricuspid valve disease development [10]. Our results show that male patients are the majority in this study, while MV diseases and tricuspid valve (TV) diseases are the predominant VHDs of this study. It is also contradicted by general population studies that females more frequently suffered from VHD or females more frequently suffered from MV diseases. However, our results are consistent with a recent cohort study that found more AS patients developed mitral valve disease than aortic valve disease [10]. Thus, we infer that the interaction between HLA-B27 and male sex plays an important part in impacting the prevalence of VHD in axSpA. In addition, stratified analyses further confirm the interaction between HLA-B27 and male sex in impacting VHD. However, limited to the current sample of the axSpA study, we could not stratify VHD by MV, TV, AV, and PV, and minor differences between them could not be discovered.

Disease duration was primarily discovered to be a risk factor in univariate analyses but was denied by multivariate analyses. After transforming the disease duration from a continuous viable to a binary variable with 24 months as the cutting point, we discovered the interaction between HLA-B27 and categorical disease duration even after adjusting for age. Both periodontitis and axial spondyloarthritis are chronic inflammatory conditions. Evidence from these studies suggests that periodontitis is associated with an increased risk of VHD and may contribute to its development, with age being a significant factor influencing this association [30]. Additionally, another genetic study supports that HLA-B27 positive patients tend to have an earlier onset and diagnosis age. The research also suggests that this age correlation may link genetically to ERAP1 and ERAP2 [31], which seemingly implies that longer disease duration contributes to the occurrence of VHD in axSpA patients [3]. Time was not the only reason for VHD in axSpA, but HLA-B27 made sense. Research concerning the gut microbiome and its metabolites in AS has discovered that HLA-B27 induces an endoplasmic reticulum stress response and promotes autophagy/unfolded protein responses (UPR). Consequently, the induction of UPR genes results in the release of tumor necrosis factor-α (TNF-α) [32]. Another study further reported that the mechanism by which gut microbiome dysbiosis caused by HLA-B27 may induce the production of inflammatory cytokines, including IL-17, IL-23, IL-22, TNF-α, and IFN-γ, ultimately leading to new bone formation and bone erosion that leads to bone fusion in AS [31]. Study upon mice have demonstrated that the overexpression of TNF in the TghuTNF (Tg197) arthritis model, in addition to chronic polyarthritis, drives the development of spontaneous left-sided VHD, which mainly leads

to valvular thickening with some degree of stenosis and occasionally to valve insufficiency, comorbid pathologies often observed in patients with SpA. Comorbid heart valve disease and chronic polyarthritis share common TNF/TNFR1-mediated mesenchymal cell-specific aetiopathogenic mechanisms [33]. In AS patients, the risk of spinal fusion increased with longer diseases duration [34]. Meanwhile, the risk of VHD increased with longer disease duration. The possible pathway is that HLA-B27 prompts the production of TNF-α by inducing UPR through the gut microbiome and its metabolites. Thus, in categorical disease duration (> 24 months), HLA-B27 is a risk factor for VHD in axSpA. According to the common TNF/TNFR1-mediated mechanisms that VHD and sacroilitis share, the therapy of TNF inhibitors might change the outcome of VHD with long disease duration.

The limitation of this study was due to a relatively small sample size. First, due to the sample size of 444 patients, the confidence intervals of this study were wide. For samples of more than 500, the average widths of confidence intervals were within reasonable ranges. For samples of less than 500, however, the average widths of confidence intervals were abnormally wide in many cases [35]. Second, due to the relatively small sample size, we could not stratify the types of VHD for further analyses between different VHD types and risk factors.

6 | Conclusion

Age was the only independent risk factor of VHD in axSpA. Evidence showed that HLA-B27 interacted with sex and disease duration to increase the risk of VHD in axSpA patients. HLA-B27 increased the risk of VHD in male axSpA patients, and HLA-B27 also increased the risk of VHD in patients with over 24 months of disease duration. In summary, we presented a definite association between HLA-B27 and VHD in axSpA. Our results will probably promote more sophisticated research in HLA-B27 subtype in axSpA. Rheumatologists will pay more attention to male patients in the onset of disease duration, and earlier therapy will be given to them.

Author Contributions

Yupeng Lai and Yanpeng Zhang developed the outline and objective of the article. Shaozhen Mo, Yihong Huang, Jiaming Huang, and Suo Zhang assisted in the preparation of figures and writing. Zhongfeng Lou, Haoliang Li, Jing Li, and Xingjiao Liu assisted in data collection and literature review. Yupeng Lai wrote the first draft of the article. Meiying Wang, Shuo Cheng, and Yanpeng Zhang revised the article critically for its intellectual content. All authors approved the final version to be published and take responsibility for the integrity of the content covered in this article.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

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LETTER TO THE EDITOR

Case Report: Nutcracker Syndrome Triggered by Rapid Weight Loss in a Patient With Systemic Lupus Erythematosus

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ABSTRACT

Nutcracker syndrome (NCS) is a rare vascular disorder characterized by compression of the left renal vein, typically causing hematuria, proteinuria, and flank pain. Although NCS is often linked with weight loss and anatomical variations, no previous reports have connected it to systemic lupus erythematosus (SLE). We describe a 44-year-old male with SLE who developed NCS after rapid weight loss, presenting with abdominal pain, hematuria, and proteinuria. Imaging confirmed left renal vein compression between the aorta and the superior mesenteric artery. This finding suggests that significant weight reduction in SLE may trigger NCS by altering retroperitoneal fat and vascular structures. A literature review reveals a consistent association between NCS and marked weight loss, as well as possible coexistence with other vascular compression syndromes, such as superior mesenteric artery syndrome. Clinicians should consider NCS in SLE patients with sudden weight changes to ensure timely diagnosis and prevent complications.

1 | Introduction

Nutcracker syndrome (NCS) is a rare vascular condition characterized by the compression of the left renal vein (LRV) between the abdominal aorta and the superior mesenteric artery (SMA) [1]. This condition can lead to hematuria, proteinuria, and flank pain, significantly impacting renal function and overall health [2]. Factors contributing to NCS include anatomical variations and significant weight loss, which can elevate intra-abdominal pressure.

Systemic lupus erythematosus (SLE) is an autoimmune disease that often leads to weight fluctuations due to the disease itself or its treatments. Rapid weight loss in SLE patients may exacerbate existing vascular compression syndromes, including NCS [3].

Here, we present a 44-year-old male patient with SLE who developed NCS following marked weight loss. This case emphasizes the need for heightened clinician awareness when managing patients with autoimmune disorders who experience sudden weight changes. Additionally, we discuss literature findings on the interplay between weight loss and NCS, focusing on the clinical and therapeutic implications in SLE patients. Recognizing weight loss as a possible precipitating factor for NCS can

Chen Li and Yu-wei Wang contributed equally to this study.

improve diagnostic accuracy and guide management decisions, ultimately reducing associated morbidity.

2 | Case Presentation

A 44-year-old male patient presented with a seven-year history of recurrent, low-grade fever (not exceeding 38°C) and polyarthritis, along with 3 months of abdominal pain. He also reported fatigue and recurrent oral ulcers, typically arising three to five times per year, each lasting 1–2 weeks and frequently triggered by stress.

The patient's joint involvement included intermittent swelling and pain of the proximal interphalangeal joints, metacarpophalangeal joints, and wrists, with morning stiffness of less than 30 min. Laboratory tests at a local hospital demonstrated significant proteinuria and hematuria, alongside positive antinuclear antibodies and anti-dsDNA antibodies, leading to a clinical diagnosis of SLE and lupus nephritis. His initial treatment plan involved prednisone, hydroxychloroquine, and leflunomide; however, inconsistent adherence led to fluctuating disease activity.

About 6 months prior to admission, the patient experienced an unexplained weight loss of approximately 20 kg, accompanied by a reduced appetite. Three months before hospitalization, he developed persistent abdominal pain primarily localized to the right upper quadrant. The pain, which worsened postprandially and occasionally radiated to the back, was relieved by flexing forward and sometimes necessitated a fetal position for comfort. During this period, he also had intermittent fever, reaching up to 38°C. He denied vomiting or diarrhea. Presumptive treatment for possible cholelithiasis and cholecystitis proved ineffective, prompting further investigation.

On admission, the patient appeared cachectic, with a body mass index (BMI) of 22.3 kg/m^2 (down from 29.4 kg/m^2 3 months earlier). He maintained a flexed posture. His abdomen was flat, without visible gastrointestinal waves, peristalsis, or abdominal wall varices. There was mild right upper abdominal and periumbilical tenderness without rebound. Neither the liver nor the spleen was palpable. Bowel sounds occurred about three times per minute.

Laboratory tests revealed a white blood cell count of 6.34×10^9/L (neutrophils: 88.6%), hemoglobin 110 g/L, and platelets 237×10^9/L. Fecal occult blood was negative. Urinalysis showed proteinuria (24-h protein excretion of 3.02 g) and hematuria. Urinary N-acetyl-β-D-glucosaminidase (NAG) was within the normal range, while microscopic examination indicated predominantly dysmorphic red blood cells. Complement levels were low (C3: 0.53 g/L, C4: 0.13 g/L). Immunoglobulin tests demonstrated IgA at 2.24 g/L, IgG at 11.33 g/L, and IgM at 0.36 g/L. Anti-nuclear antibody titer was 1:640, and anti-dsDNA antibodies were positive; all anti-ENA antibodies were negative. The erythrocyte sedimentation rate was 30 mm/h, and C-reactive protein rose to 33.9 mg/L. Liver and renal function tests were normal, with unremarkable serum amylase levels.

An abdominal ultrasound revealed gallstones, whereas the vascular ultrasound showed patent blood flow in the SMA and mesenteric vein. Gastroscopy and colonoscopy were unremarkable. CT angiography indicated a considerably reduced angle between the SMA and the aorta (30°, normal: 40°–60°) and a 0.6 cm distance at the narrowed segment, leading to a distinct "sandglass" appearance of the LRV (Figure 1).

Given the patient's intermittent joint pain, recurrent oral ulcers, eyelid and lower limb edema, urinary protein excretion, and positive anti-nuclear and anti-dsDNA antibodies—alongside reduced complement levels—a definitive diagnosis of SLE and lupus nephritis was confirmed. Further evaluation concluded that the persistent abdominal pain and fever could not be fully explained by infection, active lupus nephritis, or gallstones alone. Rather, part of the clinical presentation was consistent with NCS, where mechanical compression of the LRV causes venous hypertension, contributing to hematuria and proteinuria.

After discharge, the patient was treated primarily with nutritional support, along with daily prednisone at 15 mg and hydroxychloroquine. Due to financial constraints, he could not return to our institution for follow-up. During 6 months of

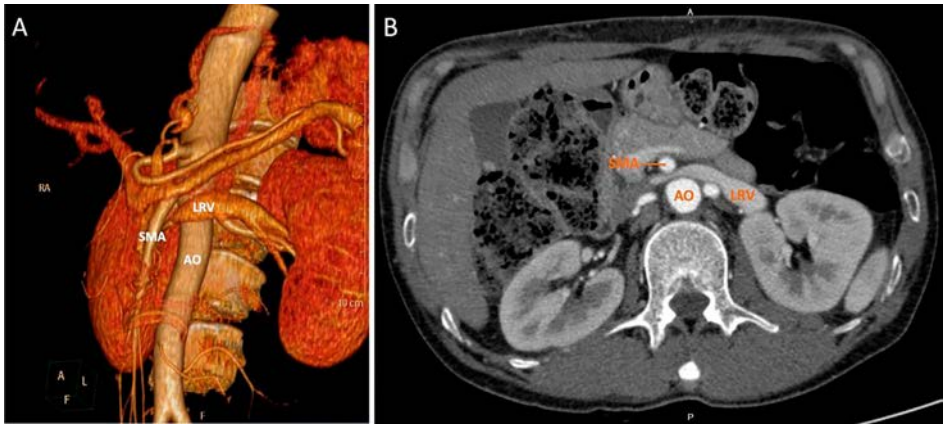

FIGURE 1 | (A) CT angiography showing the reduced angle between SMA and AO, indicative of potential vascular compression. (B) Detailed imaging demonstrating the compression and post-stenotic dilation of the left renal vein characteristic of Nutcracker syndrome.

TABLE 1 | Summary of nutcracker syndrome cases associated with rapid weight loss.

Author/year [Ref. No.]	Gender	Age (year)	Onset of symptoms	Weight loss factor	Symptoms and systemic involvement	Treatment approaches	Treatment effect
Barsoum et al./2008 [2]	Female	29	6 months prior	Severe weight loss due to duodenal obstruction from superior mesenteric syndrome, with a BMI of 13.8	Abdominal pain, and hematuria	Conservative management and nutritional support	Improved
Daily et al. /2012 [3]	Female	19	13 months prior	Losing weight, dropping from 115 to 90 lbs	Nausea, poor appetite, lower abdominal pain, and syncope	Nutritional support and stenting of her left renal vein	Weight stabilization, symptoms improved
Shi et al./2019 [4]	Famale	32	1 months prior	A rapid weight loss of approximately 10 kg with a BMI from 21 to less than 18 over the last 2 months	Gradually severe bloating, epigastric pain, left flank ache, nausea and occasional vomiting	Conservative management and dietary support	Symptoms managed effectively
Quevedo et al./2014 [5]	Famale	22	No mention	Cesarean section 3 years ago, and ecreased oral intake and 10-pound weight loss	Intractable nausea, bilious vomiting, left upper quadrant abdominal pain	Intervention with a self-expandable SMART stent, following full anticoagulation with low molecular weight heparin	Symptoms managed effectively
Oh et al./2017 [6]	Male	23	3 days prior	Body weight decreased from 83 to 63 kg during his military service, with a BMI of 20.7	Severe nausea and vomiting, but no abdominal pain and haematuria	Laparoscopic duodenojejunos- tomy was performed and nutritional support was given	Improved after surgical intervention
Nishio et al./2019 [7]	Female	12	5 days prior	Weight loss because of intestinal malrotation, BMI, 18.4	Abdominal pain and vomiting, no proteinuria but microscopic haematuria	Surgical intervention for intestinal malrotation, without any direct intervention for NCS	Improved after surgical intervention
Neirouz et al./2024 [8]	Male	32	3 months prior	Rapid weight loss (BMI drop from 25 to 18) following a series of complicated biliary surgery	Persistent low back pain	Anticoagulants with nutritional support	Symptoms resolved
Michael et al./2017 [9]	Male	28	2 days prior	Intentional dieting and weight loss of approximately 15 kg over the last 2 years with a BMI of 17	Severe vomiting, epigastric pain and bloating	Nutritional support	Condition improved

(Continues)

TABLE 1 | (Continued)

Author/year [Ref. No.]	Gender	Age (year)	Onset of symptoms	Weight loss factor	Symptoms and systemic involvement	Treatment approaches	Treatment effect
Lao et al./2023 [10]	Male	16	Several years prior	Weight loss (3.2 kg) a week before	Periumbilical pain and a new onset of bilious emesis	A nasal duodenal-jejunal junction tube was used for enteral nutrition	Symptoms alleviated within 2 weeks
Inal et al./2014 [11]	Male	28	4 years prior	Rapid weight loss contributing to symptoms	Intermittent abdominal pain, and 1-day vomiting, epigastric pain, and bloating	Nutrition support with a jejunostomy feeding tube	Symptoms improved
Farina et al./2021 [12]	Female	62	1 month prior	Excessive weight loss, BMI: 14.5	Recurrent postprandial vomiting, microhematuria, and pain in the left flank	High-calorie liquid diet	Symptoms improved
Farina et al./2020 [13]	Male	27	3 months prior	Rapid weight loss(10 kg in 3 months), with a BMI of 17.4	Painful post-prandial crises at the sub-acute onset, located at the epigastrium	Conservative management with weight gain focus	Weight gain and symptom relief achieved
Farina et al./2021 [14]	Male	69	6 month prior	Significant weight loss with a BMI of 17.0	Abdominal pain	High-calorie diet with surgical treatment	Symptom relief
Diab et al./2020 [15]	Male	18	2 months prior	Weight loss of 25 kg (32% of his body weight)	No abdominal pain, no hematuria or proteinuria	Nutritional support	Symptoms alleviated

Abbreviations: BMI, body mass index; unit, kg/m^2.

telephone follow-up, he regained 8 kg, his abdominal pain almost completely resolved, and repeat 24-h urine protein measurement at a local facility decreased to 0.85 g, with hematuria improving to a single positive (+).

3 | Discussion

This case illustrates the rare occurrence of NCS precipitated by rapid weight loss in a patient with SLE. Rapid weight loss has been identified as a common triggering factor for NCS across various patient profiles (Table 1) [2–15]. For example, cases reported by Barsoum et al. and Diab et al. highlight significant weight reductions—13.8 BMI in Barsoum's case and a 25 kg weight loss in Diab's case—that led to the onset of abdominal and gastrointestinal symptoms similar to those of our patient [2, 15].

NCS results from LRV compression between the aorta and SMA, leading to symptoms such as hematuria, proteinuria, and flank pain. The literature suggests that anatomical factors combined with weight loss can exacerbate the narrowing of the aortomesenteric angle, as seen in our patient who experienced substantial weight loss following active SLE symptoms.

In conditions like SLE, substantial weight reduction often depletes retroperitoneal fat, a key protective cushion for the LRV. Consequently, the renal vein is more prone to compression, leading to elevated venous pressure and the hallmark features of NCS [3, 8]. Several studies support the correlation between weight loss and the onset of NCS. For instance, Neirouz et al. describe a patient who developed NCS following surgery-induced weight loss, underscoring the impact of reduced body mass on vascular structures [8].

Although the coexistence of SLE and NCS is not widely reported, autoimmune diseases like SLE can induce substantial weight fluctuations via chronic inflammation, dietary changes, and medication side effects. These changes in body composition, in turn, can predispose patients to vascular compression syndromes. Oh et al. described a similar scenario in which a patient developed both NCS and superior mesenteric artery syndrome (SMAS) after losing weight during military training, mirroring our patient's experience of rapid weight reduction amid active SLE [6].

A review of treatment options highlights the need for individualized management based on symptom severity. Conservative approaches, such as nutritional support and weight gain, often serve as first-line treatments. Patients in the literature who pursued nutritional intervention, including Farina et al., experienced relief from symptoms after restoring body weight [9, 12–15]. However, for cases with persistent symptoms or severe compression, endovascular stenting or surgical decompression might be necessary. Endovascular therapy, such as stenting, has shown success in alleviating LRV compression and has become a viable alternative in recent years [5].

NCS may present concurrently with other vascular compression syndromes, such as SMAS. Identifying overlapping compressions requires careful imaging and multidisciplinary expertise. Studies by Inal et al. and Diab et al. underscore the importance of detailed CT angiography or Doppler ultrasound for accurate diagnosis, emphasizing that multimodal imaging is crucial in patients with sudden weight loss and persistent abdominal symptoms [11, 15].

4 | Conclusion

In summary, this case reinforces the significance of considering NCS in patients with rapid weight loss, especially those with underlying autoimmune disorders. The case also underscores the importance of individualized treatment, from conservative nutritional support to endovascular intervention in refractory cases. Given the potential for symptom overlap with other vascular syndromes, comprehensive imaging and a multidisciplinary approach are essential in ensuring accurate diagnosis and effective management for patients with NCS and related vascular compression syndromes.

Author Contributions

Sheng-Guang Li, Chen Li and Yu-wei Wang designed this study. Chen Li, Yu-wei Wang and Di Jin wrote the manuscript and processed images. Han Sheng and Zi-xuan Shu were responsible for images collection. All authors approved the final manuscript.

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Ethics Statement

The tients' legal guardian in this manuscript has given written informed consent to publication of their case details.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Unraveling the Dual Role of circ-CBLB and ETS-1 in Rheumatoid Arthritis: Biomarkers and Therapeutic Targets

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ABSTRACT

Objective: To investigate the effects of circ-CBLB and ETS-1 on the proliferation, apoptosis, and inflammatory cytokine expression in the fibroblast-like synoviocytes (FLSs) from patients with rheumatoid arthritis (RA).

Methods: Peripheral blood was collected from 15 pairs of healthy controls (HCs) and patients with RA to isolate peripheral blood mononuclear cells (PBMCs). mRNA expression of circ-CBLB and ETS-1 was determined using qRT-PCR. Levels of the inflammatory markers (ESR, CRP, CCP, and RF) were determined, and the 28-joint Disease Activity Score (DAS28) was calculated. For in vitro experiments, human FLS and RA-FLS were cultured, and constructs (pcDNA3.1/siRNA-circ-CBLB, pcDNA3.1/siRNA-ETS-1) were transfected into RA-FLS. Cotransfection of pcDNA3.1-circ-CBLB and siRNA-ETS-1 was undertaken to explore their combined effects. Levels of the key inflammatory cytokines (interleukin [IL]-4, IL-23, IL-13, and tumor necrosis factor [TNF]-α) were evaluated using qRT-PCR and enzyme-linked immunosorbent assays. Functional assays (CCK-8) were used to assess cell viability, apoptosis (flow cytometry), and migration. Western blotting was used to determine protein expression.

Results: In vivo analysis showed significant downregulation of circ-CBLB and ETS-1 in PBMCs from patients with RA compared with the HCs, as confirmed using qRT-PCR. Correlation analysis indicated a positive association among circ-CBLB, ETS-1, and IL-4, while circ-CBLB and ETS-1 were negatively correlated with inflammatory markers (ESR, CRP, RF, CCP, DAS28, IL-23, and TNF-α). Receiver operating characteristic curve analysis suggested circ-CBLB and ETS-1 as potential biomarkers for high disease activity in RA. In vitro, circ-CBLB overexpression increased IL-4 levels while decreasing IL-23, IL-13, and TNF-α levels. Additionally, circ-CBLB inhibited the apoptosis of RA-FLS, prolonged the cell cycle, and reduced cell migration. ETS-1 negatively regulated circ-CBLB, indicating a feedback loop.

Conclusion: circ-CBLB and ETS-1 are downregulated in RA and correlate with inflammation and disease activity. They regulate each other bidirectionally. circ-CBLB reduces RA-FLS viability, promotes apoptosis, and inhibits migration by modulating cytokines. ETS-1 has similar effects, and interfering with its expression reverses the impact of circ-CBLB.

Lei Wan and XiaoJun Zhang are equally contributed to this study.

1 | Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by progressive synovial inflammation, cartilage degradation, and bone destruction [1]. It affects approximately 1% of the global population, with significant regional variations, and women are more commonly affected than men [2]. Multiorgan involvement may occur with disease progression. RA often follows a classical circadian pattern with morning symptoms such as joint swelling and stiffness [3]. The precise pathogenesis of RA remains unclear despite extensive research, and without timely treatment, the systemic pathophysiological changes in RA can lead to severe complications, including disability and even death [4]. Early diagnosis and intervention are critical in mitigating these outcomes. In the synovial lining, fibroblast-like synoviocytes (FLSs) are the primary effector cells that drive inflammation, and their abnormal activation in RA-FLS is considered a key initiator of synovitis and bone destruction [5]. Interestingly, even after multiple generations of in vitro culture, activated RA-FLSs continue to exhibit abnormal cellular viability and proliferation, showing tumor-like properties [6]. These cells also secrete various proinflammatory cytokines and chemokines, further exacerbating the pathological progression of RA [7]. Therefore, targeting the excessive proliferation of RA-FLS and inducing their apoptosis have been identified as potential therapeutic strategies to treat RA. Circular RNAs (circRNAs) and transcription factors (TFs) are important gene-regulatory elements involved in cellular processes [8–10]. Among them, circRNA Cbl proto-oncogene B (circ-CBLB), initially characterized as a proto-oncogene, has now been identified as a crucial regulator of immune function [11]. circ-CBLB is an E3 ubiquitin ligase that plays a pivotal role in regulating both innate and adaptive immune cells, contributing to an immunosuppressive tumor microenvironment [12]. E26 transformation-specific sequence-1 (ETS-1), a member of the ETS family of TFs, can induce the transcription of several downstream target genes involved in cell proliferation, survival, invasion, and angiogenesis [13–16]. However, the roles of circ-CBLB and ETS-1 in the proliferation and apoptosis of RA-FLS are largely unexplored. This is the first study to investigate the impact of the circ-CBLB/ETS-1 axis on the viability, apoptosis, cell cycle regulation, migration, and inflammatory cytokine secretion of RA-FLSs, aiming to provide new molecular targets to treat RA.

2 | Materials and Methods

2.1 | Clinical Data

Fifteen patients with RA were included in this study from the outpatient and inpatient departments of the Rheumatology Department of Anhui Provincial Hospital of Traditional Chinese Medicine. Fifteen healthy controls (HCs; HC group) were recruited from the physical examination center of the same hospital. The enrollment period was from January 2021 to December 2021, and all participants signed informed consent forms. The diagnosis of RA was based on the 2010 American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) criteria and the revised 1987 ACR classification criteria and scoring system for RA. Patients were included if they met the ACR and EULAR classification criteria, were between 18 and 60 years of age, provided signed informed consent, and demonstrated good compliance. The exclusion criteria were as follows: Patients with severe comorbidities affecting the heart, brain, lungs, liver, kidneys, or hematopoietic system; those with other metabolic diseases; pregnant or lactating women; and individuals with severe joint deformities (Table 1). Test indicators: After collecting 4 mL of fasting venous blood from the forearm in an erythrocyte sedimentation rate (ESR) tube (black tube), peripheral blood mononuclear cells (PBMCs) were extracted using Ficoll and stored in a freezer at −80°C. ESR was determined using an automatic ESR analyzer (Vital Monitor-20). Biochemical indicators, including C-reactive protein (CRP), rheumatoid factor (RF), and anti–cyclic citrullinated peptide antibody (CCP), were measured by collecting 5 mL of venous blood in a biochemistry tube (yellow tube). After low-temperature coagulation at 3°C–5°C, the samples were centrifuged at 4000 rpm for 15 min to separate the serum, which was then stored in a freezer at −80°C.

TABLE 1 | Clinical baseline data of patients.

	RA ($n=15$)	HC ($n=15$)	$Z/t/\chi^2$	p
Age (years)	46.98 ± 4.00	43.69 ± 5.23	1.940	0.063
Gender (male/female)	4/11	4/11	0.000	1.000
Disease course (months)	5 (4, 8)	—	—	—
Height (cm)	159.60 ± 6.90	161.20 ± 8.77	−0.556	0.583
Weight (kg)	53.27 ± 8.06	54.07 ± 8.02	−0.273	0.787
Body temperature (°C)	36.47 ± 0.18	36.51 ± 0.12	−0.826	0.416
Heart rate (per min)	71.20 ± 7.26	70.67 ± 7.53	0.197	0.845
Respiration (per min)	15.00 ± 3.57	17.47 ± 4.90	−1.577	0.126
Systolic pressure (mmHg)	112.60 ± 17.07	119.47 ± 27.00	−1.104	0.279
Diastolic pressure (mmHg)	76.67 ± 8.85	75.80 ± 11.88	0.227	0.822
History of RA-related medication treatment (yes/no)	0/0	0/0	—	—

An automatic biochemical analyzer (HITACHI7600-020) was used to analyze the collected samples. The 28-joint Disease Activity Score (DAS28) was calculated for patients with RA. This study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Anhui University of Chinese Medicine (No. 2019AH-12).

The formula to calculate DAS28 is as follows:

$$\mathrm{DAS28} = 0.56 \times \sqrt{\mathrm{TJC28}} + 0.28 \times \sqrt{\mathrm{SJC28}} + 0.70 \times \ln(\mathrm{ESR}) \times 1.08 + 0.16,$$

where TJC28 = Tender joint count for 28 joints, SJC28 = Swollen joint count for 28 joints, ESR = Erythrocyte sedimentation rate (mm/h), and GH = Patient's Global Health assessment (on a scale of 0–100, usually scored by the patient).

The natural logarithm (ln) of ESR is used in the formula.

2.2 | Cell Culture

FLS and RA-FLS (iCell, Shanghai, China) were removed from the incubator, and the medium (iCell, Shanghai, China) was discarded. Cells were washed twice with phosphate-buffered saline (PBS; Hyclone, Shanghai, China) before the addition of 1 mL of trypsin (Beyotime, Shanghai, China) and digestion at 37°C for 2 min. Once the cells had shrunk and were rounded, the digestion was stopped by the addition of fresh medium. The cells were collected in a 15-mL centrifuge tube and centrifuged at 1000 rpm for 5 min. The cells were then passaged at a 1:1 or 1:2 ratio based on cell density, with passages conducted every 2 days. Cells were used for subsequent experiments after the seventh passage.

2.3 | Cell Transfection

RA-FLSs were digested, centrifuged, and washed twice with PBS. The cells were resuspended in the medium, seeded into a 6-well plate at a density of 5×10^5 cells per well, and incubated overnight at 37°C in an incubator flushed with 5% CO_2. Cells were transfected once they were 70%–80% confluent. Lyophilized pcDNA3.1/siRNA-circ-CBLB, ETS-1, and their negative controls (pcDNA3.1-NC/si-NC) were reconstituted with 125 μL of DEPC water (Generay Biotech, Shanghai, China) to a concentration of 20 μmol/L. A volume of 5 μL of 20 μmol/L pcDNA3.1/siRNA/NC was mixed in 250 μL of serum-free medium. Separately, 5 μL of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was diluted in 250 μL of serum-free medium and incubated at room temperature for 5 min. The two solutions were then mixed and incubated at room temperature for 20 min. A total of 500 μL of the transfection mixture was added to each well, and transfection efficiency was determined after 24 h using qRT-PCR. The cells were digested with 2.5 g/L trypsin and washed twice with PBS before the collection of cell pellets and storage at −80°C for further analysis. Overexpression plasmids of circ-CBLB and ETS-1, as well as siRNA and their negative controls (pcDNA3.1-circ-CBLB-NC, pcDNA3.1-ETS-1-NC, siRNA-circ-CBLB-NC, and siRNA-ETS-1-NC), were constructed by Shanghai GenePharma.

2.4 | qRT-PCR for Gene Expression

Total RNA from cells was extracted following the manufacturer's instructions in the TRIzol kit (Life Technologies, Waltham, MA, USA). Genomic DNA was removed and 1 μg of RNA, 2.0 μL of 5× gDNA Eraser buffer, and 1.0 μL of gDNA Eraser (TaKaRa, Dalian, China) were added, and the total volume was made up to 10 μL with RNase-free water in a 0.2-mL Eppendorf tube. The contents were gently mixed and centrifuged. The mixture was incubated at 42°C for 2 min in a PCR machine, followed by treatment for 1 min in an ice bath. Next, 10 μL of the reaction mixture was added, including 1.0 μL of PrimeScript RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China), 1.0 μL of RT Primer Mix, 4.0 μL of RevertAid M-MuLV reverse transcriptase (TaKaRa, Dalian, China), and 4.0 μL of RNase-free water to make up the total volume to 20 μL. The sample was incubated for 15 min at 37°C and then heated to 85°C for 5 s to terminate the reaction. The resulting cDNA was stored at −80°C until subsequent use in PCR. For PCR amplification, 1.0 μL of cDNA was used as a template. The following program was used: 95°C for 1 min (95°C for 20 s, 60°C for 1 min) ×40 cycles. The PCR reaction system (10 μL) consisted of 5 μL of 2× SYBR Green mix (Novoprotein, Suzhou, China), 1 μL each of 10 μmol/L forward and reverse primers, 1 μL of cDNA, and 2 μL of RNase-free water (Generay Biotech, Shanghai, China). Relative expression was calculated using the $2^{-\Delta\Delta C_t}$ method. The primers used in this study are listed in Table 2.

TABLE 2 | Primer sequences.

Gene	Amplicon size (bp)	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
β-Actin	96	CCCTGGAGAAGAGCTACGAG	GGAAGGAAGGCTGGAAGAGT
circ-CBLB	195	TCCTGCTAGAAGGTTACCAG	TTGCTAACGGACCAGTACAC
ETS-1	127	CCATTCTGGAGAGGGACTTC	TGCTGTAAAACCCAGAGTGT
IL-4	106	GCAGTTCCACAGGCACAA	CTGGTTGGCTTCCTTCAC
IL-23	174	TGAACAACTGAGGGAACCAA	AGCAGCAACAGCAGCATTAC
IL-13	162	ATAAGGGGCGTTGACTCAC	GTTGATGCTCCATACCATGC
TNF-α	199	CGAGTCTGGGCAGGTCTA	GAAGTGGTGGTCTTGTTGC

2.5 | CCK-8 Assay to Determine Cell Viability

Cells from each group were seeded in 96-well flat-bottom plates and cultured in an environment of 5% CO_2 for 0, 24, 48, and 72 h. Cell morphology was observed using an inverted microscope. A total of 10 μL of CCK-8 solution (BIOSS, Beijing, China) was added to each well, and incubation was continued for 1–4 h. Blank wells were set up, and the absorbance (A) values of each well were measured at 450 nm using a microplate reader.

2.6 | Flow Cytometry for Cell Cycle Analysis and Apoptosis Detection

2.6.1 | Cell Cycle Analysis

Cells were washed with precooled PBS, digested with 2.5 g/L of trypsin, and centrifuged at 2000 rpm for 5 min. The cells were resuspended and washed with precooled PBS. Approximately 50 μL of residual liquid remained after aspirating the supernatant. The cell pellet was mixed with 1 mL of precooled ethanol and fixed at 4°C for at least 2 h or overnight. After centrifugation, the supernatant was discarded, and the fixed cells were washed twice with precooled PBS. After discarding the PBS, 500 μL of PBS was added to resuspend the pellet. The cells were gently tapped to disperse them and avoid clumping. In 0.5 mL of staining buffer, 25 μL of propidium iodide (PI)-staining solution (20×) and 10 μL of RNaseA (50×) were mixed. The cell suspension was incubated in 0.5 mL of PI staining solution at 37°C in the dark for 30 min, followed by flow cytometry.

2.6.2 | Apoptosis Detection

Cells were digested with EDTA-free trypsin, centrifuged, and washed with cold PBS. A total of 1–10 $\times 10^5$ cells were collected. The 5× binding buffer was diluted with double-distilled water to a 1× working solution, and 500 μL of 1× binding buffer was used to resuspend the cells. To each tube, 5 μL of Annexin V-FITC (LianKe Bio, Hangzhou, China) and 10 μL of PI were added. The mixture was gently vortexed and incubated at room temperature in the dark for 5 min, followed by flow cytometry. The four quadrants (Q1, Q2, Q3, and Q4) represent necrotic cells, late apoptotic cells, normal cells, and early apoptotic cells, respectively.

2.7 | Cell Migration Count

Cells from each group were seeded into a 24-well plate, and after 24 h, the cells were collected. After resuspending the cells in serum-free medium, the density was adjusted to 2×10^5 cells per mL. A total of 100 μL of cell suspension was added to the Transwell chamber (Corning, 08416047, USA) and cultured for 24 h. The chambers were removed, fixed with 4% paraformaldehyde at room temperature for 30 min, stained with 0.5% crystal violet for 15–30 min, and rinsed with water several times. Cells on the membrane of the bottom of the upper chamber were carefully wiped off using a wet cotton swab. Fields were chosen at random using microscopy, and the cells were photographed and counted.

2.8 | Enzyme-Linked Immunosorbent Assay (ELISA) to Determine Cytokine Levels

ELISA was used to determine the levels of the inflammatory cytokines interleukin (IL)-4, IL-23, IL-13, and tumor necrosis factor (TNF)-α in the peripheral blood and RA-FLS culture supernatants of participants following the manufacturer's instructions in the respective assay kits (Gene Me, Wuhan, China).

2.9 | Western Blotting to Determine Protein Expression

Cell samples from each group were collected, and total protein was extracted using RIPA lysis buffer containing PMSF (Beyotime, Shanghai, China). Proteins were separated using sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE). SDS (Solarbio, Beijing, China) and PAGE gel accelerator (Solarbio, Beijing, China) were used for electrophoresis, and the proteins were transferred onto polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). The transfer time for IL-4 (17 kDa), IL-23 (21 kDa), IL-13 (12 kDa), TNF-α (26 kDa), and ETS-1 (49 kDa) ranged from 25 to 50 min. After the transfer, the membranes were blocked with 5% skim milk (Beyotime, Shanghai, China) at room temperature for 2 h to block nonspecific binding and then incubated overnight at 4°C with the following primary antibodies: IL-4 (Rabbit Anti; Abcam, Biotechnology, Cambridge, UK), IL-23 (Rabbit Anti; Abcam), IL-13 (Rabbit Anti; Abcam), TNF-α (Rabbit Anti; Bioss, Beijing, China), and ETS-1 (Rabbit Anti; Bioss). After incubation, the membranes were washed three times with PBST buffer (Zs-Bio, Beijing, China), with each wash lasting 10 min. Then, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (diluted 1:20000; Zsbio, Beijing, China) at room temperature for 1.2 h, followed by three additional washes. Lastly, the protein bands were visualized using an enhanced chemiluminescence detection kit (Thermo Corp, Waltham, MA, USA) and proteins were quantified using ImageJ to ensure the reliability and accuracy of experimental results.

2.10 | Statistical Analysis

Data were analyzed using SPSS 23.0, and graphs were generated using GraphPad 9.0. Quantitative data are expressed as mean ± standard deviation. Independent samples *t*-test was used for comparisons between two groups, whereas one-way analysis of variance was used for comparisons among multiple groups. Pearson's or Spearman's correlation tests were used for correlation analysis, and all statistical tests were two sided. Differences were considered statistically significant at $p < 0.05$.

3 | Results

3.1 | Downregulation of circ-CBLB and ETS-1 in the Peripheral Blood of Patients With RA

The results from qRT-PCR indicated a significant reduction in the expression of circ-CBLB mRNA and ETS-1 mRNA in the RA group ($p < 0.001$) compared with that in the HC group (Figure 1A); whereas, findings from ELISA indicated a decrease

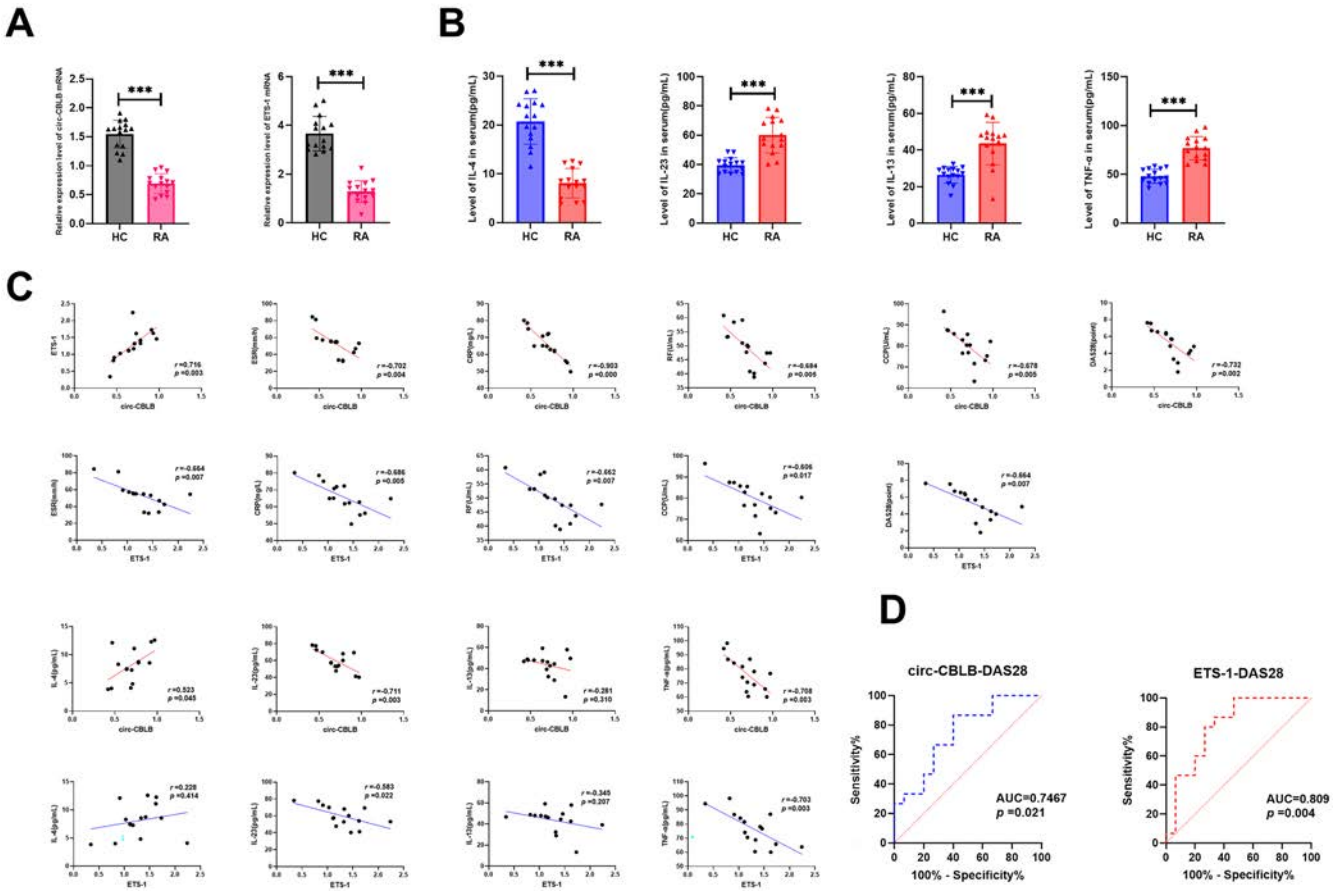

FIGURE 1 | circ-CBLB and ETS-1 expression in the peripheral blood of patients with RA. (A) qRT-PCR to determine circ-CBLB and ETS-1 expression in the RA and HC groups. HC: Healthy control, ***$p < 0.001$. (B) ELISA to determine IL-4, IL-23, IL-13, and TNF-α expression in the RA and HC groups. (C) Correlation analysis between circ-CBLB, ETS-1, and clinical indicators, including ESR, CRP, RF, CCP, and DAS28, in patients with RA. (D) ROC curve of circ-CBLB and ETS-1 predicting DAS28 in patients with RA.

in IL-4 expression and an increase in IL-23, IL-13, and TNF-α expression ($p < 0.010$) (Figure 1B). Correlation analysis revealed that circ-CBLB expression in patients with RA was positively correlated with ETS-1 and IL-4 expression ($p < 0.05$, $p < 0.01$) and negatively correlated with ESR, CRP, RF, CCP, DAS28, IL-23, and TNF-α ($p < 0.01$). Similarly, ETS-1 was negatively correlated with ESR, CRP, RF, CCP, DAS28, IL-23, and TNF-α ($p < 0.05$, $p < 0.01$) (Figure 1C). When circ-CBLB and ETS-1 levels were divided into two groups based on the DAS28 scores, those below the mean were categorized as 1, and those above the mean were categorized as 2. The receiver operating characteristic (ROC) curve showed that the areas under the curve for circ-CBLB and ETS-1 were 0.747 ($p = 0.021$) and 0.809 ($p = 0.004$), respectively, with 95% confidence intervals of 0.5705–0.9229 for circ-CBLB and 0.6486–0.9692 for ETS-1, indicating their abilities as biomarkers to predict high disease activity in patients with RA (Figure 1D).

3.2 | circ-CBLB Positively Regulates ETS-1, and Both circ-CBLB and ETS-1 Upregulate IL-4 Expression and Downregulate IL-23, IL-13, and TNF-α Expression in RA-FLSs

Results from ELISA indicated that IL-4 expression in RA-FLS decreased compared with that in FLSs, whereas IL-23, IL-13, and TNF-α expression increased significantly ($p < 0.001$), which was consistent with clinical results. The overexpression plasmid pcDNA3.1-circ-CBLB and its negative control, as well as small interfering RNA (siRNA) of ETS-1 and its negative control, were constructed. IL-4 expression in the overexpression group increased significantly compared with that in the RA-FLS group ($p < 0.001$), whereas IL-23, IL-13, and TNF-α expression decreased ($p < 0.001$) (Figure 2A). IL-4 expression in the interference group increased compared with that in RA-FLSs ($p < 0.001$), and IL-23, IL-13, and TNF-α expression decreased ($p < 0.001$) (Figure 2B). Western blotting revealed that ETS-1 protein expression in RA-FLSs was significantly lower than that in FLSs ($p < 0.001$). By constructing the overexpression plasmid pcDNA3.1-circ-CBLB and its negative control, as well as the siRNA-circ-CBLB and its negative control, it was found that ETS-1 protein expression in the overexpression group was higher than that in the RA-FLS group ($p < 0.001$); whereas, in the interference group, ETS-1 protein expression was lower than that in the RA-FLS group ($p < 0.01$) (Figure 2C).

3.3 | ETS-1 Negatively Regulates circ-CBLB, and Both circ-CBLB and ETS-1 Inhibit RA-FLS Viability; Interference With ETS-1 Expression Can Counteract and Reverse the Effects of circ-CBLB on RA-FLSs

Results from the CCK-8 assay indicated an increase in RA-FLS cell viability ($p < 0.001$) compared with that of FLSs.

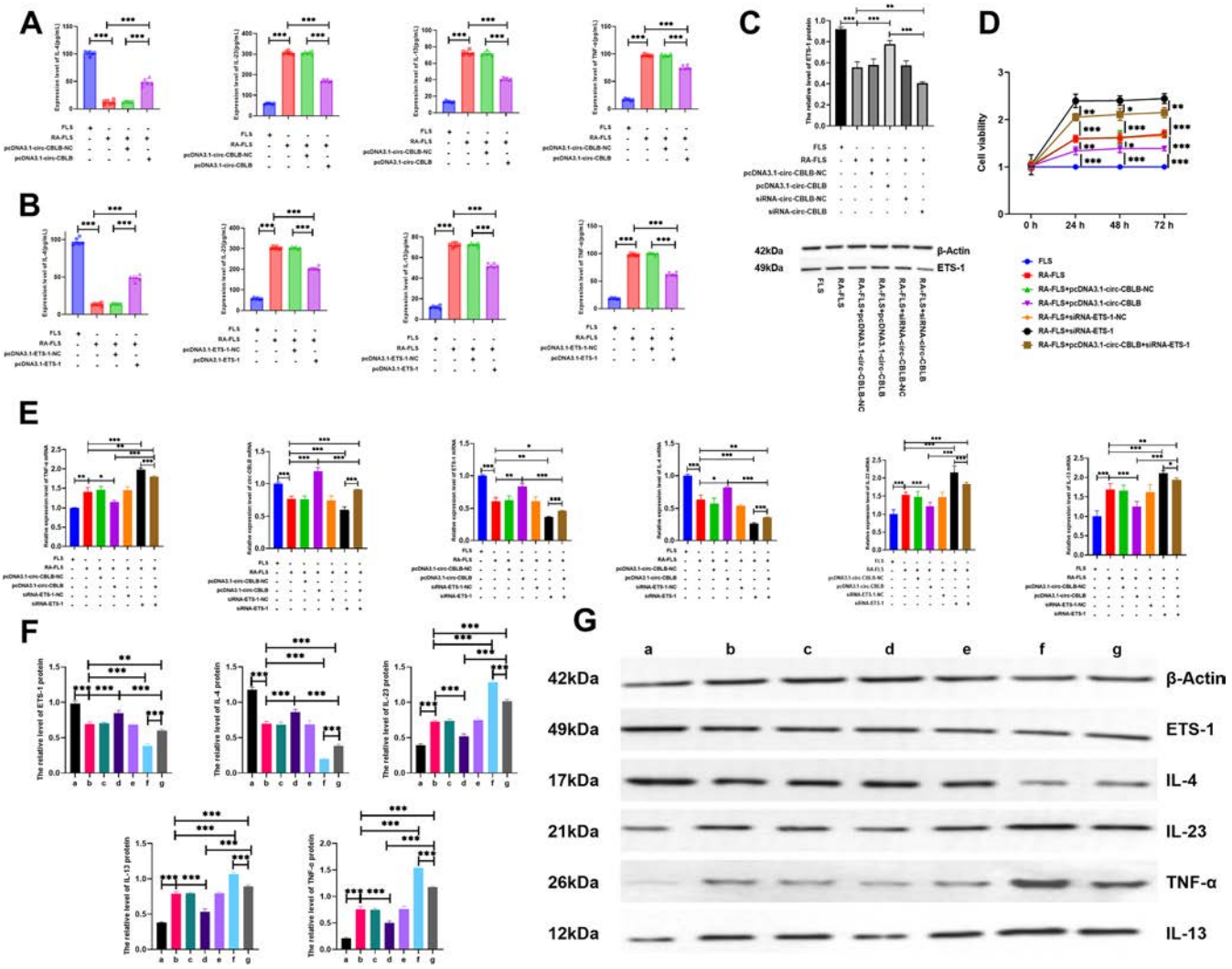

FIGURE 2 | Effects of circ-CBLB and ETS-1 on the cytokines and viability of RA-FLSs. (A) ELISA to determine the effect of circ-CBLB overexpression on IL-4, IL-23, IL-13, and TNF-α levels in RA-FLS supernatants. (B) ELISA to determine the effect of ETS-1 overexpression on IL-4, IL-23, IL-13, and TNF-α levels in RA-FLS supernatants. (C) Western blotting to determine the effect of circ-CBLB overexpression and interference on ETS-1 expression. (D) CCK-8 assay to determine the effect of circ-CBLB and ETS-1 on RA-FLS cell viability. (E) qRT-PCR to determine the effects of circ-CBLB and ETS-1 on the gene expression of IL-4, IL-23, IL-13, and TNF-α. (F) Semi-quantitative analysis of the relative protein expression of ETS-1, IL-4, IL-23, TNF-α, and L-13. (G) Western blot to determine the protein expression of ETS-1, IL-4, IL-23, IL-13, and TNF-α. a: FLS, b: RA-FLS, c: RA-FLS + pcDNA3.1-circ-CBLB-NC, d: RA-FLS + pcDNA3.1-circ-CBLB, e: RA-FLS + siRNA-ETS-1-NC, f: RA-FLS + siRNA-ETS-1, g: RA-FLS + pcDNA3.1-circ-CBLB + siRNA-ETS-1. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

By constructing the overexpression plasmid pcDNA3.1-circ-CBLB and its negative control, siRNA of ETS-1 and its negative control, and pcDNA3.1-circ-CBLB+siRNA-ETS-1, the cell viability in the overexpression group decreased at 24, 48, and 72 h ($p < 0.05$, $p < 0.01$, $p < 0.001$) compared with that in the RA-FLS group; whereas, cell viability in the interference group increased significantly at 24, 48, and 72 h ($p < 0.001$). Cell viability in the overexpression interference group increased significantly at 24, 48, and 72 h ($p < 0.001$). Compared with that in the overexpression interference group, cell viability in the interference group increased significantly at 24, 48, and 72 ($p < 0.05$, $p < 0.01$); whereas, cell viability in the overexpression group decreased significantly at 24, 48, and 72 h ($p < 0.001$) (Figure 2D). qRT-PCR revealed that the mRNA expression of circ-CBLB, ETS-1, and IL-4 decreased ($p < 0.001$) and that of IL-23, IL-13, and TNF-α increased ($p < 0.01$, $p < 0.001$) in RA-FLSs compared with that in FLSs. The mRNA expression of circ-CBLB, ETS-1, and IL-4 in the overexpression group increased significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$) and that of IL-23, IL-13, and TNF-α decreased significantly ($p < 0.05$, $p < 0.001$) compared with that in RA-FLSs. In the interference group, the mRNA expression of circ-CBLB, ETS-1, and IL-4 decreased significantly ($p < 0.01$, $p < 0.001$) and that of IL-23, IL-13, and TNF-α increased significantly ($p < 0.01$, $p < 0.001$). In the overexpression interference group, the mRNA expression of circ-CBLB increased significantly ($p < 0.001$) and that of ETS-1, IL-4, IL-23, IL-13, and TNF-α mRNA decreased significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$). The mRNA expression of circ-CBLB, ETS-1, and IL-4 in the interference group decreased significantly ($p < 0.001$) and that of IL-23, IL-13, and TNF-α increased significantly ($p < 0.05$, $p < 0.001$) compared with that in the overexpression

interference group. In the overexpression group, the mRNA expression of circ-CBLB, ETS-1, and IL-4 increased significantly ($p < 0.001$) and that of IL-23, IL-13, and TNF-α decreased significantly ($p < 0.001$) (Figure 2E). Western blotting indicated that the protein expression of ETS-1 and IL-4 in RA-FLSs decreased significantly ($p < 0.001$) and that of IL-23, IL-13, and TNF-α increased significantly ($p < 0.001$) compared with that in FLSs. The protein expression of ETS-1 and IL-4 in the overexpression group increased significantly ($p < 0.001$) and that of IL-23, IL-13, and TNF-α decreased significantly ($p < 0.001$) compared with that in RA-FLSs. In the interference group, the protein expression of ETS-1 and IL-4 decreased significantly ($p < 0.001$) and that of IL-23, IL-13, and TNF-α increased significantly ($p < 0.001$). In the overexpression interference group, the protein expression of ETS-1 and IL-4 decreased significantly ($p < 0.01$, $p < 0.001$) and that of IL-23, IL-13, and TNF-α increased significantly ($p < 0.001$). The protein expression of ETS-1 and IL-4 in the interference group decreased significantly ($p < 0.001$) and that of IL-23, IL-13, and TNF-α increased significantly ($p < 0.001$) compared with that in the overexpression interference group. In the overexpression group, the protein expression of ETS-1 and IL-4 increased significantly ($p < 0.001$) and that of IL-23, IL-13, and TNF-α decreased significantly ($p < 0.001$) (Figure 2F).

3.4 | circ-CBLB and ETS-1 Promote Apoptosis of RA-FLSs, Increase the Proportion of Cells in the S and G2 Phases, and Inhibit Cell Migration

Results from flow cytometry showed that the apoptosis rate of RA-FLSs decreased, the proportion of cells in the S and G2 phases decreased, and the number of migrating cells increased significantly compared with that in FLSs ($p < 0.001$). By constructing the overexpression plasmid pcDNA3.1-circ-CBLB and its negative control, siRNA-ETS-1 and its negative control, and pcDNA3.1-circ-CBLB+siRNA-ETS-1, it was found that the apoptosis rate in the overexpression group increased, the proportion of cells in the S and G2 phases increased, and the number of migrating cells decreased significantly compared with that in RA-FLSs ($p < 0.001$). In the interference group, the apoptosis rate decreased, the proportion of cells in the S and G2 phases decreased, and the number of migrating cells increased significantly ($p < 0.01$, $p < 0.001$). In the overexpression interference group, the apoptosis rate decreased, the proportion of cells in the S and G2 phases decreased, and the number of migrating cells increased significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$). The interference group exhibited a lower apoptosis rate, a decreased proportion of cells in the S and G2 phases, and an increased number of migrating cells compared with the overexpression group ($p < 0.01$, $p < 0.001$). The overexpression group showed a higher apoptosis rate, an increased proportion of cells in the S and G2 phases, and a decreased number of migrating cells ($p < 0.001$) (Figure 3).

4 | Discussion

During the progression of RA, FLSs undergo abnormal proliferation and expansion that are accompanied by the infiltration of immune cells, blood vessels, and osteoclasts, which lead to synovial thickening. These pathological changes contribute to joint swelling and stiffness. Notably, abnormally activated RA-FLSs acquire an invasive tumor-like phenotype and not only destroy cartilage by secreting matrix metalloproteinases (MMPs) but also regulate osteoclastogenesis, further exacerbating cartilage destruction [17]. In addition, RA-FLSs promote the infiltration of inflammatory cells into the joints, leading to the production of large amounts of inflammatory and chemotactic factors that directly or indirectly mediate cartilage destruction and worsen disease progression. The stimulation of inflammatory signals further accelerates the abnormal proliferation of FLSs, creating a vicious cycle of inflammation and tissue damage [18, 19]. RA is an autoimmune disease with an unclear pathogenesis. In addition to exploring the biological characteristics and functions of RA-FLSs, it is crucial to identify the key molecular targets that regulate RA-FLS activity. circRNAs and TFs play vital roles in regulating cellular phenotypes and functions [10, 20, 21] Among TFs, the E26 transformation-specific (ETS) family consists of 28 members, all of which share a highly conserved DNA-binding ETS domain, with many family members being linked to cancer [22]. ETS-1, in particular, is a major oncogenic driver that is associated with various malignancies including FL1 in Ewing's sarcoma, ETS in leukemia, and *ERG* gene fusion in prostate cancer [23, 24]. In the in vivo phase of this study, qRT-PCR findings revealed the downregulation of circ-CBLB and ETS-1 in the PBMCs of patients with RA compared with those of the HCs. Correlation analysis indicated that circ-CBLB was positively correlated with ETS-1 and IL-4 and negatively correlated with ESR, CRP, RF, CCP, DAS28, IL-23, and TNF-α. Furthermore, ROC curve analysis suggested the reliability of circ-CBLB and ETS-1 as biomarkers in predicting high disease activity in patients with RA.

Abnormal cell cycles and insufficient apoptosis are major contributors to the pathological proliferation of RA-FLSs [25, 26]. The cell cycle is primarily divided into two stages, namely, interphase and the mitotic (M) phase. During interphase, cells progress through the G1 phase (pre-DNA synthesis), S phase (DNA synthesis), and G2 phase (post-DNA synthesis). The integrity of the S phase is critical as it determines whether or not a cell will proceed to the M phase. A shortened cell cycle leads to accelerated cell proliferation. Apoptosis or programmed cell death is a gene-regulated process that is essential in maintaining cellular homeostasis. Abnormal accumulation of cells occurs when apoptosis is insufficient, contributing to disease progression. Another key factor in the pathology of RA is the migratory capacity of RA-FLSs, which drives chronic inflammation and joint destruction. RA-FLSs migrate into the joint cavity, where they release inflammatory mediators and attract immune cells, perpetuating a vicious cycle of inflammation and damage [6]. Our findings demonstrated that, compared with normal FLSs, RA-FLSs exhibited increased cell viability, decreased apoptosis rates, shortened S and G2 phases of the cell cycle, and enhanced migration. These abnormalities suggest that disrupted apoptosis and proliferation of RA-FLSs will exacerbate joint inflammation [27]. Cell grouping and transfection experiments were conducted to further determine the role of the circ-CBLB/ETS-1 axis in the tumor-like behavior of RA-FLSs. Our findings indicated that circ-CBLB overexpression decreased RA-FLS viability, increased apoptosis, prolonged the S and G2 phases of the cell cycle, and

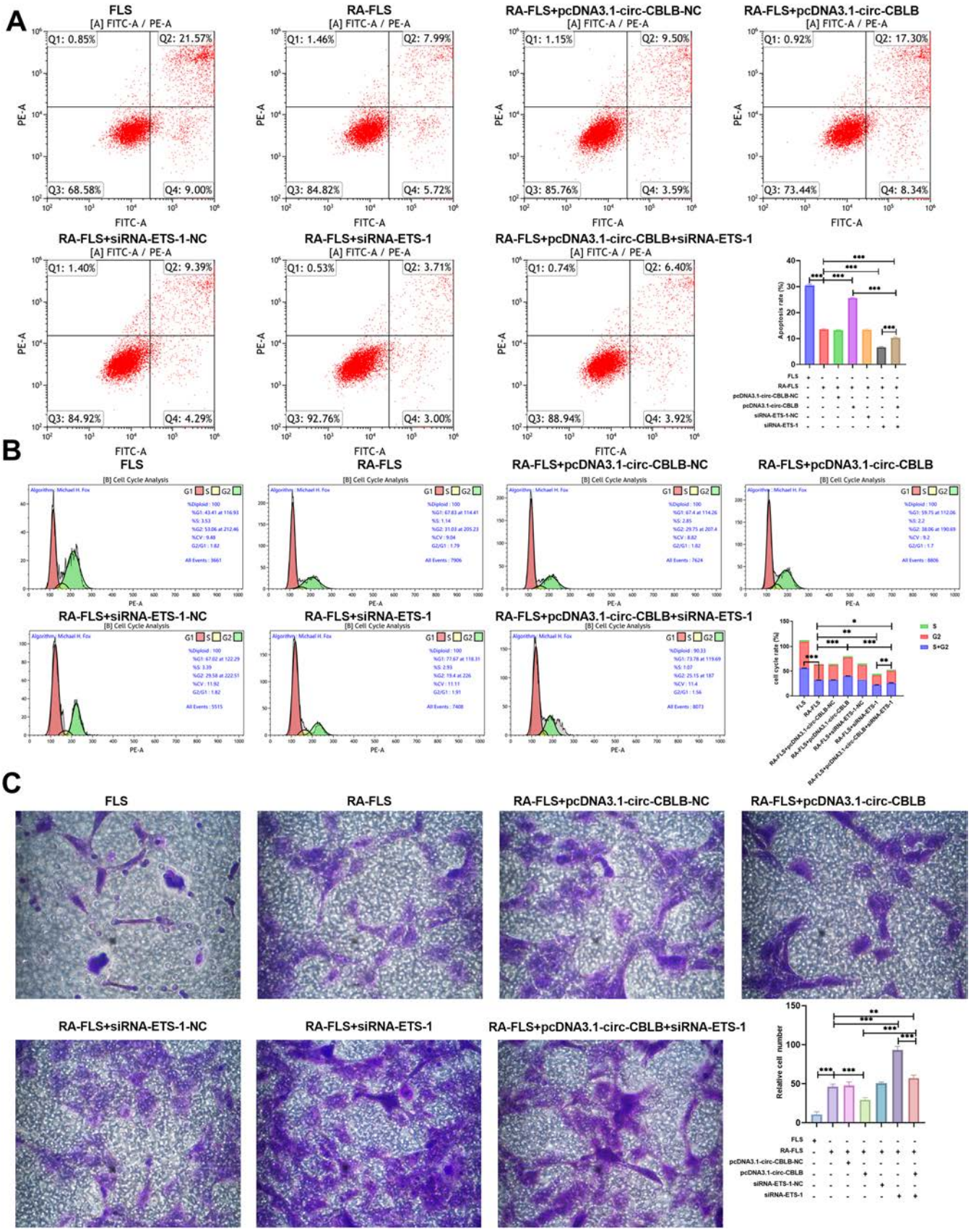

FIGURE 3 | Effect of circ-CBLB overexpression and ETS-1 interference on the apoptosis, cell cycle, and migration of RA-FLSs. (A) Flow cytometry was used to determine apoptosis in RA-FLSs, and semi-quantitative analysis of the apoptosis rate was conducted. (B) Flow cytometry was used to determine the RA-FLS cell cycle, and semi-quantitative analysis of the cell cycle was conducted. (C) Changes in cell migration were observed, and semi-quantitative analysis of cell migration counts was conducted. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

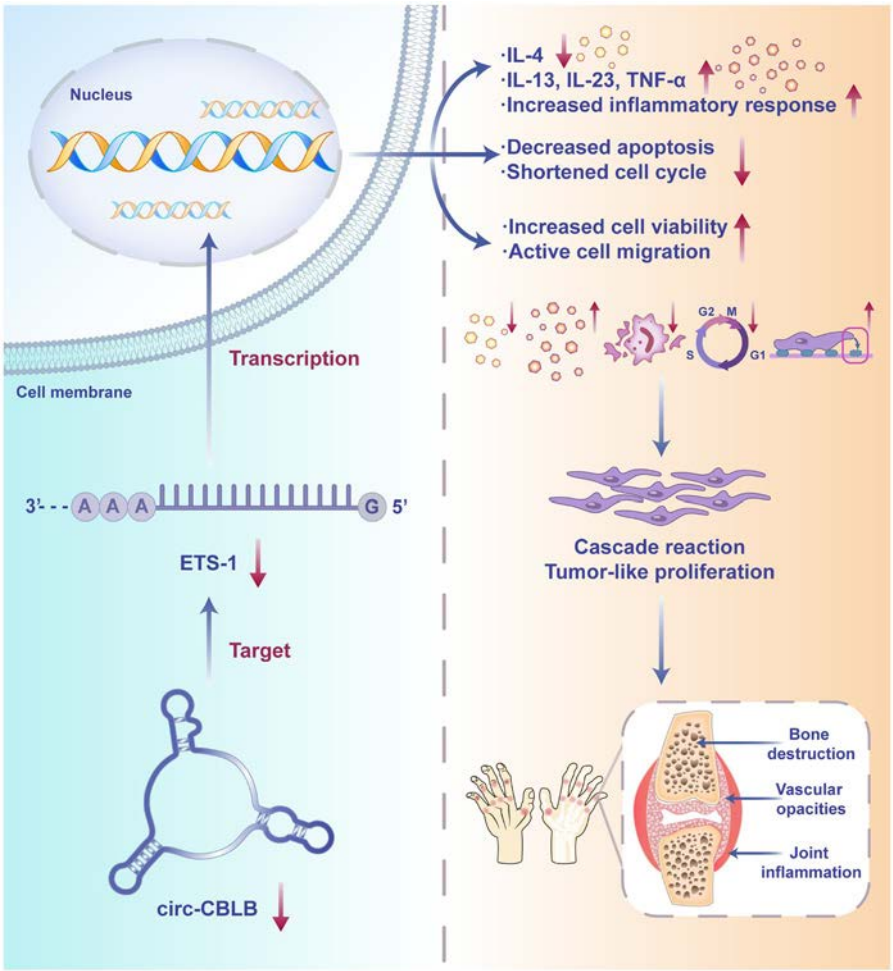

FIGURE 4 | circ-CBLB/ETS-1 causes irreversible damage to joints in RA by affecting inflammation, apoptosis, cell cycle, and migration of RA-FLSs, leading to tumor-like proliferation and a cascade reaction.

inhibited cell migration, partially restoring normal cellular function. Conversely, interference with ETS-1 resulted in the opposite effect, exacerbating the pathological characteristics of RA-FLSs.

The pathogenesis of RA involves various cytokines and complex networks [28] that trigger synovial cell proliferation and inflammatory cascades [29]. IL-4 is known to exert anti-inflammatory and antitumor effects [30]. IL-4 regulates the activity of various cell types, including RA-FLSs [7], and effectively inhibits proinflammatory factors such as TNF-α, IL-6, and IL-8. In addition, it suppresses prostaglandin E2 production, thereby controlling inflammation and protecting joint tissues including cartilage and subchondral bone. IL-23 is a proinflammatory cytokine that drives the differentiation and activation of Th17 cells, which subsequently produce IL-17A, IL-17F, IL-6, IL-22, and TNF-α. Dysregulation of IL-23 can exacerbate chronic immune-mediated inflammation [31]. IL-13 and IL-4 are members of the Th2 cytokine family, and both play key roles in allergic inflammation [32]. However, IL-13 induces immunoglobulin (Ig)G4 and IgE synthesis in human B cells independently of IL-4 and also induces CD23 expression [33]. Studies have shown that IL-13 concentrations are higher in patients with severe and moderate early RA (eRA) compared with those with the mild form of the disease and HCs, suggesting that IL-13 may be more useful than RF and CCP in predicting disease activity in eRA [34]. TNF-α is a key proinflammatory cytokine in RA [35, 36] that plays a central role in the inflammatory response, directly driving inflammation via the induction of inflammatory gene expression and indirectly driving inflammation and disease progression by inducing cell death. Western blotting in our in vitro study indicated that circ-CBLB positively regulated ETS-1. Findings from qRT-PCR, ELISA, and immunofluorescence assays were consistent and indicated that circ-CBLB overexpression increased IL-4 expression and decreased IL-23, IL-13, and TNF-α expression in RA-FLSs. Findings from the CCK-8 assay, flow cytometry, and cell migration experiments demonstrated that circ-CBLB overexpression could inhibit apoptosis, prolong the cell cycle, and suppress cell migration in RA-FLSs. Results from qRT-PCR indicated that ETS-1 could negatively regulate circ-CBLB. pcDNA3.1-circ-CBLB+siRNA-ETS-1, in the presence of circ-CBLB overexpression, could counteract and reverse the effects of circ-CBLB on RA-FLSs, including increasing IL-4 levels and decreasing IL-23, IL-13, and TNF-α levels as well as its effects on cell viability, apoptosis rate, cell cycle, and migration (Figure 4).

Our study has several limitations, including a small sample size, with only 15 pairs of HCs and peripheral blood samples of patients with RA that were used for in vivo analysis, which may affect the stability and generalizability of our findings. Additionally, the

study lacks long-term follow-up, preventing the assessment of how changes in circ-CBLB and ETS-1 expression correlate with the progression and treatment outcomes of RA over time. Furthermore, while the study focuses on the roles of circ-CBLB and ETS-1, it does not explore other potential molecular mechanisms or signaling pathways (e.g., JAK–STAT and NF-κB), which may limit the comprehensive understanding of the complex pathology of RA.

5 | Conclusions

circ-CBLB and ETS-1 are downregulated in RA-PBMCs and RA-FLSs, correlating with inflammation and disease activity. They regulate each other bidirectionally. circ-CBLB reduces RA-FLS viability, promotes apoptosis, and inhibits migration by modulating cytokines. ETS-1 has similar effects, and interfering with its expression reverses the impact of circ-CBLB.

Author Contributions

Shu Li: resources, software, methodology, investigation, formal analysis, writing – original draft, and writing – review and editing. **HaoXiang Fang:** methodology and validation. **Lei Wan:** conceptualization, supervision, project administration, funding acquisition, and writing – review and editing. **XiaoJun Zhang:** supervision.

Disclosure

Institutional Review Board Statement: This study was conducted after the approval of the Hospital Ethics Committee (2019AH-12).

Ethics Statement

Ethics approval was granted by the Ethics Committee of the First Affiliated Hospital of Anhui University of Chinese Medicine.

Consent

Informed consent: Written informed consent was obtained from all enrolled patients.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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REVIEW

Regulating Rheumatoid Arthritis From the Perspective of Metabolomics: A Comprehensive Review

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Keywords: biomarkers | metabolomics | RA | rheumatology | TCM

ABSTRACT

Rheumatoid arthritis (RA) is a severe inflammatory autoimmune disease with metabolic changes. RA patients have abnormalities in glycolysis, amino acid metabolism, choline metabolism, and fatty acid synthesis. The differential metabolites in individuals of RA patients and animal models were explored to find the potential biomarkers for the risk prediction, diagnosis, and prognosis of RA in the perspective of metabolism. Moreover, we discussed the changes of related metabolites after treatment with anti-rheumatic drugs, Traditional Chinese Medicine (TCM) and potential metabolites for the treatment of RA to explore promising metabolites. In addition, the immunological mechanism of TCM in the treatment of RA from the perspective of metabolism was also clarified. For the perspectives of research and application of the beneficial metabolites in clinic, relevant technologies and focuses for the future studies in the field have been proposed accordingly.

1 | Introduction

Rheumatoid arthritis (RA) is a chronic rheumatic pain disease with an unknown cause that affects 0.5%–1% of the world's population [1, 2]. RA patients will appear with wrist, metacarpophalangeal joint, proximal interphalangeal joint symmetry, persistent pain and swelling, and ultimately the performance of joint deformity [3]. The progression of RA is accompanied by related complications, including cardiovascular disease, infection, osteoporosis, lymphoproliferative malignancies, and peptic ulcer disease [4]. Patients with RA have a 50% chance of dying from cardiovascular disease [5].

In addition to joint manifestations, metabolic diseases are common in RA patients. There is a high prevalence of hypertension among RA patients. Among the 115 867 insurance claims collected from American RA patients, 76% of the patients were diagnosed with hypertension, which was much higher than the 44% incidence of the matched control group [6]. RA patients may experience changes in their lipid profile, characterized by increased levels of total cholesterol, low-density lipoprotein cholesterol, and triglycerides [7].

At present, the effective treatment strategies are mainly disease-modifying antirheumatic drugs (DMARDs), injection of biological

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DMARDs, targeted synthesis of DMARDs, and glucocorticoids (GC) [8, 9]. When inflammation persists, traditional drugs fail to treat RA effectively. This trend highlights the urgent need to study the effectiveness of alternative medicine in this context.

Metabonomics methods can promote early disease detection, treatment monitoring, and pathogenesis elucidation in different medical fields, especially in the field of rheumatic diseases [10]. Changes in metabolic pathways and metabolites play a vital role in meeting bioenergy needs, stimulating the release of inflammatory mediators, triggering joint degeneration and muscle wasting, regulating cell proliferation, identifying metabolites, and improving the accuracy of RA diagnosis and prognosis [11]. The treatment of RA can be improved by TCM through regulating metabolites, which is expected to become a new mode of treatment.

Our study investigated the correlation between RA and metabolism, examined its association with TCM, assessed the effectiveness of TCM in treating RA, and identified potential metabolites for RA treatment.

2 | Metabolic Changes in Individuals With RA and RA Animal Models

As a result of analyzing serum metabolites from RA patients and collagen-induced arthritis (CIA) rats, it was discovered that thymine, acetylcholine, and lactic acid levels increased; in addition, a decrease in proline, deoxycholic acid, and L-aspartate levels was observed. However, changes in certain metabolites were inconsistent in different studies, such as serum Alpha-ketoglutaric acid levels in RA patients [7]. There are also differences in metabolites between RA patients and model animals, such as decreased glycine in the serum of RA patients and increased glycine in the serum of CIA rats [12]. Moreover, the changes of metabolites in different animal samples were also different; the levels of arachidonic acid varied in the synovial fluid, urine, and serum of CIA rats [13] (Table S1). The differences in metabolites may be related to species differences, RA disease status, and physiological processes, and the differential metabolites need to be further studied. The pathogenesis of RA is generally associated with the metabolic pathways of glycolysis, amino acid uptake, choline metabolism, and fatty acid synthesis [14].

In clinical applications, some metabolites in metabolomics can be used as biomarkers for disease diagnosis and can be used to reveal disease stages. With the expanding collection of citrullinated proteins identified from the synovial fluid and serum of patients with RA, the term "RA citrullinome" has been introduced in recent decades. Citrulline is an α-amino acid, which is a highly specific indicator for the early diagnosis of RA, just like serum anti-citrullinated protein antibodies (ACPAs) [15]. In addition, elevated levels of plasma lactic acid, acetylated glycoproteins, cholesterol, and unsaturated lipids, and decreased levels of high-density lipoproteins have shown potential as biomarkers for disease severity [16]. CRP, erythrocyte sedimentation rate (ESR), disease activity score of CRP (DAS28-CRP) and DAS28-ESR are commonly used clinical indicators to evaluate RA inflammation and disease activity [17]. A large number of studies have been conducted to discover metabolites that reflect and predict inflammation. Fifty-one metabolites were significantly associated with DAS28-CRP, and 12 metabolites, such as leucine and nicotinamide, were significantly associated with DAS28-ESR [18, 19]. Butyrate is a metabolite of the gut microbiota that inhibits osteoclasts and autoantibodies, balancing the immune response of systemic T and B lymphocytes to inhibit bone erosion and inflammation [20]. The content of butyrate in the feces of AIA mice decreased [21]. The difference in metabolite levels can be used as an indicator for clinical evaluation of RA inflammation and bone destruction and their prognosis.

3 | RA-Related Metabolic Pathways

There is a link between RA pathogenesis and glycolysis, amino acid uptake, choline metabolism, and fatty acid synthesis. Proteins involved in these pathways lead to macrophage infiltration, Fibroblast-like synoviocytes (FLS) expansion, osteoclast differentiation, and inflammatory infiltration cells leading to joint damage by stimulating the above metabolic pathways (Figure 1). Targeted therapy of these proteins can effectively treat RA [22].

3.1 | Glycolysis

The inflammatory response of arthritis is caused by a crowded environment with reduced oxygen supply. Under hypoxic conditions, glycolysis is the preferred source of ATP [23]. Glycolytic inhibitors reduced the expansion and migration of FLS cells in the mouse model of arthritis, significantly reducing the severity of the disease. It is believed that glucose metabolism is involved in the pathogenesis of RA [24].

The hypoxia-inducible factor 1 (HIF1α) plays an important role in glycolysis. RA patients with abundant HIF1α in synovial fluid experience inflammation, apoptosis, oxidative damage, and cartilage erosion [25]. In arthritis mice and FLS cells, glucose transporter 1 (GLUT1) messenger RNA was expressed [24]. Expression of GLUT1 may be related to RA pathogenesis since it is regulated by HIF1α transcription [26]. As part of the pathogenesis of RA, there may also be an increase in hexokinase II (HK2), a glycolysis regulator downstream of HIF1α. Overexpression of HK2 can promote migration and invasion of FLS cells, while inhibition of HK2 protects mice from bone damage due to arthritis [27, 28].

In addition to HIF1α, another key enzyme for glycolytic decomposition of glucose is the bifunctional fructose-6-phosphate-2-kinase/fructose-2,6-bisphosphatase (PFKFB) enzyme. Recent studies revealed that inhibition of PFKFB3 activity could reduce lactic acid production and inhibit inflammation by inhibiting FLS activity, indicating that there is a link between PFKFB3 and RA pathogenesis [29].

3.2 | Amino Acid Uptake

Amino acids can promote metabolism and protein synthesis and play a crucial role in initiating appropriate inflammatory responses. Serine metabolism is involved in the transcriptional regulation of IL1β in macrophages [30]. Leucine is involved in the glycolytic recombination induced by mtorc1 in macrophages [31]. Arginine can be used by nitric oxide synthase (NOS)

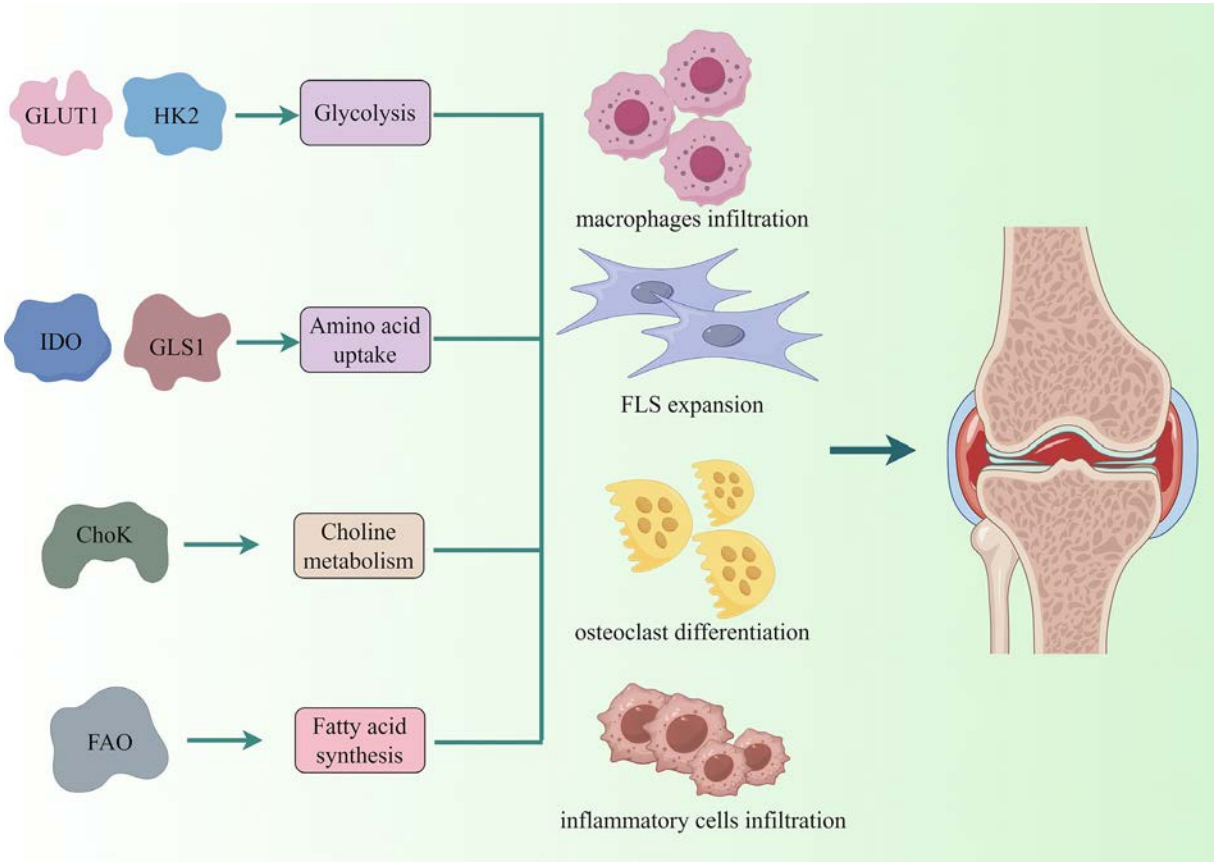

FIGURE 1 | Relationship between RA and metabolic pathways. GLUT1, HK2, IDO, GLS1, ChoK, and FAO lead to macrophage infiltration, FLS expansion, osteoclast differentiation, and inflammatory infiltration cells, through Glycolysis, Amino acid uptake, Choline metabolism, and fatty acid synthesis pathways, eventually leading to joint damage. ChoK, choline kinase; FAO, fatty acid oxidation; FLS, fibroblast-like synoviocytes; GLS1, glutaminase1; GLUT1, glucose transporter 1; HK2, hexokinase II; IDO, indoleamine 2,3-dioxygenase.

overexpressed in M1 macrophages to produce nitric oxide (NO), thereby killing microorganisms [32].

An enzyme known as Indoleamine 2,3-dioxygenase (IDO) has received a great deal of attention. IDO plays a key role in converting tryptophan into kynurenine. The decrease of tryptophan level and the strong expression of the related gene IDO1 in peripheral monocytes can be observed in RA patients [33]. The IDO molecule has also been shown to induce regulation of dendritic cells and inhibit osteoclastic differentiation in bone marrow cells [34]. A decrease in tryptophan level and a rise in IDO expression indicate that the tryptophan metabolic pathway participates in RA pathogenesis.

In addition, the glutamine catabolism pathway is associated with the pathogenesis of RA. In the glutamine catabolism pathway, glutamine is converted into glutamate by glutaminase1 (GLS1). The expression of GLS1 is up-regulated in RA-FLS. In SKG mice, glutamine deprivation reduces the expansion of RA-FLS cells, and GLS1 inhibition inhibits this expansion and improves inflammatory arthritis symptoms [35].

3.3 | Choline Metabolism

The enhancement of cell proliferation is usually related to the increase of phospholipid synthesis. The phosphatidylcholine kinase enzyme (ChoKα) is a vital enzyme for cell proliferation as well as phosphatidylcholine biosynthesis. The expression levels of ChoKα and phosphocholine in FLS can be increased by tumor necrosis factor (TNF) and platelet-derived growth factor (PDGF), while ChoKα inhibition can inhibit the activity of FLS and reduce joint destruction. The pathogenesis of RA is associated with ChoKα and phosphocholine. It is possible that targeting metabolomics could be a new treatment strategy for RA [22]. In the study of inflammatory macrophages, choline is rapidly phosphorylated by ChoKα within inflammatory macrophages, promoting the synthesis of phospholipids necessary to maintain optimal membrane fluidity and composition through the Kennedy pathway, thereby enhancing the production and secretion of cytokines [36].

Another important enzyme in phosphatidylcholine metabolism is phospholipase A2, and inflammation occurs through the hydrolysis of phosphatidylcholine by phospholipase A2. In the secondary lesion stage of adjuvant arthritis, the concentration of lysophosphatidylcholine increases, which stimulates the infiltration of macrophages and enhances their phagocytic ability [37].

3.4 | Fatty Acid Synthesis

A dynamic metabolic process of anabolism and catabolism takes place in fatty acid metabolism. Carnitine

palmitoyltransferase IA (CPT1A)-mediated fatty acid oxidation (FAO) metabolism promotes the fusion of osteoclast precursor (OCP) in RA and participates in the joint destruction of RA [38]. Synovial macrophages are mainly considered to have pro-inflammatory effects in RA [39]. Macrophage fatty acid synthase deficiency results in plasma membrane cholesterol depletion, affecting GTPase-dependent cellular functions [40]. Fatty acid metabolism in the synovium stimulates the production of FLS and macrophage inflammatory molecules, which play a role in arthritis.

4 | Treatment of Rheumatoid Arthritis by Regulating Metabolism

The persistence of inflammation in RA patients leads to poor efficacy of conventional drug therapies. Compared with conventional anti-rheumatic drugs, biological agents show rapid and effective treatment results, but their cost is high and they have potential patient compliance challenges. This trend highlights the urgent need to study alternative medicine in this context.

4.1 | Conventional Anti-Rheumatic Drugs Treated RA by Regulating Some Metabolic Ways

Leflunomide is a commonly used conventional drug for the treatment of RA. Its metabolite teriflunomide is produced in the treatment of RA, and teriflunomide is an inhibitor of dihydronicotinic dehydrogenase, which is used to treat RA by inhibiting pyrimidine biosynthesis [41]. In addition, other conventional anti-rheumatic drugs can also play a role by regulating metabolic pathways. Methotrexate inhibits the synthesis of purine and pyrimidine, promotes the release of adenosine, and is related to nucleotide metabolism [42, 43]. There was a reduction in succinic acid, taurine, lactic acid, pyruvic acid, and aspartic acid levels in RA patients' serum as a result of rituximab treatment [44]. As a result of hydroxychloroquine's effects on lipid metabolism, total cholesterol, low-density lipoproteins, and triglycerides were reduced, and high-density lipoproteins were increased, which linked to lipid metabolism [45, 46].

4.2 | Traditional Chinese Medicine, as Promising Therapies, is Utilized to Regulate the Disorder of Metabolic Ways

As a treasure of Chinese traditional culture, there are unique advantages of TCM, including its long-lasting curative effect and low occurrence of side effects [47]. The ancient practice of TCM has been used to treat a wide range of diseases since ancient times. TCM and some bioactive compounds derived from herbs are effective in reducing arthritis pain and stiffness [48]. Moreover, it can be used in combination with a variety of analgesic drugs such as non-steroidal anti-inflammatory drugs to enhance efficacy and reduce toxicity.

Metabolism is holistic, dynamic, and real-time, which coincides with the holistic view and constant motion view advocated by TCM. Different TCM can produce different effects on the body. By detecting metabolites and analyzing the changes in the metabolic spectrum, the biological effects of different TCM on the body can be evaluated to guide rational drug use in clinics.

TCM inhibits the inflammatory response by regulating inflammatory genes and inflammatory signaling pathways. TCM treatment methods usually include Chinese herbal medicine treatment, compound treatment, and TCM extract treatment. To begin with, Chinese herbal medicine can be used to treat RA and affect the metabolism of the body. Daphnes Cortex inhibits the TLR4/NF-κB/NLRP3 signaling pathway and affects amino acid metabolism and energy metabolism [49]; the mechanism of Daphnes Cortex in the treatment of RA may be related to its effect on RA metabolism. The anti-RA effect of Tian-Ma and Fu-Zi may be related to the inhibition of PTGS2 transcription and the inhibition of the arachidonic acid metabolic pathway [50]. Furthermore, in the treatment of RA with traditional Chinese medicine compound prescription, the effect of Ershiwuwei Lvxue Pill and Shentong Zhuyu Decoction may be related to the regulation of fatty acid metabolism [51, 52]. Huanglian Jiedu Decoction could adjust the metabolic level of chondrocytes under inflammatory reaction by affecting the citric acid cycle, lipid metabolism, and oxidative damage, and improve the symptoms of CIA rats and RA patients [53, 54]. In addition, some Chinese herbal extracts can also be used for the treatment of RA. Acanthopanax senticosus polysaccharides inhibited the activation of the NLRP3 inflammasome and reprogrammed the arthritic progression triggered by dysbiosis, enhanced the expression of γ-glutamylcysteine (GGC) synthetase, and enriched the serum concentration of GGC [55]. Curcumin and Morin can also alleviate the symptoms of RA by regulating fatty acid metabolism [56, 57]. Atractylodes macrocephala, *Salvia miltiorrhiza* extract, and Saposhnikoviae Radix decoction could regulate the biosynthesis of bile acids [58, 59] (Table S2). TCM treatment of RA may play a role by regulating the body's metabolism (Figure 2).

5 | Potential Metabolites on Treating RA

With the advancement of metabolomics technology, some potential metabolites for the treatment of RA have been discovered. Specific metabolites may be therapeutic drugs for RA [60]. This study focused on tryptophan metabolism, glucose metabolism, the tricarboxylic acid cycle, fatty acid metabolism, and lipid metabolism to identify metabolites that may be useful in treating RA.

Tryptophan is metabolized in the liver and other tissues primarily through the tryptophan-kynurenine pathway, tryptophan-5-hydroxytryptamine pathway, and tryptophan-indole pathway. Kynurenine (KYNA) is the product of the tryptophan metabolic kynurenine pathway. 3-Hydroxyanthranilic acid (3-HAA) is a metabolite produced by the IDO pathway. PI3K/Akt/mTOR activation and downstream NF-κB activation are inhibited by 3-HAA in LPS-induced macrophage inflammatory mediators, suggesting 3-HAA may be a new chemotherapeutic drug [61]. The final product of 5-hydroxytryptamine is 5-hydroxyindoleacetic acid (5HIAA). There is evidence that Saposhnikovia divaricata (SD) reduces the content of 5HIAA in rat serum, suggesting that SD might regulate tryptophan metabolism [62]. Simiao pill is a commonly used prescription for the

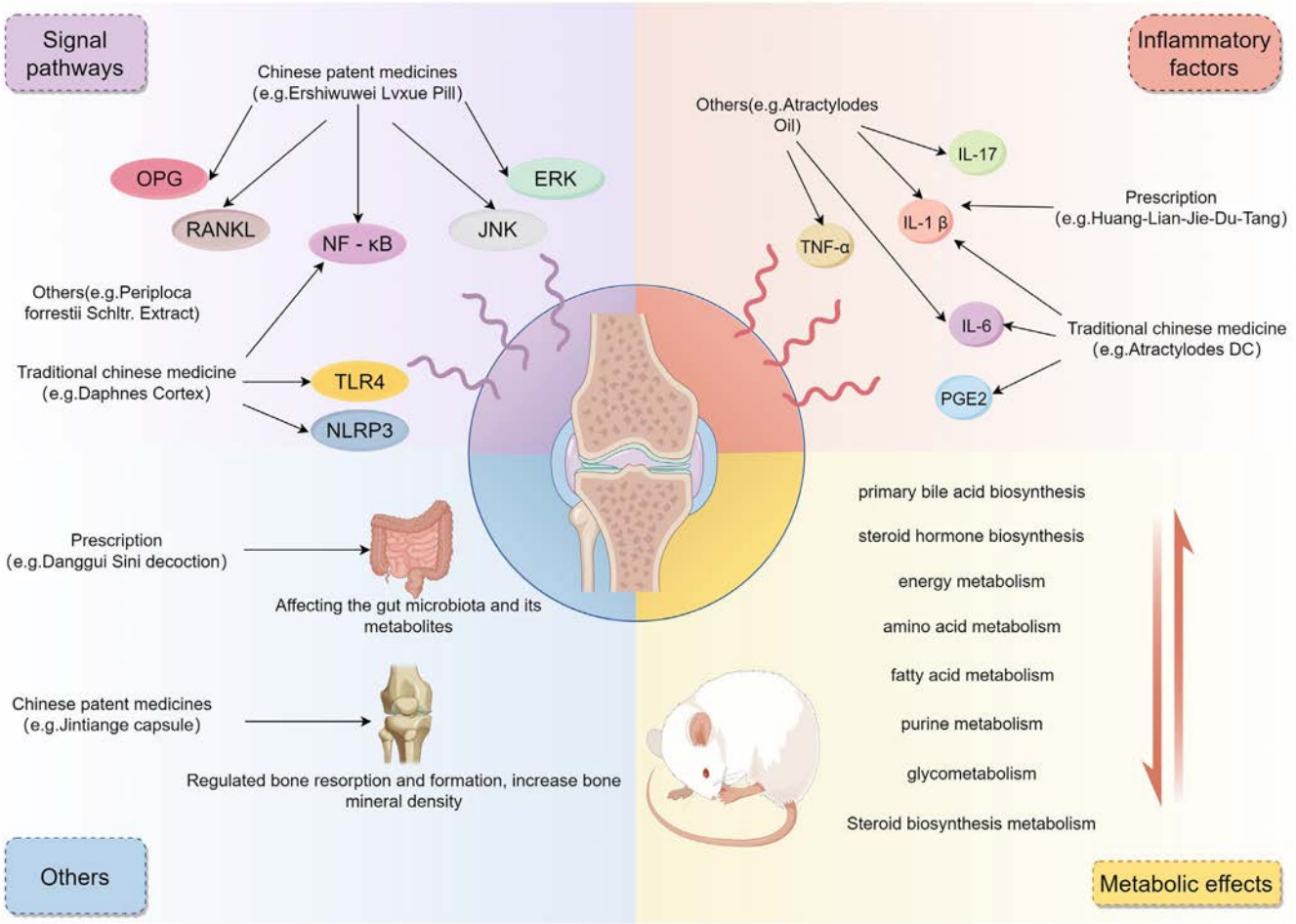

FIGURE 2 | TCM can alleviate RA by regulating various pathways.

treatment of RA. Metabolomics showed that, as well as regulating the metabolism of tryptophan and pyrimidine, Simiao pill can enhance the metabolism of linoleic acid and promote thymus and spleen recovery [63], a variety of metabolic pathways can be regulated through the treatment of RA, which is closely linked to tryptophan metabolism.

In the glucose metabolism pathway, HIF1α plays a key role as a regulator of glycolysis. α-mangostin (MAN) is the main bioactive compound derived from the main component of mangosteen. As a result of the treatment, glucose 6-phosphate, fructose 6-phosphate, 3-phosphoglycerate, and phosphoenolpyruvate were reduced, and pyruvate synthesis was restored in rats with adjuvant-induced arthritis. The effect of MAN on HIF-1α-mediated angiogenesis can be reduced by inhibiting aerobic glycolysis in adjuvant-induced arthritis rats [64]. MAN, a compound derived from TCM, can alleviate the clinical symptoms and inflammatory factors of AIA rats by regulating the glucose metabolism pathway. The enzymes involved in the glucose metabolism pathway can be used to treat RA.

There is succinate in the synovial fluid of patients with RA, which is an intermediate in the tricarboxylic acid cycle [65]. By inhibiting succinate dehydrogenase (SDH), succinate inhibits the HIF-1α/VEGF axis, showing potential to reduce arthritis angiogenesis. The SUCNR1/GPR91 receptor functions as a G protein-coupled sensor for extracellular succinate [66, 67]. SUCNR1/GPR91 allows dendritic cells to be recruited into lymph nodes, increasing Th17 cells and exacerbating experimental arthritis induced by antigens. The elimination of SUCNR1 inhibited inflammation and reduced the expansion of Th17 cells [68]. By inhibiting succinic acid, the tricarboxylic acid cycle can be blocked, offering new possibilities for treating RA in the clinic. Hypoxia TGF-β1-induced and succinate-associated NLRP3 inflammasome activation in RA rats is inhibited by clematanin AR (C-AR) inhibition of succinate dehydrogenase. Consequently, inflammation and fibrosis are prevented from crosstalking, preventing myofibroblast activation. Targeting synovium succinate may be a new method to prevent arthritis fibrosis [69].

Inhibition of fatty acid synthesis to delay adipogenesis reduces tissue inflammation, corrects tissue invasion of RA T cells, and reverses arthritis-inducing behavior [70]. The anti-inflammatory effects of ω-3 fatty acids have been demonstrated [71]. The therapeutic effects of ω-3 fatty acids have been evaluated for decades in clinical studies. In the study, ω-3 fatty acids reduced joint swelling and LTB4 production [72]. Anti-inflammatory effects have been observed with some fatty acids in RA.

The cytoplasmic phospholipase A2α (cPLA2α) enzyme is highly conserved and widely expressed in human and rodent cells from a variety of tissues [73]. cPLA2α releases arachidonic acid and then produces prostaglandins and leukotrienes [74]. This has

been shown to be a key factor in LPS-induced osteoclast formation and bone resorption [75]. When cPLA2α deficient mice and effective leukotriene B4 receptor antagonists were used, the severity of arthritis was significantly reduced [76]. Triptolide (TWG) can effectively improve arthritis in CIA rats and correct the significant increase of ceramide and lipid abnormalities such as lysophosphatidylcholine [77]. TCM treatment of RA by regulating lipid metabolism is a new idea for clinical treatment.

6 | Conclusion

This study reviews metabolic pathways and mechanisms potentially linked to RA pathogenesis. TCM may become an alternative therapy combined with anti-rheumatic drugs to treat RA by regulating the metabolism. Finally, the potential metabolites for the treatment of RA were explored. These metabolites can regulate the body's tryptophan metabolism, glucose metabolism, tricarboxylic acid cycle, fatty acid metabolism, and lipid metabolism. It is expected to become new methods for RA treatment by targeting metabolites or metabolic pathways.

However, there are limited studies on spatial metabolome in current metabolic studies, and clinical research on potential metabolites for RA treatment is lacking. Further research may require the establishment of domestic or international randomized and blinded clinical trials on the treatment of RA with metabolites. Finally, we anticipate the development of beneficial drugs and treatments for rheumatoid arthritis metabolites as more large cohorts emerge.

Author Contributions

W.W. contributed to the conception of the study. Y.L. and Y.C. wrote the first draft of the manuscript. J.J., M.L., and X.W. performed the literature research. W.W. and L.X. supervised the work and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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ORIGINAL ARTICLE

Exploration of Circadian Clock-Related Genes in the Pathogenesis of Psoriatic Arthritis to Identify Potential Therapeutic Targets From Multi-Omics Insight: A Mendelian Randomization Study

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Keywords: circadian clock-related genes | multi-omics | psoriatic arthritis | summary-data–based Mendelian randomization | therapeutic targets

ABSTRACT

Background: Circadian rhythms have been shown to play a significant role in the etiology and progression of immune-related morbidities, including cancer and autoimmune diseases. As an autoimmune disorder, psoriatic arthritis (PsA) has been under-explored in the context of circadian rhythms. This study aimed to explore the relationship between circadian rhythm and PsA.

Methods: We conducted a Summary-data–based Mendelian randomization (SMR) analysis, obtaining summary data on gene methylation, expression, and protein abundance levels of circadian clock-related genes (CRGs) from European ancestry individuals, with a total of 1749 genes selected from the GeneCards database using "biological clock" as the search term. The discovery cohort was obtained from the GWAS catalog database, and the replication cohort was obtained from the FinnGen database. Candidate genes related to circadian rhythm and PsA were identified through research, and their pharmacological potential and molecular docking were further validated as drug targets.

Results: After integrating multi-omics data, we identified 11 methylation sites in three genes (*HLA-DQB1*, *ITPR3*, and *GABBR1*) of CRGs that were causally related to PsA, and the effects produced were not consistent. The three gene expressions of CRGs (*IL4*, *HLA-DQB1*, and *OPI-AIS5*) were related to PsA. At the protein level, we identified three proteins (GCKR, STAT3, and CSNK2B) of CRGs related to PsA. The top 20 drug candidates underwent drug prediction screening, resulting in the identification of 12 compounds that demonstrated effective outcomes with three (HLA-DQB1, STAT3, and IL-4) specific therapeutic targets through molecular docking.

Abbreviations: CRGs, circadian clock-related genes; DSigDB, Drug Signatures Database; eQTL, expression quantitative trait loci; FDR, false discovery rate; GTEx, genotype-tissue expression; GWAS, genome-wide association study; HEIDI, heterogeneity in the dependent instrument; IVs, instrumental variants; LD, linkage disequilibrium; MAF, minor allele frequency; mQTL, methylation quantitative trait loci; pQTL, protein quantitative trait loci; PsA, psoriatic arthritis; SMR, Summary-data–based Mendelian randomization; SNP, single nucleotide polymorphism.

Aimei Liu and Wuda Huoshen contributed equally to this study.

Conclusion: This study suggests altered expression of circadian clock genes and proteins, including *HLA-DQB1*, *ITPR3*, *GABBR1*, *IL4*, *OIP5-AS1*, GCKR, CSNK2B, and STAT3, as factors contributing to the increased risk of PsA.

1 | Introduction

Psoriatic arthritis (PsA) is a chronic, immune-mediated inflammatory arthropathy, which occurs in approximately 30% of patients with psoriasis [1], characterized by varying degrees of peripheral and axial joint involvement, and is associated with an increased mortality rate from cardiovascular disease [2]. The pathogenesis of PsA is complex, involving genetic predispositions, immune-mediated inflammation, and environmental factors (such as infection, trauma, stress, obesity, and smoking) [3]. The specific details are not yet clear. Improving our understanding of the pathogenesis of PsA could help to establish effective diagnostic biomarkers, develop precise drugs, and predict which patients will respond to which therapy. A previous study has suggested that circadian rhythms may play a key role in the pathogenesis of psoriasis, as sleep and melatonin may lead to changes in the immune system, leading to the incidence and progression of psoriasis [4]. It has been shown that the circadian clock plays a role in the pathogenesis and clinical manifestations of inflammatory arthritis [5]. In models characterized by significant disruptions in circadian rhythms and melatonin release, such as in night-shift workers or in patients with sleep-related disorders [6], the risk of developing various rheumatic conditions, including psoriasis [4], The circadian rhythm, also referred to as the circadian clock, is an internal clock system that regulates a multitude of biological and behavioral processes, including sleep, hormone production, body temperature, and immune system function. The circadian rhythm has the ability to regulate immune system function by governing the circulation of lymphocytes, natural killer (NK) cells, antibody production, complement levels, cytokine synthesis, host–pathogen interactions, and induction of both innate and adaptive immunity [7]. Therefore, the immune system is affected by the circadian rhythm, and a disruption of the circadian cycle may lead to the occurrence of inflammatory diseases such as PsA. In addition, previous studies have suggested that disruption of the circadian clock may contribute to PsA progression, influence disease severity, and affect DLQI scores [8]. In order to better understand PsA, it is essential to study the relationship between circadian rhythm and PsA.

Mendelian randomization (MR) is not only a reliable method using genetic variants (single-nucleotide polymorphisms [SNPs]) to explain observational bias, but also a quasi-randomized controlled natural trial, due to variants are naturally and randomly allocated during meiosis [9]. MR uses genetic information as instrumental variables (IVs) to analyze the associations between exposures and outcomes. Because the genetic variants are usually unrelated to confounding factors, the difference in outcomes between individuals who carry the mutations and those who do not can be explained by differences in risky factors or disease susceptibility. Therefore, MR overcomes the drawbacks of confusion or reverse causality in traditional observational research [10]. Summary-data–based MR (SMR) is an extension of MR, in which the expression quantitative trait loci (eQTL) or DNA methylation quantitative trait loci (mQTL) for summary analysis, mainly used to analyze the association between genotype, gene expression, and phenotype [11].

To the best of our knowledge, currently, no MR study has investigated the possible causal relationship between circadian rhythm disorders and PsA. Therefore, this study aims to explore the causal association between circadian rhythm disruption and PsA using a comprehensive MR approach, characterized by the genetic susceptibility of circadian rhythm–related genes.

2 | Methods

2.1 | Study Design

We conducted the SMR analysis using publicly available summary-level data of quantitative trait loci (QTLs) and GWAS studies (Figure 1 and Tables S1–S18). All participants provided informed consent, and this study was approved by the ethics committee review board.

2.2 | Data Sources of Circadian Clock-Related Genes (CRGs)

We used "biological clock" as the main search term from the GeneCards (https://www.genecards.org/) to obtain 1749 CRGs (Table S2).

2.3 | Selection of Genetic Instruments

We used publicly available data for research. The eQTL summary-level data were collected from the eQTLGen Consortium (https://www.eqtlgen.org/) and the GTEx Consortium V8 (https:// gtexportal.org/), further details are outlined in the Tables S1–S18. We selected the MR mQTL instruments based on genetic variations that exhibit stable correlations with gene methylation related to circadian rhythm. Using blood mQTL summary data from a meta-analysis of two cohorts totaling 1980 individuals is a solid approach for identifying genetic instruments for studies [12]. We used the file from Price et al. to annotate the closest genes to DNA methylation probes [13]. MR protein QTL (pQTL) instruments were extracted from summary data provided by Ferkingstad E et al. ($n = 35\,559$) [14] and Sun et al. ($n = 54\,219$) [15]. In the analysis of eQTL, mQTL, and pQTL data, only probes that are linked to the expression of circadian clock–related genes and have at least one common cis-QTL (minor allele frequency [MAF] > 2%) surpassing the genome-wide significance threshold ($p < 5.0E\text{-}08$) were considered in the SMR analyses. The cis region was defined as within a 1 Mb range of a probe in both directions.

We conducted another instrument selection method to verify the observed correlation using eQTL as instruments. Specifically,

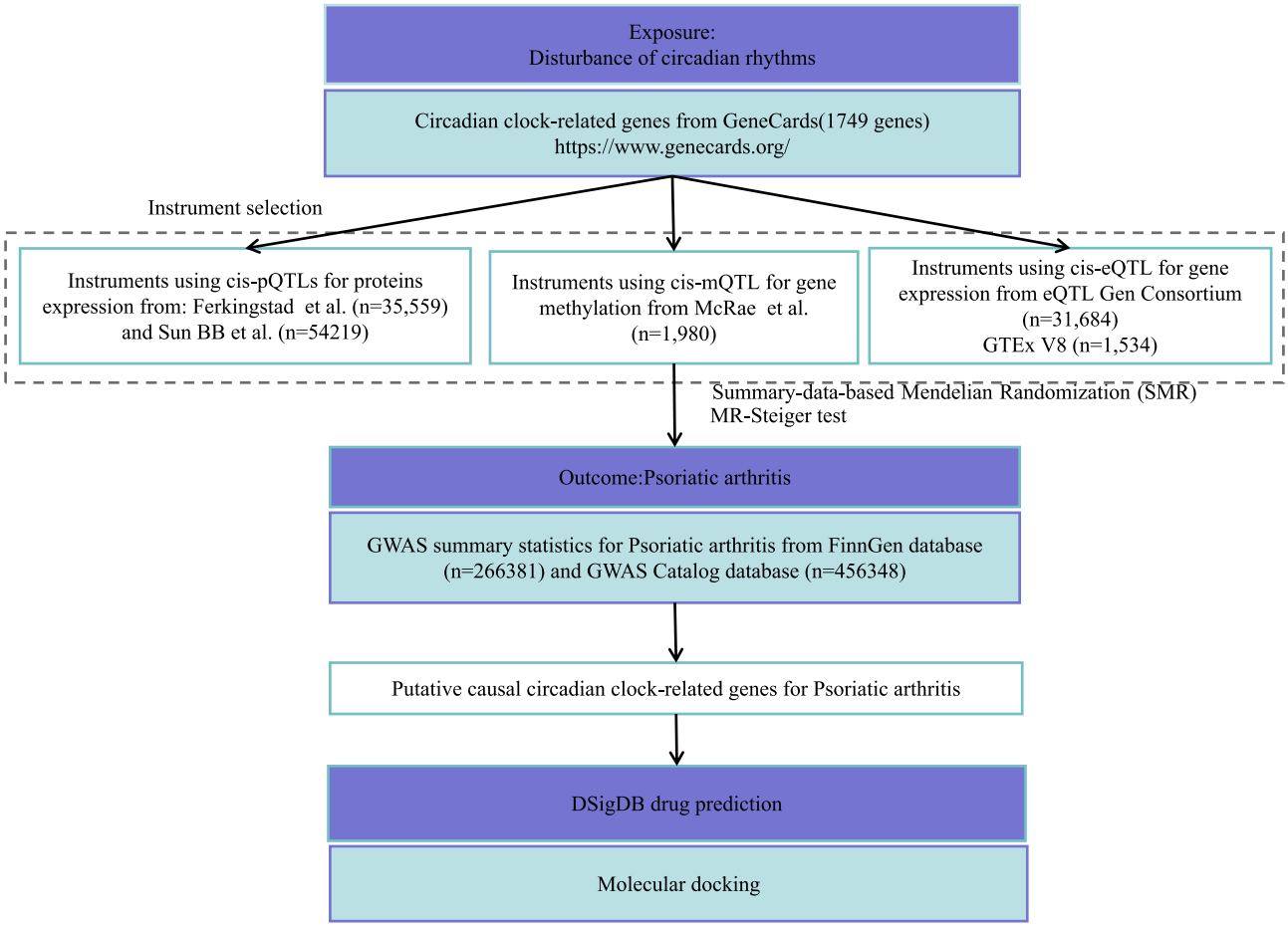

FIGURE 1 | Flowchart of this study. GWAS, genome-wide association studies; IVW-MR, inverse-variance-weighted MR; QTL, quantitative trait loci; SNP, single nucleotide polymorphism.

we selected single nucleotide polymorphisms (SNPs) located within 100 kb windows of the target gene, which had been associated with the circadian clock at a genome-wide significance threshold ($p < 5.0E-08$). These obtained SNPs were considered substitutes for the circadian clock-related exposures.

2.4 | Outcome Sources

GWAS summary statistics for PsA in discovery cohorts were collected from the FinnGen database, which included 266 381 individuals of European ancestry [16]. On the other hand, GWAS summary statistics for PsA in replication cohorts were obtained from the GWAS Catalog database, consisting of 456 348 individuals [17].

2.5 | Statistical Analyses

In our study, the SMR approach was employed using cis-QTLs as instruments to estimate the effect sizes. The SMR method was employed to investigate the association between gene expression and the outcome of interest, utilizing summary-level data from both GWAS and the investigations of cis-QTL [11]. For allele harmonization and analysis, the SMR software (version 1.03, https://yanglab.westlake.edu.cn/software/smr/#Overview) was used. To calculate the odds ratio (OR) estimates of circadian rhythm disorder on the risk of PsA, the following formula was applied: $OR = \exp.(\beta)$, where OR represents the odds ratio estimate per 1-ln increase in circadian clock genome levels, and exp. is the base of the natural logarithm.

The SMR software was used to perform the heterogeneity in the dependent instrument (HEIDI) test for the SMR technique. This test helped to assess whether the observed correlation between gene expression and the outcome of interest can be attributed to a linkage scenario [11]. For the calculation of linkage disequilibrium (LD), Genomes of European ancestry obtained from the 1000 Genomes Project Consortium were used as a reference [18]. The HEIDI test with $p > 0.01$ shows the presence of linkage imbalance [19]. Indeed, horizontal pleiotropy occurs when a single genetic variant is associated with the expression or function of multiple genes. In our study, we identified neighboring genes within a 1 Mb window that exhibited a strong correlation with the instrumental mutation. To investigate the potential presence of horizontal pleiotropy, we performed SMR analysis to examine whether the expression of the genes was also associated with the outcomes related to PsA. In addition, we conducted a direction test through Steiger filtering in MR analysis to ensure the stability of our SMR results. This measure helped to evaluate the direction of causality.

The Benjamini–Hochberg method was used to adjust the p values and control the false discovery rate (FDR) at $a = 0.05$.

2.6 | Integrating Results From Multi-Omics Levels of Evidence

To elucidate the multifaceted relationship between the regulation of clock-related genes and PsA, we synthesized findings from two complementary tiers of gene regulation, providing a comprehensive understanding of their interconnections. Genes exhibiting associations with PsA across multiple omics layers were classified into Tier 1. In contrast, genes showing associations with PsA at a single omics level were classified into Tier 2.

2.7 | Drug Prediction

We utilized protein–drug interactions to investigate whether target genes could be potential drug targets by using the Drug Signatures Database (DSigDB), which is available at http://amp.pharm.mssm.edu/Enrichr. DSigDB contains a large collection of 22 527 gene sets and 17 389 unique compounds that interact with 19 531 genes. By uploading the identified target genes to DSigDB, this study allows for the prediction of potential drug candidates and the assessment of the medicinal properties of these targets. In our study, we uploaded the genes from our SMR results to the DSigDB section of Enrichr. This enabled us to identify candidate drugs and evaluate the therapeutic potential of the target genes based on the enriched gene sets. Default parameter settings were applied throughout the drug prediction. Potential drug targets were then selected based on an adjusted p value of less than 0.05.

2.8 | Molecule Docking

Small molecule ligand drug structures were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), while protein structures were obtained in pdb format from the PDB database (https://www.rcsb.org/). We utilized the CB-Dock2 platform (https://cadd.labshare.cn/cb-dock2/) to validate and visualize the interactions between the small molecule ligands and protein structures, which can help assess the feasibility and potential efficacy of the candidate drugs in targeting specific proteins.

3 | Results

3.1 | Clock Gene Methylation and PsA

In the SMR analysis of the whole genome cis-eqtl of the circadian clock and PsA, we matched 2835 unique genetic loci. To reduce the linkage disequilibrium response and the whole genome type I error, we removed the association of $P_{HEIDI} < 0.01$ and by the marginal significance ($p < 0.05$). Through multiple corrections, after integration with the UK Biobank and the FinnGen data, we determined that 11 CpG loci of the three genes *HLA-DQB1*, *ITPR3*, and *GABBR1* were causal with PsA. CpG loci within the same gene displayed varying effects on PsA risk. Specifically, we found that elevated methylation levels at the *HLA-DQB1* gene loci cg01745539, cg00995368, cg09555323, and cg03202060 were significantly associated with an increased risk of PsA (OR 1.26, 95% CI 1.20–1.33; OR 1.60, 95% CI 1.38–1.86; OR 2.57, 95% CI 1.76–3.74; OR 3.29, 95% CI 1.79–6.06). Reduced methylation at cg22984282 and cg11986643 was associated with an increased risk of PsA (OR 0.82, 95% CI 0.78–0.86; OR 0.74, 95% CI 0.67–0.81). Similarly, an increase in *ITPR3* cg18558423 promoted the risk of PsA (OR 1.26, 95% CI 1.11–1.42), while cg07713849 was just the opposite (OR 0.68, 95% CI 0.55–0.83). Elevated methylation levels at the *GABBR1* locus cg09577455 were associated with an increased risk of PSA (OR 1.02, 95% CI 1.01–1.03) (Figure 2a). The detailed SMR analysis results of clock gene methylation and PsA were presented in Tables S3 and S4. All evaluations successfully passed the Steiger test in gene methylation level and were presented in Table S15.

3.2 | Clock Gene Expression and PsA

eQTL was matched to 585 and 1285 genes from eQTLGen Consortium and GTExV8, respectively. The gene expression of HLA-DQB1, akin to methylation, exhibited a causal association with the manifestation of PsA. After integration, we identified the following three genes: the increased expression of the HLA-DQB1 gene was associated with an increased risk of PsA (OR 1.26, 95% CI 1.18–1.34). There was also strong evidence of a correlation between IL4 and PsA. The decreased expression of IL4 led to an increased risk of PsA (OR 0.28, 95% CI 0.17–0.46). In contrast, the increase of OPI-AIS5 promoted the risk of PsA (OR 1.75, 95% CI 1.31–2.34) (Figure 2b). The results of the SMR analysis examining the relationship between clock gene expression and PsA are presented in Tables S5–S8. All evaluations met the significance threshold according to the Steiger test for gene methylation level, with findings presented in Table S16.

3.3 | Clock Encoded Protein and PsA

PQTL were matched to 625 and 363 proteins from Ferkingstad et al. and Sun et al. respectively. After integration, three proteins were identified: GCKR, CSNK2B, and STAT3. GCKR also had a strong positive correlation with the occurrence of PsA (OR 3.29, 95% CI 1.76–6.06). The increased expression of STAT3 also led to the evaluated risk of PsA (OR 1.18, 95% CI 1.08–1.29). CSNK2B was just the opposite (OR 0.98, 95% CI 0.97–0.99) (Figure 2c). The SMR results of clock-encoded protein and PsA were summarized in Tables S9–S12. All evaluations satisfied the Steiger test criteria for gene methylation level, with results presented in Table S17.

3.4 | Integrating Results From Multi-Omics Level of Evidence

After integrating evidence from multiple omics layers, we identified one gene (*HLA-DQB*1) that was classified into tier 1 based on both gene expression and gene methylation data. The remaining genes (*ITPR3*, *GABBR1*, *IL4*, *OIP5-AS1*, GCKR, CSNK2B, and STAT3) were not classified into Tier 1, as they showed associations with PsA only at a single omics level.

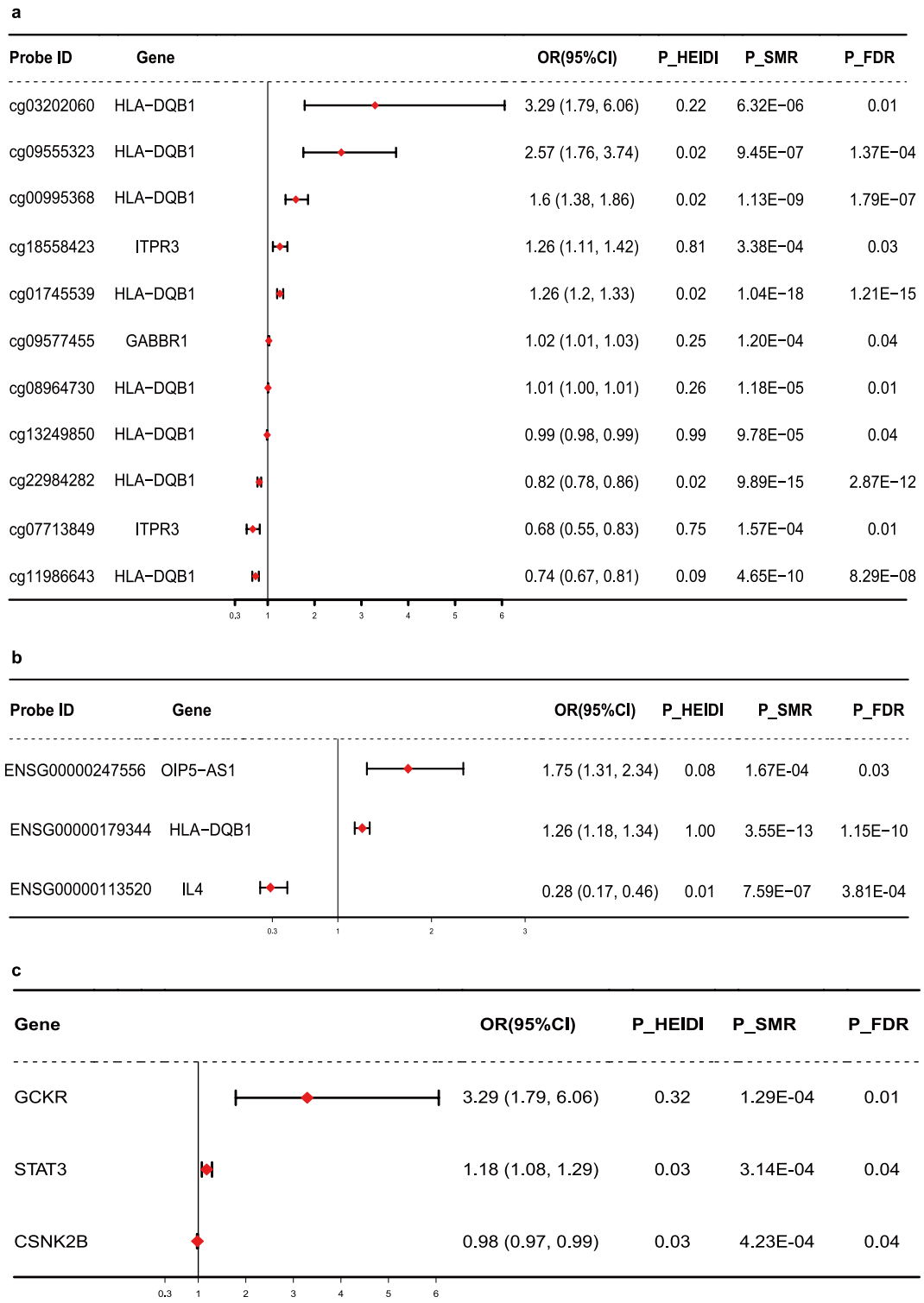

FIGURE 2 | Summary-data–based Mendelian randomization (SMR) results for the association between the multi-omic level of circadian clock-related genes and PsA. OR, odds ratio; PsA, psoriatic arthritis; SMR, Summary-data–based Mendelian randomization. (a) The SMR results for the association between the methylation of circadian clock–related genes and PsA. (b) SMR results for the association between the expression of circadian clock-related genes and PsA. (c) SMR results for the association between the encoded protein of circadian clock–related genes and PsA.

3.5 | Drug Candidate Prediction

The DsigDB database was used to predict the possible effective intervention drugs. According to the adjusted *p* value, the top 20 potential compounds were listed (Table S18 and Table 1). Results showed that DL-Phenylalanine BOSS and L-glutamine BOSS affected IL4, STAT3, and ITPR3 (Table 1). IL4 was linked to 2,4-Diisocyanato-1-methylbenzene CTD 00006908 and HLA-DQB1 (Table 1). Ouabain BOSS was linked to STAT3 and ITPR3. Trichloroethylene CTD 00006932 was linked to IL4 and GABBR1. The rest were linked to IL4 and STAT3.

TABLE 1 | Candidate drug predicted using DSigDB.

Drug names	*p*	Adjusted *p*	Genes
DL-Phenylalanine BOSS	5.90E-06	0.003	IL4; STAT3
L-glutamine BOSS	4.56E-05	0.008	IL4; STAT3
Cladribine CTD 00007175	5.65E-05	0.008	IL4; STAT3
2,4-Diisocyanato-1-methylbenzene CTD 00006908	6.47E-05	0.008	IL4; HLA-DQB1
Pulmicort Nebuamp BOSS	7.35E-05	0.008	IL4; STAT3
Kynurenine BOSS	1.20E-04	0.01	IL4; STAT3
124020-07-1 CTD 00007038	1.20E-04	0.01	IL4; STAT3
Azathioprine CTD 00005457	2.06E-04	0.012	IL4; STAT3
Ferrous sulfate CTD 00001009	2.21E-04	0.012	IL4; STAT3
105156–22-7 CTD 00005867	2.21E-04	0.012	IL4; STAT3
Ouabain BOSS	2.53E-04	0.013	STAT3; ITPR3
Sodium sulfate BOSS	2.88E-04	0.013	IL4; STAT3
2-Fluoroadenosine BOSS	4.25E-04	0.016	IL4; STAT3
Trichloroethylene CTD 00006932	4.46E-04	0.016	IL4; GABBR1
Pentadecane BOSS	4.69E-04	0.016	IL4; STAT3
Acetoacetic acid BOSS	4.92E-04	0.016	IL4; STAT3
Etretinate BOSS	5.39E-04	0.016	IL4; STAT3
Dichloromercury CTD 00006273	5.39E-04	0.016	IL4; STAT3
Chlorophenothane CTD 00005755	5.51E-04	0.016	IL4; STAT3
Eicosapentaenoic acid BOSS	5.63E-04	0.016	IL4; STAT3
Eicosapentaenoic acid BOSS	5.63E-04	0.016	IL4; STAT3

3.6 | Molecular Docking

The assessment of the affinity between drug candidates and their targets is essential for evaluating the efficacy of drug intervention on the target. Molecular docking was performed in this study. Using the binding sites and interactions of the top 20 drug candidates with the corresponding gene-encoded proteins, each drug candidate was connected to its protein target through visible hydrogen bonds and strong electrostatic interactions. By calculating the binding energy of each interaction, a total of 12 drug candidates were found to have effective docking results with their drug targets (Table 2). Specifically, 2,4-Diisocyanato-1-methylbenzene CTD 00006908 was found to be associated with HLA-DQB1 and IL-4, while several other drugs were connected to IL-4 and STAT3. Notably, Pulmicort Nebuamp BOSS, Etretinate, Cladribine CTD 00007175, and 2-Fluoroadenosine exhibited stronger associations. Among the compounds targeting STAT3 gene expression, ouabain demonstrated the most significant association (Figure 3). A lower binding energy corresponds to a more favorable binding interaction and increased affinity.

4 | Discussion

In our study, we leveraged multi-omics data integration to analyze GWAS signals and perform molecular docking in order to explore the relationship between genetic prediction of CRGs methylation, expression, protein, and PsA. The following causative genes or proteins were identified: *HLA-DQB1*, *ITPR3*, *GABBR1*, *IL4*, *OIP5-AS1*, GCKR, CSNK2B, and STAT3. Molecular docking was performed on the corresponding gene-encoded proteins, which further proves the value of these gene-targeted drug developments.

Circadian clock–related gene HLA-DQB1 belongs to the HLA-II antigen β para-system, which is composed of β chains and forms a functional complex protein with DQA1 composed of α chains [20]. Its primary role is to process and present exogenous antigens for CD4 + T lymphocytes, facilitating immune regulation [20]. The involvement of HLA-DQB1 in the pathogenesis of various diseases, including rheumatoid arthritis, has been demonstrated by previous studies [21], and different gene loci also play different roles. The DQB1*06:02 allele is considered to confer susceptibility to multiple sclerosis [22]. HLA-DQB1*05:03 and DQB1*03:02 are substantially increasing the incidence of pemphigus; HLA-DQB1*02, DQB1*06:01, and DQB1*03:03 are negatively correlated [23]. HLA-DQB1*02 is a risk factor for psoriasis, and DQB1*0202 is an important allele for the response to Acitretin drug therapy in patients with psoriasis [24]. Participants with the HLA-DQB1*06 allele produced more antibodies in their bodies after Coronavirus disease 2019 (COVID-19) vaccination [25]. The aforementioned studies have demonstrated that HLA-DQB1 plays a pivotal role in immune

TABLE 2 | Docking results of available proteins with small molecules.

Target	PDB ID	Drug	PubChem ID	Binding energy
HLA-DQB1	2NNA	2,4-Diisocyanato-1-methylbenzene CTD 00006908	11443	−5.5
IL4	1BBN	Pulmicort Nebuamp BOSS	5281004	−6.4
IL4	1BBN	Cladribine CTD 00007175	20279	−6.1
IL4	1BBN	Kynurenine BOSS	846	−6
IL4	1BBN	124020-07-1 CTD 00007038	12133445	−6
IL4	1BBN	Azathioprine CTD 00005457	2265	−5.9
IL4	1BBN	DL-Phenylalanine BOSS	994	−5.6
IL4	1BBN	2,4-Diisocyanato-1-methylbenzene CTD 00006908	11443	−5.6
IL4	1BBN	Etretinate	5282375	−7.1
IL4	1BBN	2-Fluoroadenosine	8975	−6.6
IL4	1BBN	Eicosapentaenoic acid	446284	−5.8
IL4	1BBN	Chlorophenothane	3036	−5.7
STAT3	6NJS	Ouabain	439501	−9.0
STAT3	6NJS	Pulmicort Nebuamp BOSS	5281004	−7.7
STAT3	6NJS	2-Fluoroadenosine	8975	−7.1
STAT3	6NJS	Cladribine CTD 00007175	20279	−6.8
STAT3	6NJS	Etretinate	5282375	−6.4
STAT3	6NJS	Azathioprine CTD 00005457	2265	−6.3
STAT3	6NJS	Kynurenine BOSS	846	−6.2
STAT3	6NJS	Chlorophenothane	3036	−6.1
STAT3	6NJS	124020-07-1 CTD 00007038	12133445	−5.8
STAT3	6NJS	Eicosapentaenoic acid	446284	−5.4
STAT3	6NJS	DL-Phenylalanine BOSS	994	−5.2

activity. The aforementioned studies have demonstrated that the role of HLA-DQB1 in immune activity is well documented. Our study revealed a significant association between HLA-DQB1 gene methylation and gene expression with the occurrence of PSA. Specifically, increased methylation at cg01745539, cg00995368, cg09555323, and cg03202060 was linked to an elevated risk of PsA, whereas decreased methylation at cg22984282 and cg11986643 was associated with a reduced risk.

ITPR3 encodes the inositol 1,4,5-triphosphate receptor, which is a widely existing endoplasmic reticulum calcium channel protein related to apoptosis [26]. The upregulation of this receptor is implicated in the development of various tumors, including cervical squamous cell carcinoma and cholangiocarcinoma [27]. The expression in the salivary glands of Sjogren's syndrome and autoimmune extra-glandular diseases related to secretion defects is observed to be reduced [28]. The upregulation of methylation levels at the ITPR3 locus cg18558423 may be associated with an increased risk of PsA. The human genes that encode IL-4 and IL-13 are situated at chromosome 5q31. Both IL-4 and IL-13 belong to the T helper cytokine 2 family and share a receptor known as the type II receptor [29]. The cytokine IL-4 plays a crucial role in both the initiation and therapeutic management of psoriasis. Notably, IL-4 has been identified as a psoriasis-associated gene in the CASP (Collaborative Association Study of Psoriasis) through a comprehensive GWAS study involving individuals of European descent [30]. The skin treated with dupilumab, an antibody that targets the IL-4 and IL-13 receptors in the treatment of atopic dermatitis, exhibited psoriasis-like alterations [31]. Our study suggests that the decrease of IL4 gene expression may lead to the occurrence of PsA. The long non-coding transcript OIP5-AS1 exhibits high expression in the nervous system as a pivotal role in tumor transformation. The involvement of this lncRNA in the regulation of cell cycle transformation at various stages is evident. OIP5-AS1 is elevated in almost all types of tumor tissues except for multiple myeloma [32]. Further, it was found that OIP5-AS1 is associated with the occurrence and development of rheumatoid arthritis; the knockdown of RNA OIP5-AS1 can inhibit the proliferation and inflammation of FLSs [33]. The overexpression of OIP5-AS1 may be associated with the occurrence of PsA.

GCK is a key liver enzyme for glucose metabolism. The expression of glucose kinase regulatory protein (GCKR) is primarily

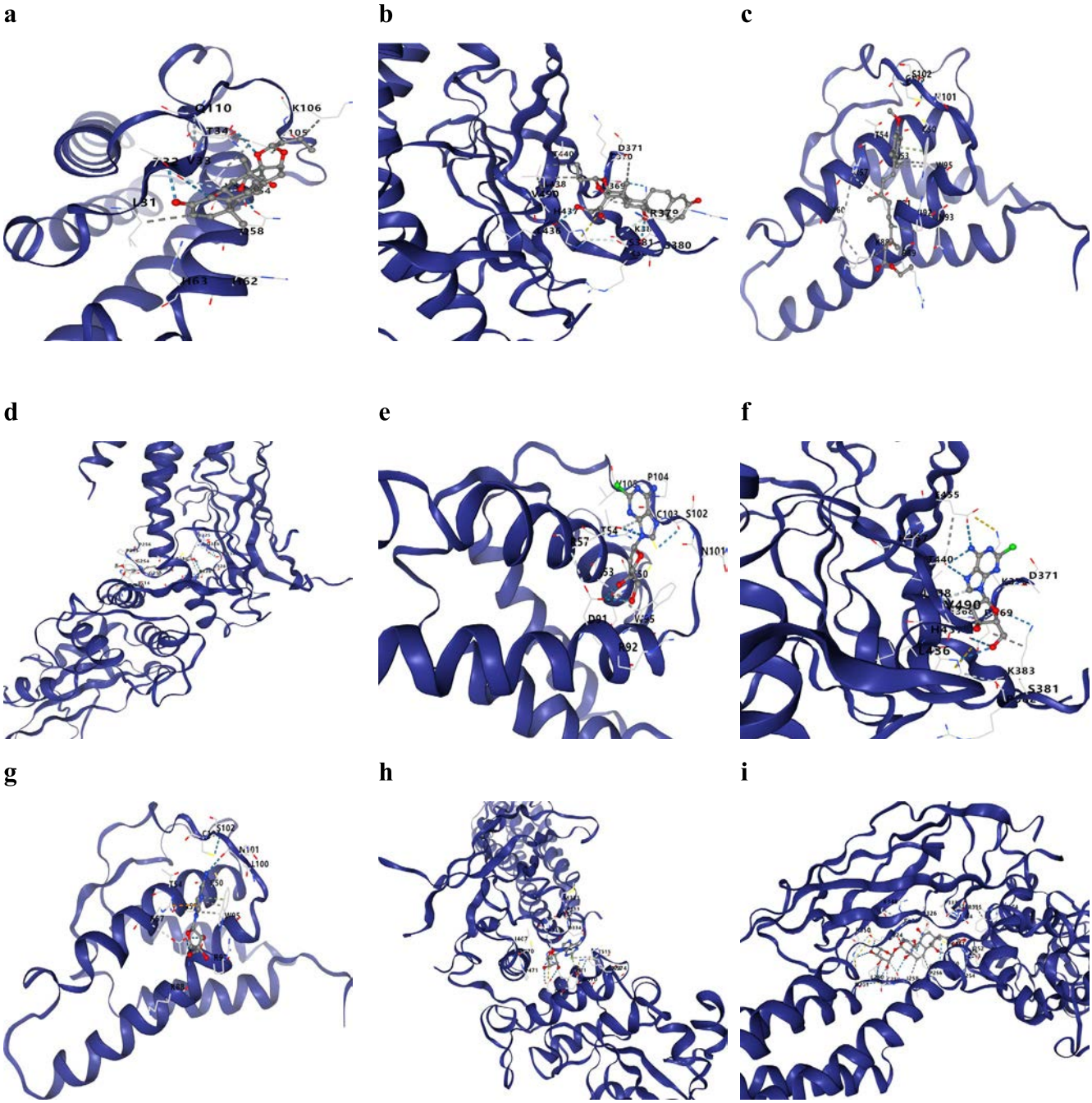

FIGURE 3 | Docking results of protein-small molecule interactions. (a) IL4 docking Pulmicort Nebuamp BOSS, (b) STAT3 docking Pulmicort Nebuamp BOSS, (c) IL docking etretinate, (d) STAT3 docking etretinate, (e) IL4 docking Cladribine CTD 00007175, (f) STAT3 docking Cladribine CTD 00007175, (g) IL4-2 docking fluoroadenosine, (h) STAT3 docking 2-fluoroadenosine, (i) STAT3 docking ouabain.

limited to hepatocytes. GCKR is a glucose kinase regulatory protein that can act as an inhibitor of glucose kinase (GCK) activity [34]. By influencing metabolism, GCKR has been linked to many metabolic diseases, most notably NAFLD [35], which is considered to be a predictor of the transformation of asymptomatic hyperuricemia to gout and a genetic marker for end-stage and high-risk kidney disease (ESKD) population in type 2 diabetes patients [36]. GCKR has not been studied in relation to psoriasis and PsA for the time being. The findings of our study suggest that the upregulation of the protein encoded by the GCKR gene may potentially contribute to the pathogenesis of PsA. In summary, we can see that among the genes affecting circadian rhythm, the genes associated with PsA include immunity, cell proliferation, and metabolism. CSNK2B and STAT3 have been implicated in the pathogenesis of PsA, although the relationship between these genes and the disease remains modest.

Furthermore, this study successfully predicted and conducted molecular docking for drugs targeting the identified genes, providing additional evidence to support their potential as therapeutic targets in disease treatment. Pulmicort Nebuamp BOSS contains Budesonide as its active ingredient, which has been shown in studies to improve clinical scores and cell proliferation in psoriasis when used for a duration of 1–3 weeks

[37]. The pharmacologically active metabolite of Etretinate is Acitretin, which has been widely utilized for treating PsA [38, 39]. Cladribine CTD 00007175 (2-chlorodeoxyadenosine) is currently extensively employed in multiple sclerosis treatment [40], there was one clinical case report demonstrating relief from PsA after oral administration of this drug [41]. Therefore, it can be inferred that these aforementioned drugs may exert their effects on treating psoriasis or PsA by modulating the expression of IL-4 and STAT3 genes. Previous studies have demonstrated the potential of ouabain as an effective anti-psoriasis medication due to its capacity to induce cytotoxicity in psoriatic keratinocytes [42]. However, there have been no clinical trials conducted thus far to investigate the efficacy of ouabain in treating PsA. These pharmaceuticals influence genes associated with the circadian rhythm and have therapeutic potential for PsA or psoriasis. Consequently, our study is reciprocally validated, suggesting that genetic alterations in the circadian clock may influence psoriatic arthritis.

This study boasts several strengths. Notably, it is the first to explore the causal relationship between circadian rhythm and the onset of PsA using SMR, integrating multi-omics data to elucidate GWAS signals. This approach effectively mitigates confounding variables and reverse causality, thereby enhancing the precision of our findings. Furthermore, the GWAS data utilized for IV selection feature a large sample size and high accuracy. Additionally, we employed molecular docking and drug prediction analyses to uncover the therapeutic potential of the identified genes.

To minimize the impact of confounding factors, we utilized a MR study design. Furthermore, we implemented rigorous measures to optimize the validity of our instrumental variables, thereby mitigating potential biases to the greatest extent possible. However, some sources of inaccuracy and bias may still exist. Unconsidered potential factors may still significantly impact the research outcomes. First, the generalizability of the study is limited by its predominant inclusion of individuals of European descent. Second, the existing eQTL and mQTL datasets lack information on genetic variation associated with gene expression or methylation levels in the X and Y chromosomes. In addition, in order to explore more potential relationships between PsA and CRGs as an exploratory study, we included the CRGs verified in only one cohort. In future studies, we plan to extend our research to more diverse racial and ethnic populations to address the limitation that the current data are predominantly derived from individuals of European descent.

Nevertheless, we provide a comprehensive catalog of potential therapeutic agents that merit further validation through experimental and population-based studies.

5 | Conclusion

Multi-omics studies have revealed altered expression of circadian clock genes and proteins, including HLA-DQB1, ITPR3, GABBR1, IL4, OIP5-AS1, GCKR, CSNK2B, and STAT3, which are associated with an increased risk of PsA. Notably, these genes and proteins also emerge as potential therapeutic targets. Furthermore, in silico drug prediction indicates a potential therapeutic prospect and significance.

Author Contributions

Zhen Qin, Aimei Liu, Yifei Wang, and Wuda Huoshen contributed to the conception and design of the study. Zhen Qin, Aimei Liu, and Wuda Huoshen conducted data analysis. Zhen Qin and Yifei Wang drafted the manuscript, and Aimei Liu performed editing. Sha Yi provided valuable guidance on writing style. All authors reviewed and approved the final manuscript.

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Ethics Statement

This study draws on published studies and coalitions that have made their publicly accessible summary statistics, which have obtained approval from their respective ethical review boards. So, a new ethical review and approval was not required in our study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets presented in this study can be found in online repositories. The names of the repositories can be found in the article or Supporting Information.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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ORIGINAL ARTICLE

Cognitive Functional Impairment Associated With Physical Function and Frailty in Older Patients With Rheumatoid Arthritis

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ABSTRACT

Introduction: Cognitive function is crucial for comprehending, adhering to, and terminating treatment regimens, and for maintaining quality of life in individuals with rheumatoid arthritis (RA). This study aimed to investigate the factors associated with cognitive functional impairment in Japanese patients with RA.

Methods: A total of 452 patients with RA, aged 65 years and older, were enrolled in this study. We examined cognitive functional impairment using the Dementia Assessment Sheet for Community-based Integrated Care System 8-items (DASC-8), frailty using the Kihon Checklist (KCL), and clinical data. We defined cognitive functional impairment as a DASC-8 score ≥ 11. Patients were categorized into three groups based on the total KCL scores: robust (0–3), pre-frailty (4–7), and frailty (≥ 8).

Results: The prevalence of cognitive functional impairment was 26.5%. Using multivariate logistic regression analysis, factors associated with cognitive functional impairment included age ($p = 0.046$), Health Assessment Questionnaire-Disability Index score ($p < 0.001$), and KCL score ($p < 0.001$). The frailty rates in the groups with and without cognitive functional impairment were 85.0% and 28.6%, respectively ($p < 0.001$).

Conclusion: The study findings suggest that cognitive functional impairment is more likely to occur with age, as aging is a factor associated with cognitive impairment. Our findings highlight the importance of assessing cognitive ability in older patients with RA, particularly the elderly, who should receive management for cognitive function alongside RA disease activity, physical function, and frailty in their treatment plan.

1 | Introduction

Cognitive functional impairment leads to disability and decreased quality of life (QOL) in older adults. The prevalence of dementia is increasing in the rapidly aging rural community population in Japan [1, 2].

Rheumatoid arthritis (RA) is a systemic autoimmune disease that causes joint damage and physical dysfunction [3]. Cognitive functional impairment is a comorbidity of RA. Previous studies have reported a 31%–72% prevalence of cognitive impairment in patients with RA [4, 5]. Its pathogenesis has been linked to age, inflammation, disease activity, chronic pain, and disease-modifying anti-rheumatic drugs (DMARDs) [6–10].

Cognitive functional impairment can significantly impact patients with RA, leading to reduced physical function, diminished quality of life, lower medication adherence, and deterioration of mental health [5, 11–14]. Therefore, it is crucial to understand cognitive functional impairment in patients with RA.

Based on a nationwide database, the age of RA onset in Japan has significantly increased over the last decade [15]. The mortality rate in patients with RA is gradually increasing [16, 17], along with the growing number of elderly patients affected by RA. Frailty is an aging state characterized by a reduced ability to recover from stress, resulting in increased vulnerability, and patients with RA are at a high risk of developing frailty [18–20].

We believe that cognitive function is important for the understanding, continuation, and discontinuation of treatment, as well as the maintenance of QOL in patients with RA. Therefore, in this study, we aimed to investigate factors associated with cognitive functional impairment in Japanese patients with RA.

2 | Patients and Methods

2.1 | Patients

A total of 452 patients with RA were included in this study. Patients aged 65 years and older, diagnosed with RA according to the 1987 American College of Rheumatology classification criteria [21] or the 2010 American College of Rheumatology/European League Against Rheumatism criteria, were included [22].

This study was approved by the Ethical Review Board of this institution (Approval number: TGE00888-064) and followed the guidelines put forth by the Declaration of Helsinki. All patients willingly agreed to participate in the study and provided written informed consent.

2.2 | Clinical Data Assessment

Demographic, clinical, and laboratory data included age, sex, body mass index, disease duration of RA, presence of anti-cyclic citrullinated peptide antibody (anti-CCP Ab); use of biological disease-modifying antirheumatic drugs (bDMARDs), targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs), methotrexate (MTX), or glucocorticoids (GCs); pain intensity of 0–100 mm using the visual analog scale (pain VAS), Clinical Disease Activity Index (CDAI) [23], Health Assessment Questionnaire-Disability Index (HAQ-DI) [24]; and the Kihon Checklist (KCL) score [25]. The KCL score comprises 25 questions under seven domains: activities of daily living (5), physical function (5), nutritional status (2), oral function (3), house boundedness (2), cognitive function (3), and depressive mood (5). Each question was scored from 0 to 1, and the total score ranged from 0 to 25. Based on the total KCL score, the patients were categorized into three groups: robust (scores of 0–3), prefrailty (scores of 4–7), and frailty (scores ≥ 8) [25].

2.3 | Cognitive Assessment

Cognitive assessment was conducted using the Dementia Assessment Sheet for the Community-based Integrated Care System 8-items (DASC-8), which evaluates memory (item 1), orientation (item 2), instrumental activities of daily living (IADL) (items 3–5), and basic ADL (BADL) (items 6–8). Each item was scored from 0 to 4 (total maximum score: 32). A higher score indicated greater cognitive functional impairment. A DASC-8 score of ≤ 10 indicated no cognitive functional impairment and ADL, while a score of ≥ 11 indicated mild to severe cognitive functional impairment or IADL impairment and impairment of BADL [26].

2.4 | Statistical Analysis

Statistical analysis was performed by comparing groups with and without cognitive functional impairment using analysis of variance. To identify the factors associated with cognitive functional impairment, the characteristics and clinical data of the two groups were compared using the Wilcoxon rank-sum test and Fisher's exact test (as appropriate). In the multivariate logistic regression analysis, the factors were selected based on their presumed relevance rather than solely on the *P*-value from the univariate analysis. The results of the logistic regression analysis were reported as odds ratios (OR) and 95% confidence intervals (CIs). The KCL score and HAQ-DI, CDAI and pain VAS were regarded as confounding factors and analyzed separately. The cutoff values of the HAQ-DI and KCL score in cognitive functional impairment were measured using the receiver operating characteristic (ROC) method with corresponding sensitivity and specificity and area under the curve (AUC). Multiple regression analysis was conducted using factors similar to those in the multivariate logistic regression analysis. Spearman's rank correlation analysis was performed to examine the relationship between the DASC-8 score and patient characteristics.

All analyses were performed using the R Statistical Package, version 3.3.2 (http://www.r-project.org/). A *p*-value of <0.05 was considered statistically significant.

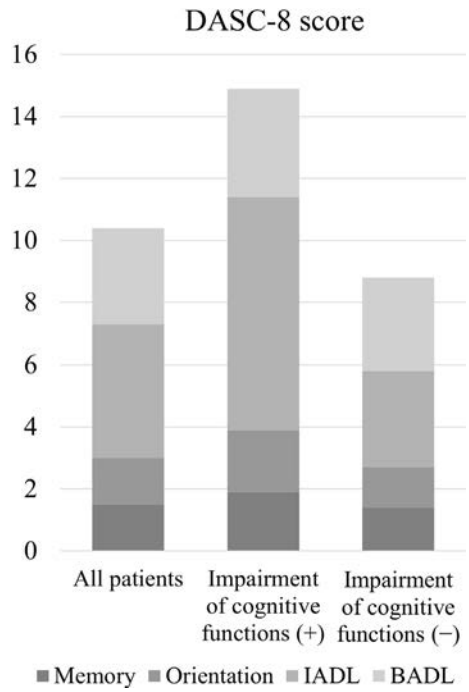

FIGURE 1 | Distribution of the DASC-8 scores.

3 | Results

In this study, the prevalence of cognitive functional impairment was 26.5%. The mean DASC-8 scores for all patients and for the groups with and without cognitive functional impairment were 10.4 ± 3.4, 14.8 ± 4.0, and 8.9 ± 0.8, respectively. The distribution of the DASC-8 scores is shown in Figure 1.

Table 1 shows the patient characteristics and clinical data of all patients and those with and without cognitive functional impairment. The variables were significantly different among the two groups were age, sex, duration of RA; b- or tsDMARD, MTX, and GC use; pain VAS, CDAI, HAQ-DI, and KCL scores using univariate analysis. Median age, HAQ-DI, and KCL score for patients with cognitive functional impairment were 79.5 years, 1, and 12, respectively, when compared to 74 years, 0, and 5, respectively, for those without impairment. Multivariate analysis identified several factors associated with cognitive functional impairment. In the model including the KCL score and CDAI, the significant factor was KCL score ($p < 0.001$; OR, 1.360; 95% CI, 1.263–1.464). In the model including the HAQ-DI and CDAI, the significant factors included age ($p < 0.001$; OR, 1.080; 95% CI, 1.037–1.125) and HAQ-DI ($p < 0.001$; OR, 7.061; 95% CI, 4.288–11.628) (Table 2). In the model including the KCL score and pain VAS, the significant factor was the KCL score ($p < 0.001$; OR, 1.360; 95% CI, 1.264–1.464). In the model including the HAQ-DI and pain VAS, the significant factors were age ($p < 0.001$; OR, 1.079; 95% CI, 1.036–1.124) and HAQ-DI ($p < 0.001$; OR, 6.401; 95% CI, 3.993–10.260) (Table S1). The cutoff values of HAQ-DI and KCL score in cognitive functional impairment calculated using the ROC method were 0.5 (sensitivity: 72.5%, specificity: 85.5%, AUC: 0.848) and 8 (sensitivity: 78.3%, specificity: 78.9%, AUC: 0.856), respectively. In addition, in patients with HAQ-DI ≤0.5, the factors associated with cognitive functional impairment included age ($p = 0.002$; OR, 1.098; 95% CI, 1.034–1.165) and KCL score ($p < 0.001$; OR, 1.255; 95% CI, 1.127–1.398) (Table S2).

TABLE 1 | Patient characteristics and clinical data.

Median (Q1, Q3) Mean ± SD	All ($n = 452$)	Impairment of cognitive functions (+) ($n = 120$)	Impairment of cognitive functions (−) ($n = 332$)	p
DASC-8 score	9 (8, 11)	13.5 (12, 17)	9 (8, 10)	< 0.001
	10.4 ± 3.4	14.8 ± 4.0	8.9 ± 0.8	
Memory (item 1), mean	1.5 ± 0.6	1.9 ± 0.8	1.4 ± 0.5	
Orientation (item 2), mean	1.5 ± 0.7	2.0 ± 0.8	1.3 ± 0.5	
IADL (items 3–5), mean	4.3 ± 2.4	7.5 ± 2.8	3.1 ± 0.4	
BADL (items 6–8), mean	3.1 ± 0.7	3.5 ± 1.3	3.0 ± 0.1	
Age, years	76 (71, 81) 75.5 ± 8.2	79.5 (75, 84) 79.5 ± 7.2	74 (69, 79) 74.1 ± 8.1	< 0.001
Female, *n* (%)	367 (81.2)	106 (88.3)	261 (78.6)	0.020
Body mass index	21.8 (19.8, 23.9) 22.1 ± 3.6	21.1 (19.3, 23.4) 21.6 ± 3.7	22.0 (19.9, 24.2) 22.3 ± 3.5	0.091
Duration of RA, years	14 (9, 22) 16.9 ± 11.6	18.5 (10.8, 29) 19.7 ± 12.8	14 (9, 20) 15.8 ± 10.9	0.001
Anti-CCP Ab positive, *n* (%)	343 (75.9)	95 (79.2)	248 (74.7)	0.384
b or tsDMARDs use, *n* (%)	281 (62.2)	92 (76.7)	189 (56.9)	< 0.001
MTX use, *n* (%)	255 (56.4)	49 (40.8)	206 (62.0)	< 0.001
Glucocorticoid use, *n* (%)	73 (16.2)	28 (23.3)	45 (13.6)	0.020
Pain VAS	19 (5, 46) 26.7 ± 26.1	32 (11, 57.3) 36.5 ± 28.3	14 (3, 34.8) 23.1 ± 24.4	< 0.001
CDAI	3.3 (1.0, 7.4) 4.6 ± 4.3	4.9 (2.4, 9.2) 6.0 ± 4.6	2.8 (0.7, 6.7) 4.0 ± 4.0	< 0.001
HAQ-DI	0.15 (0, 0.75) 0.5 ± 0.7	1 (0.5, 1.8) 1.2 ± 0.8	0 (0, 0.38) 0.3 ± 0.4	< 0.001
KCL score	7 (4, 10) 7.2 ± 4.7	12 (9, 15) 11.7 ± 4.3	5 (3, 8) 5.6 ± 3.7	< 0.001

Abbreviations: anti-CCP Ab, anti-cyclic citrullinated peptide antibody; bDMARDs, biological disease-modifying antirheumatic drugs; CDAI, Clinical Disease Activity Index; DASC-8, Dementia Assessment Sheet for Community-based Inte grated Care System 8-items; HAQ-DI, Health Assessment Questionnaire Disability Index; KCL, Kihon Checklist; MTX, methotrexate; Q1, 25% quantile; Q3, 75% quantile; RA, rheumatoid arthritis; SD, standard deviation; tsDMARDs, targeted synthetic disease-modifying antirheumatic drugs; VAS, visual analog scale.

TABLE 2 | Factors associated with cognitive functional impairment in the model incorporating (a) KCL score and CDAI, and (b) HAQ-DI and CDAI.

(a)	*p*	Odds ratio (95% CI)
Age	0.054	1.041 (0.999–1.084)
Sex	0.480	1.323 (0.609–2.874)
Body mass index	0.627	0.982 (0.911–1.058)
Duration of RA	0.225	1.014 (0.992–1.036)
b or tsDMARDs use	0.200	1.495 (0.809–2.763)
MTX use	0.290	0.738 (0.420–1.296)
Glucocorticoid use	0.395	1.343 (0.681–2.647)
CDAI	0.263	1.037 (0.973–1.104)
KCL score	<0.001	1.360 (1.263–1.464)
(b)	***p***	**Odds ratio (95% CI)**
Age	<0.001	1.080 (1.037–1.125)
Sex	0.448	1.344 (0.626–2.886)
Body mass index	0.670	0.983 (0.909–1.063)
Duration of RA	0.732	0.996 (0.973–1.019)
b or tsDMARDs use	0.119	1.634 (0.881–3.033)
MTX use	0.764	0.917 (0.522–1.611)
Glucocorticoid use	0.606	1.201 (0.600–2.403)
CDAI	0.504	0.977 (0.911–1.047)
HAQ-DI	<0.001	7.061 (4.288–11.628)

Abbreviations: bDMARDs, biological disease-modifying antirheumatic drugs; CDAI, Clinical Disease Activity Index; CI, Confidence Interval; HAQ-DI, Health Assessment Questionnaire Disability Index; KCL, Kihon Checklist; MTX, methotrexate; RA, rheumatoid arthritis; tsDMARDs, targeted synthetic disease-modifying antirheumatic drugs.

In the multiple regression analysis, the model including the KCL score and CDAI, identified the KCL score as a significant factor ($p < 0.001$). In the model with the HAQ-DI score and CDAI, significant factors included age ($p < 0.001$) and HAQ-DI ($p < 0.001$) (Table 3). The model incorporating the KCL score and pain VAS identified the KCL score as a significant factor ($p < 0.001$). In the model with the HAQ-DI score and pain VAS, significant factors included age ($p < 0.001$) and HAQ-DI ($p < 0.001$) (Table S3).

The correlation coefficients between the DASC-8 score and age, HAQ-DI, and KCL scores were 0.306 ($p < 0.001$), 0.637 ($p < 0.001$), and 0.647 ($p < 0.001$), respectively (Table 4).

The frailty rates in the groups with and without cognitive functional impairment in all patients were 85.0% and 28.6%, respectively ($p < 0.001$). The rates of frailty and pre-frailty in the groups with and without cognitive functional impairment in all patients were 95.8% and 68.1%, respectively ($p < 0.001$) (Figure 2a). The frailty rates in the groups with and without cognitive functional impairment in patients aged ≥75 years were 86.2% and 39.5%, respectively (Figure 2b). The frailty rates in the groups with and without cognitive functional impairment in patients aged <75 years were 80.8% and 18.2%, respectively (Figure 2c).

4 | Discussion

In this study, we investigated the association between cognitive functional impairment and RA. Patients with RA and impaired cognitive functions tended to be older, were mostly female, had a longer duration of RA, and were being administered b- or ts-DMARDs, MTX, and GCs. Additionally, these patients had higher pain VAS, CDAI, HAQ-DI, and KCL scores. Our results showed that age, HAQ-DI, and KCL scores were associated with cognitive functional impairment.

Factors associated with cognitive functional impairment using the Montreal Cognitive Assessment (MoCA) in patients with RA were age, female sex, education level, and disease activity of RA [5, 7]. A previous study involving patients (aged 67.6 ± 8.1 years who had a DAS28-ESR score of 3.5 ± 1.5) showed a rate of 69.6% cognitive functional impairment [5], whereas another study reported a 72% cognitive functional impairment rate in those aged 59.2 ± 11.4 years and a DAS28 score of 3.5 ± 0.8 [7]. Old age emerged as a common factor associated with cognitive functional impairment, both in the present study and in previous studies. The rates of increase of dementia in Japan were 4.9% (mean age, 74.1 ± 6.9 years) in 1997, 7.1% (mean age, 74.1 ± 6.9 years) in 2004, 9.2% (mean age, 78.0 ± 7.1 years) in 2012, and 16.3% (mean age, 77.1 ± 7.8 years) in 2016 in the general population aged 65 years and older [27]. Our results, consistent with those of earlier studies, indicate that the rate of cognitive functional impairment in patients with RA was higher than that in the general population. Our analysis of the correlation between the DASC-8 score and patient characteristics identified CDAI through multiple regression analysis. This suggests that CDAI may influence the DASC-8 score. Although age has been consistently linked to cognitive functional impairment, our findings suggest that maintaining a suppressed state of RA disease activity in the patients may play a role in mitigating cognitive functional impairment.

As observed in a systematic review, several previous studies showed a positive correlation between physical activity and the maintenance of cognitive function [28]. In another assessment, cognitive function scores using a battery of 12 standardized neuropsychological measures were significantly associated with HAQ-DI in patients with RA [29]. In this study, the HAQ-DI was independently associated with cognitive functional impairment. These results are similar to those of previous studies on the general population [30, 31]. We believe that cognitive function and physical function impairments are related in patients with RA, highlighting the importance of managing both aspects in treating RA. Our findings suggest that, to minimize cognitive functional impairment, the HAQ-DI should be maintained at 0.5 or below. The DASC-8 consists of memory, orientation, IADL, and BADL. As shown in Figure 1, the rates of BADL scores among the DASC-8 scores in the groups with and without cognitive functional impairment were 23.5% and 33.7%, respectively. The DASC-8 may be overestimated by physical dysfunction because it also reflects the impact of BADL. Cognitive functional impairment using the Mini-Mental Status Examination (MMSE) and physical frailty using the modified Fried Frailty Index are more likely to occur in patients with RA [32]. In previous reports, factors associated with frailty using the KCL score included age and disease duration of RA [19]. Age and RA disease activity

TABLE 3 | Multiple regression analysis in the model incorporating (a) KCL score and CDAI, and (b) HAQ-DI and CDAI.

(a)	Unstandardized regression coefficient (95% CI)	Standardized regression coefficient	*p*
Age	0.004 (−0.001–0.009)	0.074	0.088
Sex	0.027 (−0.062–0.116)	0.024	0.552
Body mass index	−0.002 (−0.012–0.007)	−0.019	0.633
Duration of RA	0.002 (−0.001–0.005)	0.050	0.209
b or tsDMARDs use	0.046 (−0.028–0.120)	0.051	0.219
MTX use	−0.057 (−0.128–0.014)	−0.064	0.118
Glucocorticoid use	0.046 (−0.047–0.138)	0.038	0.330
CDAI	0.003 (−0.005–0.012)	0.031	0.455
KCL score	0.047 (0.038–0.055)	0.497	<0.001
(b)	**Unstandardized regression coefficient (95% CI)**	**Standardized regression coefficient**	***p***
Age	0.008 (0.004–0.012)	0.147	<0.001
Sex	0.036 (−0.052–0.124)	0.032	0.422
Body mass index	−0.002 (−0.011–0.008)	−0.016	0.686
Duration of RA	−0.001 (−0.003–0.003)	−4.254e−03	0.916
b or tsDMARDs use	0.063 (−0.010–0.136)	0.070	0.089
MTX use	−0.023 (−0.094–0.048)	−0.026	0.524
Glucocorticoid use	0.021 (−0.071–0.113)	0.017	0.658
CDAI	−0.003 (−0.012–0.006)	−0.029	0.495
HAQ-DI	0.331 (0.275–0.387)	0.522	<0.001

Abbreviations: bDMARDs, biological disease-modifying antirheumatic drugs; CDAI, Clinical Disease Activity Index; CI, Confidence Interval; HAQ-DI, Health Assessment Questionnaire Disability Index; KCL, Kihon Checklist; MTX, methotrexate; multiple, multiple regression analysis; RA, rheumatoid arthritis; tsDMARDs, targeted synthetic disease-modifying antirheumatic drugs.

TABLE 4 | Correlation and multiple regression analyses between the DASC-8 score and patient characteristics.

	Correlation coefficient (95% CI)	*p* (correlation)
Age	0.306 (0.220–0.387)	<0.001
Body mass index	−0.057 (−0.149–0.352)	0.225
Duration of RA	0.111 (0.019–0.201)	0.018
Pain VAS	0.201 (0.111–0.288)	<0.001
CDAI	0.173 (0.083–0.262)	<0.001
HAQ-DI	0.637 (0.579–0.689)	<0.001
KCL score	0.647 (0.590–0.698)	<0.001

Note: Standardized regression coefficient is denoted by floating point expression.
Abbreviations: CDAI, Clinical Disease Activity Index; CI, Confidence Interval; DASC-8, Dementia Assessment Sheet for Community-based Inte grated Care System 8-items; HAQ-DI, Health Assessment Questionnaire Disability Index; KCL, Kihon Checklist; multiple, multiple regression analysis; RA, rheumatoid arthritis; VAS, visual analog scale.

were independently associated with frailty using the Survey of Health, Aging and Retirement in Europe Frailty Instrument in RA [33]. Moreover, physical function was associated with frailty status using the method developed by Fried et al. (Fried phenotype) [34] among adults with RA [35]. In Japanese community-dwelling older people, the proportion of robust, prefrail, and frail was 30.3%, 59.8%, and 9.9% in men and 25.3%, 64.7%, and 10.0% in women, respectively, according to the Fried phenotype. The proportion of frail people as defined by KCL ≥ 7 was 30.8% in men and 33.3% in women [36]. As the diagnosis of frailty by KCL score in this study is ≥ 8, the difference in the percentage of frailty may be minimal. This study demonstrated that cognitive functional impairment was associated with the KCL score in patients with RA. Among participants aged 65 years and older for whom the Cognitive Abilities Screening Instrument was used to diagnose dementia, the rates of frailty and pre-frailty, using the method developed by Fried et al. [34], were 54.6% and 26.8%, respectively [37]. In community-dwelling older adults with mild cognitive impairment using the MMSE, the rate of pre-frailty using the revised Japanese version of the Cardiovascular Health Study (J-CHS) was 51.2%, and physical functions such as knee extension strength and timed up and go test were declining [38]. The cutoff value of frailty defined by the J-CHS was 7 (sensitivity: 76.2%, specificity: 79.9%, AUC: 0.861) in Japanese older adults [39]. Given that a KCL score of 8 or higher indicates frailty, the diagnosis of frailty using KCL may align closely with that of J-CHS. In the present study, the frailty rate in the group

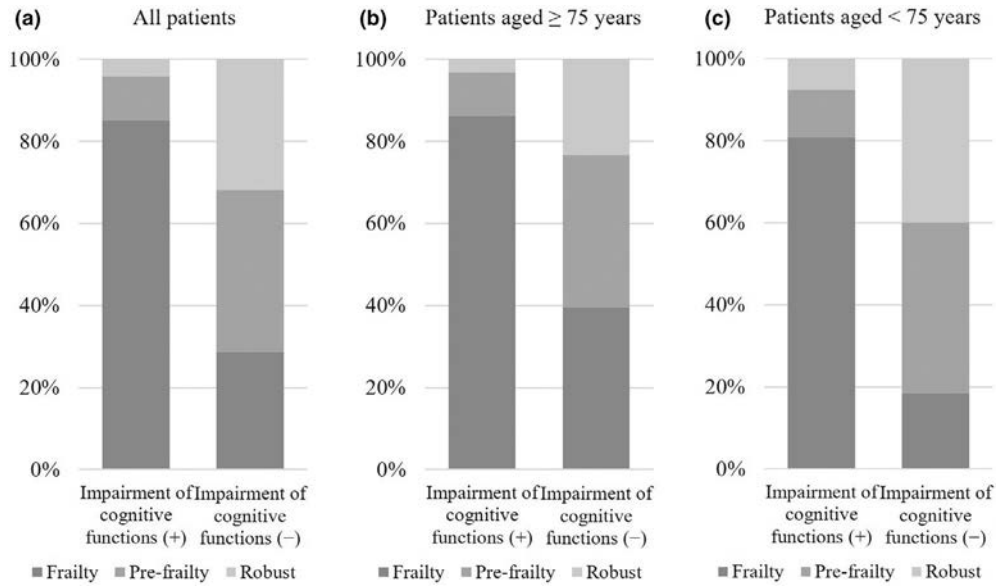

FIGURE 2 | Proportion of frailty and pre-frailty in (a) all patients and patients aged (b) ≥ 75 years and (c) < 75 years with and without cognitive functional impairment.

with cognitive functional impairment was 85.0%. Patients with RA and impaired cognitive functions are more likely to develop frailty. In this study, we found that cognitive functional impairment and frailty were related. We suggest that maintaining a KCL score of 8 or under may help in preventing cognitive functional impairment in patients with RA.

Our multivariate analysis showed that, although RA treatment medications were not identified as a significant factors for cognitive functional impairment, patients with cognitive impairment had higher rates of biologic or targeted synthetic DMARDs (b/ts DMARDs) and glucocorticoid use, and lower rates of methotrexate (MTX) use when compared to those without cognitive impairment. We believe that this trend reflects the association between the older age of patients and cognitive functional impairment. Furthermore, kidney function, as indicated by the estimated glomerular filtration rate of < 60 mL/min/1.73m^2, was identified in 53.3% and 39.5% of patients without and with cognitive functional impairment, respectively. Kidney function may have influenced drug selection. In a previous report from the United Kingdom, similar to our results, RA treatment drugs were not associated with cognitive functional impairment, as assessed by MoCA, in patients with RA [5]. In contrast, the use of tumor necrosis factor inhibitors was associated with a reduced risk of dementia (Hazard ratio, 0.64; 95% CI, 0.52–0.80) [40]. Long-term use of MTX for > 4 years gradually decreased dementia risk in patients with RA (OR, 0.37; 95% CI, 0.17–0.79) [41]. Further studies are required to clarify the relationship between cognitive functional impairment and drugs for RA treatment.

In patients with cognitive functional impairment, medication adherence is a key concern. In this study, 22 out of 44 patients receiving subcutaneous bDMARDs were self-injecting. We believe that injection use did not impact our findings, as family members or hospital staff administered injections for the patients who were unable to self-inject the bDMARDs.

This study has several limitations. First, in a previous report, cognitive functional impairment was associated with low education and income in patients with RA. However, these variables could not be evaluated in the present study. Second, owing to the cross-sectional design of our study, we could not conclude that a causal association exists between cognitive functional impairment and the factors associated with cognitive functional impairment. Accordingly, a prospective study is required to clarify whether cognitive functional impairment predicts the effects on HAQ-DI and KCL scores. Third, the patients included in this study had a prolonged duration of RA and displayed suppressed RA disease activity, as determined using CDAI. Therefore, our results may have been influenced by the clinical profiles of the patients. Finally, we used the DASC-8 to assess cognitive functional impairment. Different methods of dementia assessment may have altered the results. However, the DASC-8 highly correlated with the DASC-21 [26]. We believe that the DASC-8 is simple, easy for medical staff to use, and useful in clinical practice.

In conclusion, our results suggest that cognitive functional impairment is more likely to occur with age, as aging is an associated factor in patients with RA with prolonged duration and stable disease activity of RA. Our findings highlight the importance of investigating cognitive ability in older patients with RA, especially in elderly patients, in whom cognitive function, in addition to disease activity of RA, physical function, and frailty for treating RA, must be managed with reference to the cutoff values of HAQ-DI and KCL score obtained in this study.

Author Contributions

Study concept and design: T.M., N.O., and R.H. Acquisition of subjects and data: T.M., N.O., and R.H. Analysis and interpretation of data: K.Y. and K.I. Preparation of manuscript: T.M., K.I., and K.O.

Conflicts of Interest

Takeshi Mochizuki received honoraria for lectures from AbbVie, Astellas, Bristol-Myers, Chugai, Daiichi Sankyo, Eisai, Eli Lilly, Janssen, Mochida, Pfizer, and Teijin. Koichiro Yano received honoraria for lectures from AbbVie, Astellas, Ayumi, Bristol-Myers, Eisai, Hisamitsu, Mochida, and Takeda. Katsunori Ikari received honoraria for lectures from AbbVie, Astellas, Bristol-Myers, Chugai, Eisai, Eli Lilly, Janssen, Takeda, Tanabe-Mitsubishi, and UCB. The other authors declare that they have no conflicts of interest. The sponsors were not involved in the study design; collection, analysis, and interpretation of data; writing of the article; and/or decision to submit the results for publication.

Data Availability Statement

Research data are not shared.

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LETTER TO THE EDITOR

Rheumatoid Factor Predicts Long-Term Retention Associated With Effectiveness of Certolizumab Pegol in Patients With Rheumatoid Arthritis: A Two-Center Retrospective Study

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Dear Editor,

Rheumatoid arthritis (RA) is a systemic autoimmune disorder characterized by persistent synovitis in multiple joints, resulting in bone and cartilage destruction [1]. The widespread introduction of biological/targeted synthetic disease-modifying antirheumatic drugs (b/tsDMARDs) has resulted in substantial improvements in disease activity and inhibition of joint destruction. Certolizumab pegol (CZP) is a fragment crystallizable (Fc)-free, polyethyleneglycolylated, humanized Fab fragment targeting tumor necrosis factor-alpha (TNF-α). So far, several observational studies have reported survival rates of CZP of ≥2 years in real-world settings mainly in European countries [2–4]; however, data on long-term persistence focusing on only CZP in the Japanese population is limited. Furthermore, recent studies in Spain have indicated that clinical responses and serum drug levels are not significantly influenced by high rheumatoid factor (RF) levels in patients treated with CZP, in contrast to other TNFis with the Fc region [5, 6]. Nevertheless, there is a paucity of research on the effect of RF titers on long-term retention associated with the effectiveness of CZP in Japanese patients with RA in clinical practice.

In the current study, we aimed to clarify whether RF titers predict the long-term continuation related to the effectiveness of CZP by evaluating real-world data from Japanese patients with RA.

This retrospective observational study was conducted at two tertiary referral centers in Japan: the Tohoku University and Osaki Citizen Hospitals. Consecutive patients aged ≥18 years who were diagnosed with RA according to the 2010 classification criteria [7] and initiated CZP treatment between March 2013 and September 2021 were enrolled in the study. These patients were followed-up until June 2022 or CZP was discontinued. The primary objective was to determine whether RF influenced the continuation related to the effectiveness of CZP. This study was conducted in compliance with the Declaration of Helsinki and was approved by the Ethics Committees of the respective institutions.

Baseline demographics and clinical information of patients with RA initiating CZP were retrospectively collected from electronic medical records: age, sex, disease duration, positivity and titers of anti-citrullinated protein antibody (ACPA) and RF, frequency and dose of concomitant methotrexate (MTX) and oral glucocorticoids (GCs) equivalent to prednisolone (PSL), number and class of b/tsDMARDs administered before starting CZP, values of C-reactive protein (CRP), tender joint count, swollen joint count, duration of CZP

administration, proportion of CZP initial loading, and reasons for CZP discontinuation.

Clinical responses including effectiveness of b/tsDMARDs were assessed according to the previously described report [8]. Retention duration was defined as the period from CZP administration to withdrawal for non-response to CZP in effective analysis population, and drug survival was thus considered to be a surrogate marker representative of clinical response to CZP [8].

Continuous variables expressed as medians with interquartile ranges (IQR) were compared using the Mann–Whitney U test. Categorical variables expressed as numbers (%) were compared using the Fisher's exact test. Kaplan–Meier curves were constructed to calculate retention rates of CZP associated with drug effectiveness, and the log-rank test was used to compare the curves. The optimal cutoff value for RF titers to differentiate between the retention and discontinuation groups was determined using receiver operating characteristic (ROC) curve analysis. Cox proportional hazards models were used to determine the predictors of CZP discontinuation owing to ineffectiveness. Variables with $p < 0.1$ by univariate Cox regression analysis and potential confounders were applied to the multivariate model. The adjusted hazard ratio (HR) with a 95% confidence interval (CI) was calculated. All statistical analyses were performed using JMP version 10.2 Software (SAS Institute, Japan) and GraphPad Prism 7.03 (GraphPad Software, La Jolla, CA, USA). Statistical significance was set at $p < 0.05$.

Of the 107 patients, 9 patients had insufficient available data, and 12 patients completely discontinued CZP treatment due to reasons other than treatment response during the observational period (median: 56.0 months, IQR: 36.0–76.0 months). After excluding the 12 patients, the effectiveness analysis population was identified ($n = 86$). As shown in Table 1, the majority of patients were females (87.8%). The median age at CZP initiation and disease duration were 56.0 (42.5–64.0) and 5.0 (2.0–8.2) years, respectively. Seventy-four (75.5%) patients had positive RF with median titers of 137.7 (50.8–334.5) IU/mL. MTX was simultaneously administered to 60 (61.2%) patients at a median dose of 8.0 (8.0–10.0) mg/week. CZP was introduced as first-line biologics in 43 (43.9%) patients. After dividing the effective analysis population into continuation and discontinuation groups, we assessed the differences in clinical variables between the 2 groups. The continuation group had significantly lower RF titers and higher proportion of b/tsDMARDs-naïve status than the discontinuation group. ACPA titers were also lower in patients who continued CZP, but did not reach statistical significance.

As shown in Table S1, the multivariate model demonstrated b/tsDMARD-naive as the most influential factor related to CZP discontinuation due to ineffectiveness (HR: 0.46, 95% CI: 0.22–0.90, $p = 0.024$). Retention rates in the b/tsDMARD-naïve groups were significantly higher than those in the b/tsDMARD-switch groups (Figure 1A). Next, the Cox regression analysis was performed to explore the predictive factors for effectiveness leading to CZP continuation in patients with a b/tsDMARD-naïve background, revealing that the RF value was the strongest predictor of discontinuation of CZP (HR per 100 IU/mL increment: 1.25, 95% CI: 1.06–1.60, $p = 0.009$) (Table S2).

An ROC curve analysis was performed to determine the optimal cut-off value of RF titers, which was determined to be 79.9 IU/mL (area under the curve, 0.76; $p = 0.003$). We then calculated retention rates stratified by RF titers of 79.9 IU/mL. As shown in Figure 1B, ≥ 79.9 IU/mL groups demonstrated significantly lower retention rate of CZP compared with < 79.9 IU/mL groups in the b/tsDMARDs-naïve population, indicating the highest retention rate in the b/tsDMARD-naive profile with RF titers of < 79.9 IU/mL. ACPA titers had no significant effect on CZP continuation (Figure S1).

This study investigated the long-term persistence related to the effectiveness of CZP in a real-world setting among Japanese patients with RA. No history of molecular-targeted therapies before CZP initiation was the strongest contributor to the increasing retention rate of CZP. Particularly, in the b/tsDMARD-naïve patients, the increase in RF titers was the significant predictor of the reduced retention rate of CZP, with RF titers of ≥ 79.9 IU/mL significantly decreasing the probability of continuation of CZP compared to that of < 79.9 IU/mL.

Our multivariate analysis identified b/tDMARD-naïve status as an independent predictor, and among the b/tsDMARD-naïve patients, the majority (> 65%) showed sustained retention of > 5 years. Previous studies also demonstrated that biologics-naïve predicted significantly better outcomes of CZP in the European population [2, 3, 9]. In one study [3], the retention curve in biologics-naïve patients was almost similar to our results; however, in other studies [2, 9], retention rates of 2 years after CZP initiation as the first biologic ranged between 40.1%–60%, which was lower than that in the present study. This discrepancy is likely because retention rates in a real-world setting are affected by various factors, including adverse events, disease remission, patient preferences, and drug effectiveness. Nevertheless, one of the conditions suitable for implementing CZP is biologic-naïve, as with other TNFis [10].

RF has not been consistently shown to be predictive of clinical responses to TNFi in previous studies, in which CZP was not evaluated [11, 12]; however, in the present study, RF titers elevation significantly correlated with the discontinuation of CZP in b/tsDMARDs-naive population. Recent accumulating evidence suggests that CZP exerts better clinical outcomes than other TNFi in populations with high RF titers [5, 6, 13]. Although post hoc analysis from six clinical trials focusing only on CZP demonstrated steady efficacy across baseline RF quartiles, remission rates at week 24 were likely to be numerically lower in early and established RA patients with the highest RF than in those with lower RF levels [14]. Moreover, in an observational study, when limited to b/tsDMARD-naïve status, the median time to discontinuation of CZP was shorter in higher RF groups than that in lower RF groups (2.5 vs. 7.5 years, respectively) [5], which is consistent with our results. Furthermore, although ACPA is closely associated with RF and is a well-established marker of disease severity [15], our results, showing no correlation between ACPA and long-term retention of CZP, are consistent with a post hoc analysis of the EXXELERATE study suggesting that ACPA does not play a direct role in modulating treatment response to CZP [16]. Therefore, RF titers may be a more clinically relevant in the long-term retention associated with the effectiveness of CZP than ACPA titers. Collectively, these findings suggest that

TABLE 1 | Baseline demographics and clinical characteristics of 98 patients with rheumatoid arthritis who were administered CZP.

		Effective analysis population ($n=86$)		
	Total analysis population ($n=98$)	Continuation ($n=45$)	Discontinuation ($n=41$)	p*
Age at CZP initiation, years	56.0 (42.5–64.0)	56.0 (41.0–61.5)	55.0 (40.0–65.5)	0.39
Female, *n* (%)	86 (87.8%)	39 (86.7%)	36 (87.8%)	>0.99
Disease duration, years	5.0 (2.0–8.2)	4.0 (1.7–6.8)	5.0 (2.0–8.8)	0.34
ACPA positive, *n* (%)	73 (74.5%)	32 (71.1%)	30 (73.2%)	>0.99
ACPA titer, U/mL	115.0 (44.6–500.0)	105.6 (35.7–428.9)	241.2 (49.4–668.9)	0.21
RF positive, *n* (%)	74 (75.5%)	33 (73.3%)	32 (78.1%)	0.14
RF titer, IU/mL	137.7 (50.8–334.5)	84.0 (43.0–207.0)	206.6 (95.3–439.5)	0.004
MTX use, *n* (%)	60 (61.2%)	32 (71.1%)	21 (51.2%)	0.077
MTX dose, mg/week	8.0 (8.0–10.0)	10.0 (8.0–12.0)	8.0 (7.0–10.0)	0.22
PSL use, *n* (%)	43 (43.9%)	19 (42.2%)	20 (48.8%)	0.67
PSL dose, mg/day	5.0 (2.6–7.8)	5.0 (2.5–5.0)	5.5 (3.1–8.0)	0.17
Initial loading, *n* (%)	73 (74.5%)	37 (82.2%)	27 (65.9%)	0.091
Number of b/tsDMARDs used prior to CZP				
0, *n* (%)	43 (43.9%)	28 (62.2%)	12 (29.3%)	0.003
1, *n* (%)	31 (31.6%)	11 (24.4%)	13 (31.7%)	0.48
≥2, *n* (%)	24 (24.5%)	6 (13.3%)	16 (39.0%)	0.012
Class of b/tsDMARDs used prior to CZP				
TNFi, *n* (%)	48 (50.0%)	14 (31.1%)	27 (65.9%)	0.002
IL-6Ri, *n* (%)	18 (18.4%)	4 (8.9%)	13 (31.7%)	0.013
CTLA4-Ig, *n* (%)	12 (12.2%)	3 (6.7%)	7 (17.1%)	0.18
JAKi, *n* (%)	2 (2.0%)	0 (0.0%)	2 (4.9%)	0.22
CRP, mg/dL	1.2 (0.4–3.0)	0.91 (0.4–3.2)	1.4 (0.2–2.9)	0.54
Tender joint count	3.0 (2.0–6.0)	3.0 (1.0–5.8)	4.0 (1.5–7.5)	0.11
Swollen joint count	3.0 (1.0–6.0)	3.0 (1.0–6.0)	3.0 (2.0–8.0)	0.12

Note: Continuous variables presented as median (interquartile range) are compared using the Mann–Whitney *U* test. Categorical variables presented as frequencies number (%) are the Fisher's exact test.

Abbreviations: ACPA, anti-citrullinated protein antibody; b/tsDMARDs, biological/targeted synthetic disease-modifying antirheumatic drugs; CRP, C-reactive protein; CTLA4-Ig, cytotoxic T lymphocyte-associated antigen-4-Ig; CZP, certolizumab pegol; IL-6Ri, interleukin-6 receptor inhibitor; JAKi, Jak kinase inhibitor; MTX, methotrexate; PSL, prednisolone; RF, rheumatoid factor; TNFi, tumor necrosis factor inhibitor.

*The continuation group versus the discontinuation group by the Mann-Whitney U test or the Fisher's exact test, as appropriate.

elevated RF levels could attenuate the effectiveness of CZP, but to a lesser extent than its effect on other TNFis, regardless of ACPA levels.

Because the effect of autoantibodies on the pathogenesis of RA is an area under intensive study [17], it is valuable to speculate on the mechanisms by which RF affects the therapeutic effect of CZP. This may be explained partly by the involvement of the type I interferon (IFN-I) pathway. The expression of IFN-I signaling-related genes before and 3 months after the initiation of TNFi was significantly higher in non-responders than that in responders, respectively [18]. Furthermore, a strong positive correlation between serum IFN-α concentrations and RF titers was observed in early RA [19]. Importantly, the concept of cross-regulation between IFN-I and TNF-α in pathological conditions or in the course of disease progression has been currently proposed [20]. Taken together, elevated RF levels, potentially indicating an immune state with a pathophysiological predominance of IFN-I signaling over TNF pathways, might result in some degree of resistance to TNF inhibition.

This study has several limitations. First, this study was retrospective, and the sample size was small. Therefore, undetected confounding factors might have influenced the results, and we cannot exclude the possibility that it was underpowered to detect statistical differences, especially in the sub-analyses. Second,

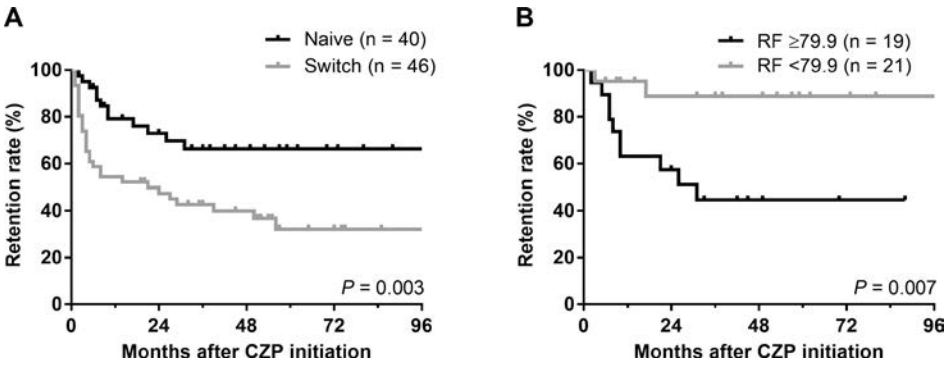

FIGURE 1 | Kaplan–Meier curves for retention rates of CZP based on (A) a history of b/tsDMARDs prior to CZP initiation in the effective analysis population and (B) RF titers of 79.9 IU/mL in the b/tsDMARD-naïve group. *p*-values were calculated using the Log-rank test. CZP, certolizumab pegol; b/tsDMARDs, biological/targeted synthetic disease-modifying antirheumatic drugs; RF, rheumatoid factor.

we did not evaluate analgesics, intra-articular injection of GCs, csDMARDs other than MTX, or adjustments in both PSL and DMRADs doses during the observational periods. Third, only two patients who received JAKi were included. Further studies are warranted to assess whether previous JAK inhibition affects subsequent treatment response to CZP. Nevertheless, this study is the first to identify the distinct determinants of sustained effectiveness of CZP based on the presence or absence of previously utilized b/tsDMARDs. These findings may help select patients suitable for CZP, resulting in personalized therapeutic strategies for RA.

In conclusion, our study highlights key factors influencing long-term retention related to clinical response to CZP in treating RA. These results indicate that the therapeutic effect of CZP is highest in patients with no history of prior molecular-targeted therapies and lower RF levels, irrespective of ACPA levels. The findings emphasize that selecting patients based on their treatment history and RF levels can improve disease outcomes, supporting the potential of personalized treatment strategies for RA.

Author Contributions

T.M. conceived the study design. T.M. and S.O. contributed to the acquisition, analysis, or interpretation of data. T.M. drafted the manuscript. All authors critically reviewed the manuscript and approved the submitted manuscript.

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Ethics Statement

This study design was approved by the Ethics Committee of Osaki Citizen Hospital (reference number: 20230313–36) and Tohoku University Graduate School of Medicine (reference number: 2023–1-047). Written informed consent was waived due to the retrospective nature of the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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LETTER TO THE EDITOR

Mechanistic Insights From A Case of Giant Cell Arteritis Polymyalgia Rheumatica Spectrum Disease

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Dear Editor,

We report a 61-year-old male with severe, treatment-refractory polymyalgia rheumatica (PMR) and, subsequently, large vessel-giant cell arteritis (LV-GCA) in the setting of multiple sclerosis (MS) treatment with daclizumab, a monoclonal antibody targeting CD25 now withdrawn from market due to immune-related adverse events (IRAEs) [1].

The patient presented in September 2017 with shoulder and hip girdle pain and stiffness, and elevated CRP (40.6 mg/L) and ESR (110 mm/h). Rheumatoid factor (RhF) and anti-cyclic citrullinated peptide (anti-CCP) antibody were negative, satisfying the 2012 EULAR/ACR Classification Criteria for PMR [2]. Daclizumab therapy had been given for 5 months prior (150 mg SC monthly). ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) confirmed a PMR diagnosis (Figure 1A), without evidence of vasculitis.

Treatment with prednisolone 15 mg daily was initiated without effect. The dose was escalated to 30 mg daily and daclizumab ceased within 2 weeks of initial presentation. Ocrelizumab, an anti-CD20 monoclonal antibody, was started for MS, while leflunomide (20 mg daily) was introduced as a steroid-sparing agent for PMR, given the efficacy of the active metabolite teriflunomide in MS [3], which the patient had previously trialed and tolerated. Despite this and confirmed B-cell depletion, clinical features and raised inflammatory markers persisted. Repeat PET/CT in October 2018 demonstrated ongoing PMR (Figure 1B). Leflunomide was ceased and methotrexate commenced (20 mg weekly). Over the ensuing period, ocrelizumab infusions continued and hydroxychloroquine (400 mg daily) was also trialed, acknowledging a lack of substantive evidence for its use in PMR [4]. Despite this, prednisolone dosing remained ≥ 7 mg daily due to persistent PMR symptoms (pain and stiffness at the shoulder and hip girdle).

Four years after presentation, the patient developed night sweats and weight loss suspicious for LV-GCA. There were no cranial features. PET/CT once again confirmed active, albeit less ^{18}F-FDG avid PMR consistent with the patient's ongoing musculoskeletal symptoms, along with new abnormal uptake in the subclavian arteries consistent with large vessel vasculitis (Figure 1C). Methotrexate and hydroxychloroquine were discontinued, while prednisolone was escalated, and tocilizumab (162 mg SC weekly) commenced. Repeat imaging 5 months later revealed improved vascular ^{18}F-FDG uptake (Figure 1D).

In February 2023, while taking prednisone 7 mg daily, together with weekly tocilizumab and 6-monthly ocrelizumab (both of which have established efficacy in rheumatoid arthritis) [5, 6], new bilateral wrist/hand symptoms developed. RhF and anti-CCP remained negative, while plain x-rays demonstrated degenerative findings. Contrast-enhanced magnetic resonance imaging later confirmed left wrist and second metacarpophalangeal joint synovitis. Tocilizumab was switched to abatacept, achieving clinical improvement in peripheral arthritis. On latest PET/CT (March 2024), vascular ^{18}F-FDG uptake had resolved completely but mild PMR remained (Figure 1E). Fortunately, no infectious or other complications related to dual biologic therapy have emerged to date.

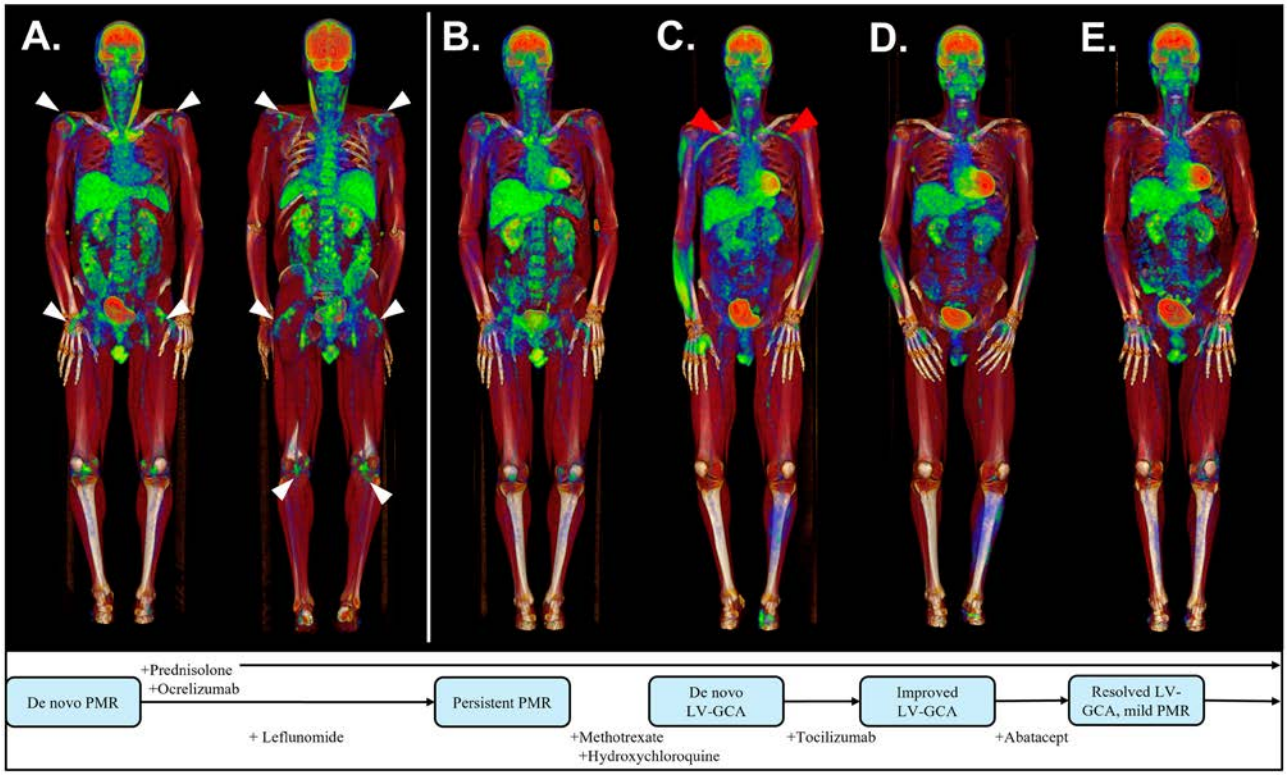

FIGURE 1 | Serial ^{18}F-FDG whole body PET/CT imaging: (A) Baseline at PMR diagnosis, with white arrows indicating characteristic abnormalities at shoulders, hips and knees (September 2017); (B) persistent PMR activity (October 2018); (C) interval development of LV-GCA indicated by red arrows (January 2022); (D) improvement in LV-GCA post tocilizumab (July 2022); (E) resolution of LV-GCA following treatment with abatacept, although mild PMR persists (March 2024).

Giant cell arteritis polymyalgia rheumatica spectrum disease (GPSD) is a new term proposed to describe the clinicopathologic continuum between PMR and GCA [7]. It also reflects a shared immunologic origin, whereby abnormal inflammatory responses target musculotendinous structures and arteries courtesy of uncontrolled innate immunity and a breakdown in self-tolerance [8]. These processes have been better studied in GCA, but the pathogenesis of PMR has proved more elusive. Although ours is a single case, where temporal correlation remains subject to chance, these observations are important for their insights into the errors of immune regulation that may propagate different disease phenotypes within GPSD.

Daclizumab binds the high-affinity α-sub-unit (CD-25) of the interleukin-2 receptor (IL2R), which is highly expressed on T-regulatory (T_{reg}) cells. T_{reg} cell numbers are subsequently down-regulated, however their bioavailability for innate lymphoid cells (ILCs) is increased [1]. Downstream, this paradoxically results in ILC precursors and natural killer (NK) cells receiving more IL-2 signal. In MS, NK cell proliferation is desirable to eliminate autologous activated T cells. Clinical trials demonstrated efficacy for daclizumab in relapsing–remitting disease, however this switch in IL-2 signaling has been linked to IRAEs including severe cutaneous reactions, fulminant hepatitis and fatal myeloencephalitis [1]. The bolstering of innate immune pathways is thought responsible. To the best of our knowledge, there are no other case reports of PMR and/or GCA developing after treatment with daclizumab.

In classical PMR, levels of soluble IL2R correlate with disease activity [9]. Similarly, a low percentage of T_{reg} cells has been reported in PMR and GCA, although their role in disease pathogenesis is thought less significant than Th1 and Th17 CD4+ subsets [8]. Recognition of IL-6 as the key cytokine to maintain T_{reg}/Th17 homeostasis is noteworthy in this context, as is the ability of tocilizumab therapy (but not glucocorticoids) to correct pathologic imbalances in this system [10]. The underwhelming impact of B-cell depletion upon disease progression in this case, particularly the development of LVV and arthritis, is also clear. While the BRIDGE-PMR study demonstrated efficacy for rituximab in achieving glucocorticoid-free remission, a causative antibody does not exist in PMR and circulating B cell numbers are reduced at diagnosis compared with healthy controls [8, 11].

PMR-like presentations among cancer patients treated with immune checkpoint inhibitor therapy further parallel daclizumab here. Agents blocking cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death-1 (PD-1) regulatory pathways elicit an anti-tumor response by promoting T-cell activation. The fact that abatacept, a CTLA-4 agonist that abrogates T-cell co-stimulation at the dendritic cell level via immune checkpoint stimulation proved most effective in this case supports the hypothesis that immunoregulatory cells are key to GPSD pathogenesis. Abatacept has previously demonstrated benefit in GCA, although the ALORS study in PMR failed to meet its ambitious primary endpoint [12]. Recent success in RA with the PD-1 agonist, peresolimab, provides additional

evidence of the therapeutic potential possessed by immunoregulation [13].

Ultimately, adverse events from targeted therapies provide a window of opportunity to better understand classical autoimmune diseases. Surely, hypothesis-generating cases like ours suggest PMR and GCA should be prime candidates for future proof-of-concept studies with immunomodulatory therapies.

Author Contributions

Author O.N. retrieved relevant data and wrote the original manuscript draft; Author A.M.T.P. provided images for the included Figure; Author D.F.L.L. assisted with the mechanistic concepts explored in the manuscript; Author C.E.O. proposed the original article and provided all editorial overview. All authors reviewed and approved the final version.

Consent

Written, informed consent was sought from the patient prior to manuscript submission.

Conflicts of Interest

Authors O.N., A.M.T.P., and D.F.L.L. declare no conflicts of interest. Author C.E.O. declares speaking honoraria from Abbvie, Fresenius Kabi, Janssen, Novartis, and Roche and consultancy for Abbvie.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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LETTER TO THE EDITOR

Tel Hashomer Camptodactyly Syndrome: Report on a New Case

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Dear Editor,

The Tel Hashomer camptodactyly syndrome (THCS), first described by Goodman et al., is a rare disease, mainly characterized by the presence of camptodactyly with muscular hypoplasia, skeletal dysplasia, facial dysmorphism, and abnormal dermatoglyphics (Online Mendelian Inheritance in Man (OMIM) #211960). Since then, 23 additional cases have been described about THCS in various countries, including Israel, India, Russia, Italy, Hungary, Arabia, and Brazil, suggesting the presence of the mutant gene in diverse populations. The genetic basis for this disorder is unknown; thus, there is a possibility that this clinical presentation may be contained within another genetic diagnosis [1–4].

We report on a case of a pediatric THCS in Portugal, whom we have followed for 8 years.

1 | Case Presentation

This is a case of a 14-year-old boy initially admitted to the Pediatrics department for investigation of poor height and weight development (both below 3rd percentile), delayed onset of puberty, and multiple bone deformities. Subsequently, he was referred to our Pediatric and Young Adult Rheumatology unit due to a one-month history of hip mechanical pain. There were no constitutional symptoms, rashes, ulcers, ocular, gastrointestinal, cardiac, or respiratory complaints. The parents were non-consanguineous and otherwise healthy, with no family history of similar manifestations or any other congenital disorder.

On physical examination, the patient exhibited distinctive characteristics, including shuffling gait, short stature (132 cm, < 3rd percentile), low weight (28.7 kg, < 3rd percentile) and global muscular atrophy. He had a peculiar facies with a rounded face, microretrognathia with limited mouth opening, and a short neck. Camptodactyly and brachydactyly were observed in both hands and feet, along with a partial absence of palmar dermatoglyphics. Additionally, vitiligo lesions with acral predominance were noted. Cervical spine and hip mobility were limited, and he had no painful or swollen joints. Laboratorial studies with full blood count, muscle enzymes, renal, and hepatic function had no changes. Bone biochemistry, including serum calcium, phosphorus, serum total alkaline phosphatase, and parathyroid hormone, was within the normal range. Endocrine workup excluded growth hormone deficiency and thyroid changes. The immunological panel was unremarkable, including negative antinuclear antibodies, rheumatoid factor, and anti-citrullinated protein antibodies. Electromyographic studies of the upper and lower limbs presented a polyphasic action potentials with an increased incidence of brief high-frequency components, suggestive of chronic myopathy. Echocardiogram was normal.

Abbreviations: BMI, body mass index; OMIM, online mendelian inheritance in man; THCS, Tel Hashomer camptodactyly syndrome.

Plain radiographs (Figure 1) revealed a diffuse osteopenia, bilateral campodactyly in the 4th and 5th fingers, more pronounced in the right hand, and severely dysmorphic feet, with subluxation and overlapping of nearly all toes. There was dorso-lumbar spine straightening and spina bifida at the S1 level. Deficient coverage of the femoral heads due to bilateral acetabular hypoplasia was evident. A hip joint ultrasound was performed, showing no synovitis. Clinical findings were compatible with a diagnosis of THCS. The patient was referred to physical rehabilitation.

Due to severe radiographic osteopenia, without fractures, bone densitometry was performed revealing low values (lumbar spine Z score−3.1). Supplementation with vitamin D and calcium was started, and later, at age 18, risedronate sodium 35 mg/week for 5 years, resulting in an improvement of the Z score value to −2.1.

The patient was referred to Genetics, where he was investigated with array-CGH and, later, trio whole-exome sequencing, which has been inconclusive. Currently, other genetic tests are ongoing, including Skeletal Dysplasia NGS Panel.

During the follow-up, there was a gradual progression of bone deformities and subsequent reduction of hands and wrists mobility. In his last check-up, at the age of 22 years, his height was 146 cm and he weighed 37 kg, BMI 17 kg/m^2. His facial features and other findings remained as described at the age of 14 years. Plain radiographs revealed a progression of campodactyly, along with wrist involvement (Figure 2).

2 | Discussion

To the best of our knowledge, this is the first case of TCHS in a Portuguese pediatric patient.

As previously described in the literature, THCS is a rare entity, essentially characterized by camptodactyly, muscular hypoplasia, bone dysplasia, distinct facial features, and abnormal dermatoglyphics creases [3, 5, 6].

Melegh et al. conducted a systematic review in 2005 and analyzed all the clinical characteristics of 18 cases, revealing consistent features such as camptodactyly, spindle-shaped fingers, and a highly arched palate in all patients. Other prevalent findings included abnormal dermatoglyphics creases and muscle hypoplasia in 94.1%, ocular hypertelorism, facial asymmetry, and a small mouth in 88.2%, and a long philtrum and winging of the scapula in 82.3%. Notably, approximately two-thirds of the patients presented with thoracic scoliosis, short stature, partial syndactyly, brachycephaly, prominent forehead, malocclusion, clinodactyly, and clubbed feet. Subsequent to this review, additional cases have been documented, totaling 23 patients according to a systematic literature review published in 2016 by Wijerathne et al. [7, 8].

Our patient, in addition to classic features, also presented a severe hip dysplasia and osteoporosis, conditions not previously associated with this syndrome.

Radiologically, the pronounced changes observed in the feet were previously described by Goodman et al. along with spina bifida; however, carpal destruction has not been reported before.

To date, there is no documented genetic basis for THCS, raising the possibility that this clinical presentation might be encompassed by another genetic diagnosis. Genetic investigations thus far have solely utilized conventional karyotyping, a limitation possibly stemming from most of the earlier reports being published over two decades ago. Exome sequencing of our patient did not identify any mutated genes, suggesting that this syndrome may not be due to a single gene mutation.

A comprehensive understanding of the pathophysiology, gaining additional significance once the responsible gene is identified, is essential for effective management, encompassing patient care, genetic counseling, and prenatal diagnosis. A multidisciplinary approach is imperative, especially considering that corrective orthopedic surgery and physical rehabilitation may be required due to multiple bone deformities.

3 | Conclusion

This case highlights the need to recognize this rare entity so that more cases could be identified and reported. Such increased awareness is essential for uncovering the genetic basis of this

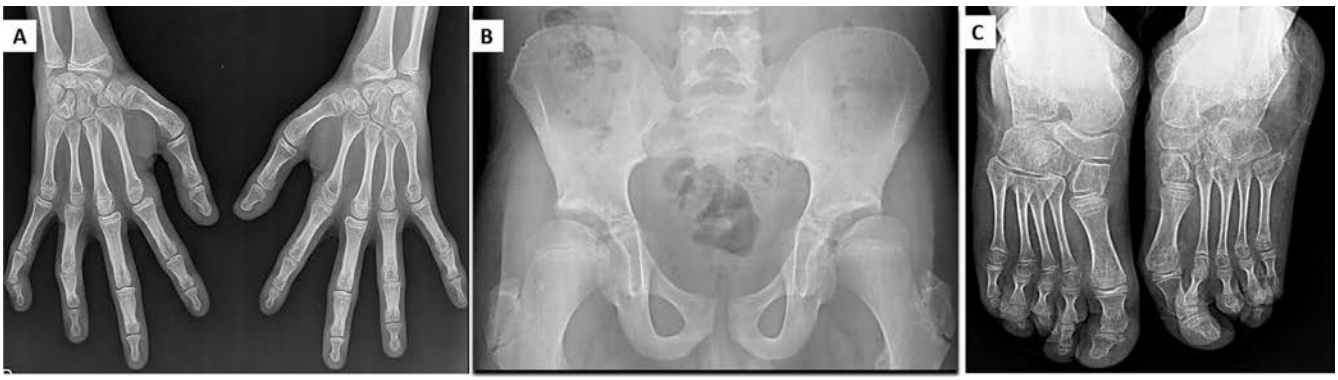

FIGURE 1 | Patient's plain radiographs of hands, pelvis and foot, at 14 years old. (A) Camptodactyly of 4th and 5th fingers, more pronounced on the right side; (B) Bilateral undercoverage of the femoral head due to acetabular hypoplasia; (C) Marked osteopenia and complete destruction of the normal anatomy of the foot, with subluxation of the various joints of the hindfoot and deformity of the navicular bone on the left. Bilateral hallux valgus and overlapping of the different fingers.

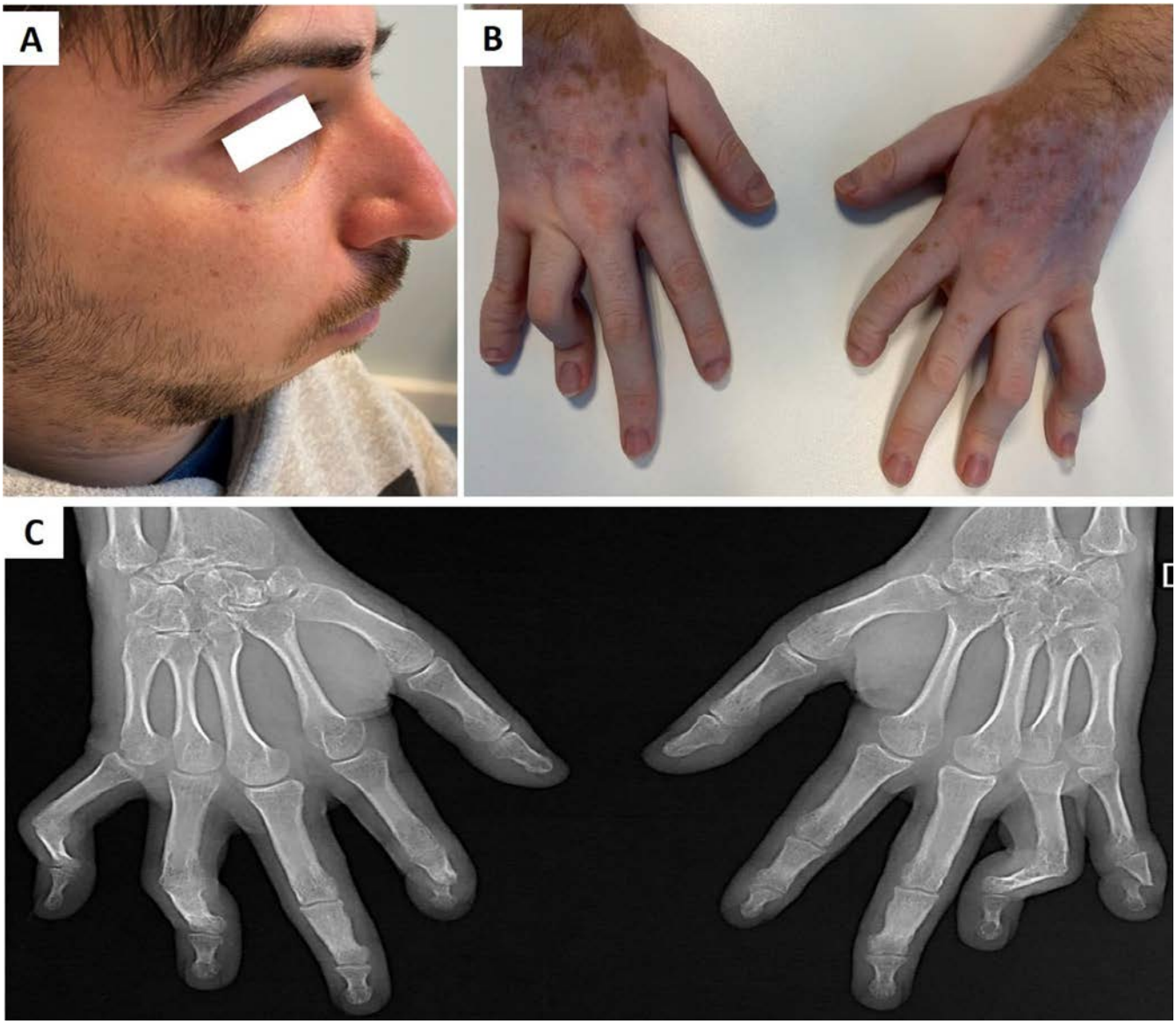

FIGURE 2 | (A) Microretrognathia and neck shortening; (B) Vitiligo lesions on the back of the hands, bilateral camptodactyly of the 4th and 5th fingers, and on the right hand, the 2nd finger; (C) Ankylosis of the proximal and distal interphalangeal joints in the fingers, with campodactyly and carpals destruction, particularly pronounced on the right side.

syndrome and improving the approach to these patients and their families.

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Author Contributions

The author takes full responsibility for this article.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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ORIGINAL ARTICLE

Canakinumab Treatment in Familial Mediterranean Fever Patients: With/Without Colchicine

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ABSTRACT

Objectives: To compare the differences in attack characteristics, acute-phase reactants, and renal outcomes in patients using canakinumab with or without colchicine treatment.

Methods: FMF patients treated with canakinumab for ≥3 months were retrospectively reviewed. Patients unable to continue colchicine for various reasons were identified and grouped as those receiving the canakinumab + colchicine combination (CAN + CLC) and canakinumab monotherapy (CANmono). Attack frequency, C-reactive protein (CRP), urine protein–creatinine ratio (UPCR), and kidney function tests were recorded before and after canakinumab treatment.

Results: Fifty-five patients receiving canakinumab treatment were included in the study. Thirty-one patients (56.4%) used CAN + CLC and 24 (43.6%) CANmono. With both CAN + CLC and CANmono treatment, there was no significant change in UPCR of patient groups with GFR > 60 and < 60 mL/min/1.73 m^2. Amyloid A (AA) amyloidosis was present in 21 (38.2%) patients. In patients with AA amyloidosis receiving CAN + CLC, there was a nonsignificant decrease in UPCR and increased creatinine levels after treatment ($p = 0.214$ and $p = 0.051$, respectively). Median GFR decreased significantly from 69 (IQR, mg/dl, 45–95) to 44 (IQR, mg/dl, 28–75) with CAN + CLC treatment ($p = 0.021$). In the CANmono group, compared to baseline values, there was no significant change in posttreatment UPCR, serum creatinine, and GFR values.

Conclusion: It is difficult to make a recommendation regarding the discontinuation or continuation of colchicine treatment in all FMF patients who initiated anti-IL-1 treatment. Canakinumab can be continued as monotherapy in patients who cannot continue colchicine treatment due to side effects and patient noncompliance.

1 | Introduction

Familial Mediterranean fever (FMF) is a monogenic autoinflammatory disease that develops secondary to mutations in the Mediterranean fever (MEFV) gene located on the short arm of chromosome 16 [1]. FMF is characterized by self-limiting attacks of fever, arthritis, and serositis. However, in some patients, subclinical inflammation continues during the attack-free period [2]. In cases where the disease is not well controlled, there is a risk of developing amyloid A (AA) amyloidosis, which is the primary cause of morbidity and mortality in FMF disease.

Colchicine, the cornerstone of FMF treatment for years, has been proven effective in preventing febrile attacks and reducing the risk of AA amyloidosis [3, 4]. However, in some patients, colchicine cannot be used in effective doses due to side effects such as diarrhea, increased liver enzyme activity, or myopathy. Another problem is patients' compliance with colchicine treatment, which is used in multiple daily doses throughout life. The

full compliance rate with colchicine treatment varies between 40% and 65% [5–7]. On the other hand, despite the use of colchicine at the highest tolerated doses, up to 5% of patients do not respond to colchicine [8]. In such issues, agents other than colchicine are needed to prevent FMF attacks and the development of AA amyloidosis.

After understanding the role of IL-1β in the pathogenesis of FMF, which increases due to inflammasome activation, the importance of IL-1β inhibitors in treatment is increasing [9]. In recent studies in FMF patients, IL-1β inhibitors anakinra (IL-1Ra antagonist) and canakinumab (IgG-targeting IL-1β) have been shown to reduce the number of acute attacks, disease activity, and acute-phase response [9, 10]. The ability to achieve FMF treatment goals, such as preventing acute attacks and subclinical inflammation, with IL-1β inhibitors raises the question of whether continuing to use colchicine in patients has an additional contribution to IL-1β monotherapy in preventing the development of AA amyloidosis.

In this study, we aimed to compare the differences in attack characteristics, acute-phase reactants, and renal outcomes in patients using canakinumab with or without colchicine treatment.

2 | Materials and Methods

2.1 | Study Design

Patients diagnosed with FMF according to the Tel-Hashomer criteria [11] or the New Eurofever/PRINTO classification criteria [12] at the Rheumatology Clinic of Akdeniz University between January 2014 and January 2023 were retrospectively reviewed. Patients treated with canakinumab for ≥3 months were included in the study. Patients with a history of pregnancy during follow-up were excluded from the study.

During canakinumab treatment, patients who did not use colchicine due to toxicity (e.g., elevated liver enzymes, leukopenia, or myopathy), intolerance (e.g., abdominal pain, nausea, or diarrhea) or noncompliance with treatment (use of the drug only during an attack despite warnings) were identified. Patients were grouped as those receiving the canakinumab + colchicine combination (CAN + CLC) and canakinumab monotherapy (CANmono).

Demographic characteristics, attack types, duration of the first symptoms and FMF diagnosis, comorbid diseases, MEFV gene mutations, family history of FMF, and patients with biopsy-proven AA amyloidosis were determined. Attack frequency in the last 3 months and C-reactive protein (CRP), serum creatinine, urine protein-to-creatinine ratio (UPCR), and glomerular filtration rate (GFR) levels before and after canakinumab treatment were recorded. Serum amyloid A (SAA) was not evaluated because most patients' regular measurements were unavailable. Renal outcomes were evaluated in patients who received canakinumab treatment for ≥6 months and did not receive hemodialysis replacement therapy (HDRT).

The study was approved by the Ethics Committee of the Akdeniz University Faculty of Medicine (Protocol No: KAEK-200).

2.2 | Statistical Analysis

IBM SPSS 23 (Statistical Package of Social Science) was utilized for statistical analysis. The general characteristics of the study population were analyzed with descriptive statistics. Data were shown as total numbers and percentiles, numeric data as mean ± standard deviation, or as median value (minimum and maximum). The chi-square test or Fisher's exact test was used to compare the categorical data of the groups, and the Student's T test was used to compare numeric (continuous) variables. The Mann–Whitney U test was used to compare parametric values that are not normally distributed. Paired sample T test or Wilcoxon signed-rank test, whichever is appropriate, was used to compare pre- and posttreatment results. p-Value <0.05 was considered to be statistically significant.

3 | Results

3.1 | Baseline Characteristics of the Study Patients

Fifty-five patients receiving canakinumab treatment were included in the study. The mean age of the patients was 36.8 ± 9.6 years, and 26 (47.3%) patients were females. AA amyloidosis was present in 21 (38.2%) patients (renal in 21, cardiac in 7, and gastrointestinal in 4). Of the patients, 9% had comorbidities due to increased inflammation associated with FMF (two axial spondyloarthropathy, one polyarteritis nodosa, one psoriasis, and one inflammatory bowel disease), 5.5% had diabetes mellitus, and 36.4% had hypertension. Canakinumab treatment was started in 14/55 patients due to newly developed proteinuria, 9/55 patients due to colchicine toxicity/intolerance, and 32/55 patients due to colchicine resistance. The median follow-up period for canakinumab was 25 (3–86) months. Before canakinumab, anakinra was used in 18 (32.7%) patients, and it was discontinued due to ineffectiveness in 8 (14.5%) patients, side effects in 6 (11%), and noncompliance in 4 (7.3%).

Thirty-one patients (56.4%) used CAN + CLC and 24 (43.6%) CANmono. The demographic characteristics and genetic mutation details of the patients are given in Table 1. The reason for not using colchicine following canakinumab was due to toxicity/severe intolerance in 9 (16.4%) and patient noncompliance in 15 (27.3%).

After treatment, 74.2% of patients using CAN + CLC and 79.2% using CANmono had no attack in the last 3 months, and there was no statistical difference between the groups regarding the number of attacks ($p = 0.687$) (Table 1). In the third month of canakinumab treatment, both groups had a significant decrease in CRP levels (CAN + CLC, $p < 0.001$; CANmono, $p = 0.003$). There was no statistically significant difference in CRP levels in the 3rd month after treatment and at the last follow-up (Table 1). The median CRP levels for both groups at baseline, 3rd, 6th, 12th, and 24th months are shown in Figure 1.

3.2 | Renal Outcomes

Three of the 55 patients were excluded from the analysis because they received canakinumab treatment for less than 6 months, and three were excluded because they received HDRT. Renal outcomes were analyzed for 49 patients.

TABLE 1 | Demographic and clinical characteristics of the patients taking colchicine + canakinumab and canakinumab monotherapy.

Characteristics	Canakinumab + Colchicine ($n = 31$)	Canakinumab ($n = 24$)	p
Female sex, (%)	42.0	54.2	0.368
Age, years (mean ± SD)	35.1 ± 9.3	39.0 ± 9.7	0.135
Age at diagnosis of FMF, years			
mean ± SD	18.3 ± 10.1	24.7 ± 10.5	0.156
median, min–max	18 (2–40)	21.5 (5–54)	
Duration from the onset of FMF symptoms, years (mean ± SD)	27.6 ± 8.3	26.2 ± 12.0	0.624
Duration from the diagnosis of FMF, years (mean ± SD)	16.8 ± 6.4	14.4 ± 8.5	0.228
Follow-up time of canakinumab therapy, months			
mean ± SD	30.6 ± 20.3	26.0 ± 20.2 24.5 (3–86)	0.337
median, min–max	30 (3–72)		
Regular use of canakinumab, n (%)	20 (64.5)	20 (83,3)	0.141
Previous anakinra usage, n (%)	12 (38.7)	6 (25)	0.283
Colchicine dosing, milligram (median, min–max)	1.25 (0.5–3)	—	
Attack types, n (%)			
Fever	26 (83.9)	20 (83.3)	1.000
Serositis	29 (93.5)	21 (87.5)	0.643
Peritonitis	26 (83.9)	17 (70.9)	0.246
Pleuritis	17 (54.9)	13 (54.2)	1.000
Joint involvement	22 (71.0)	18 (75.0)	0.739
Amyloidosis, n (%)	12 (38.7)	9 (37.5)	0.927
Mutation, n (%)[a]			
M694V mutation at least one copy	24 (92.3)	16 (84.2)	0.636
—M694V homozygous	13 (50.0)	10 (52.6)	0.862
Exon 2 mutation at least one copy	9 (34.6)	8 (42.1)	0.609
—R202Q mutation at least one copy	8 (30.8)	5 (26.3)	0.745
GFR, (mL/min/1.73 m2), n (%)			
≥ 90	18 (58.1)	15 (62.5)	0.252
90–60	3 (9.7)	1 (4.2)	
60–30	0	3 (12.5)	
30–15	1 (3.2)	1 (4.2)	
< 15	1 (3.2)	1 (4.2)	
HDRT or kidney transplantation, n (%)	9 (29.0)	5 (20.8)	0.489
Comorbidities, n (%)			
Diabetes mellitus	2 (6.4)	1 (4.2)	1.0
Hypertension	10 (32.3)	10 (41.2)	0.472
ACEi/ARB usage, n (%)	5 (16.1)	7 (29.2)	0.246
FMF attacks in last 3 months, n (%)			
None	23 (74.2)	19 (79.2)	0.687
One attack	5 (16.1)	2 (8.3)	
Two attack	2 (6.5)	2 (8.3)	
Two or more attacks	1 (3.2)	1 (4.2)	
CRP levels, mg/L (mean ± SD)			
Pretreatment	54.0 ± 56.1[b]	38.0 ± 46.6[c]	0.260
3rd month after treatment	7.70 ± 16.4[b]	4.20 ± 5.43[c]	0.306
Last visit	6.19 ± 9.7	4.72 ± 5.2	0.506

Abbreviations: ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; CRP, C-reactive protein; GFR, glomerular filtration rate; HDRT, hemodialysis replacement therapy; SD, standard deviation.

[a]FMF gene analysis is available in 45 patients.

[b]Patients using Canakinumab + Colchicine pretreatment vs. 3rd month after treatment; $p < 0.001$ (paired-samples test).

[c]Patients using Canakinumab monotherapy pretreatment vs. 3rd month after treatment; $p = 0.003$ (paired-samples test).

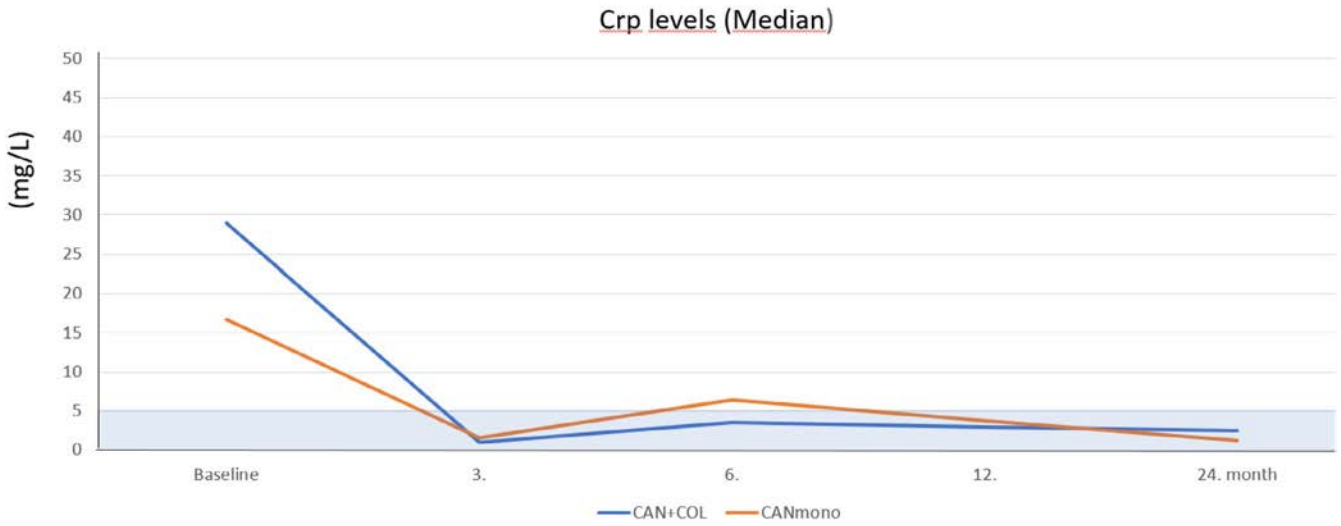

FIGURE 1 | Changes in CRP levels over time with CANmono and CAN + CLC treatment. CAN + COL, canakinumab + colchicine; CANmono, canakinumab monotherapy; CRP, C-reactive protein.

Twenty-seven patients were treated with CAN + CLC and 22 with CAN monotherapy. There was no significant change in protein levels after treatment with CAN + CLC and CANmono treatment in both patient groups with GFR > 60 mL/min/1.73 m^2 and < 60 mL/min/1.73 m^2 (Table 2). In patients with AA amyloidosis, in the CAN + CLC group, there was a decrease in urine protein levels and increased creatinine levels with treatment, but the differences were not statistically significant ($p = 0.214$ and $p = 0.051$, respectively). Median GFR was decreased significantly from 69 (IQR, mg/dl, 45–95) to 44 (IQR, mg/dl, 28–75) with CAN + CLC treatment ($p = 0.021$). In the CANmono group, compared to baseline values, there was no significant change in posttreatment urine protein, serum creatinine, and GFR values (Table 2). Additionally, in kidney transplant patients, no significant differences were observed in urine protein, serum creatinine, and GFR levels after treatment compared to baseline values in both the CAN + CLC and CANmono groups (Table 2). There was no significant difference between CAN + CLC and CANmono treatments in terms of changes in urine protein, serum creatinine, and GFR levels (data not shown).

The characteristics and renal outcomes of patients with AA amyloidosis receiving CAN + CLC ($n = 12$) and CANmono ($n = 9$) are detailed in Tables 3 and 4, respectively. Colchicine treatment was not used in 9 of 21 patients with AA amyloidosis (42.8%) due to side effects or patient noncompliance.

In patients with AA amyloidosis who received CAN + CLC, eight (66.7%) were kidney transplant patients. During the follow-up period, two of eight patients developed graft rejection, and one patient with allograft AA amyloidosis had an increase in proteinuria and a decrease in GFR. In three of the other four patients, despite the significant decrease in proteinuria, there was an increase in serum creatinine level and a reduction in GFR level (Table 3).

Of the patients with AA amyloidosis who received CANmono, three (33.3%) were kidney transplant patients and one of them developed recurrent AA amyloidosis of the allograft during follow-up (Patient 8) (Table 4). Of the other six patients, four had no change in proteinuria (one had a decrease in GFR), while two patients had an increase in proteinuria and a decrease in GFR. In a patient with renal, cardiac, bladder, and gastrointestinal AA amyloidosis, a severe increase in proteinuria and subsequent end-stage kidney disease (ESKD) developed, and HDRT was started (Patient 3) (Table 4).

Two AA patients receiving CAN + CLC therapy died during follow-up. The first patient died due to invasive pneumonia and sepsis during HDRT, and the other patient, who had a history of kidney transplantation, died after hip arthroplasty surgery.

4 | Discussion

The main aim of FMF treatment is to control attacks and subclinical inflammation and prevent the development of AA amyloidosis. Canakinumab and anakinra have recently been frequently used in FMF diseases and have been shown to reduce the number of attacks and acute-phase proteins [13]. However, data showing the effects of these agents on proteinuria and renal functions is limited and contradictory. There is also insufficient data on the effects of anti-IL-1 monotherapy on renal functions in patients who cannot use colchicine due to reasons such as intolerance, toxicity, and patient noncompliance. In this study, the effects of CANmono and CAN + CLC treatment were similar in terms of the number of acute attacks, acute-phase reactants, and renal outcomes.

Akar S et al. reported that 47 of 157 FMF patients had proteinuria and that after anti-IL-1 treatment (151 with anakinra and 21 with canakinumab), there was a significant reduction in proteinuria, a decrease in daily protein excretion in 77% of 47 patients, no proteinuria in 21%, and also reported that there was no significant change in serum creatinine level [14]. In another study, it was stated that in 17 patients with FMF-related amyloidosis, there was a significant decrease in proteinuria in 10/17 patients, while there was no difference in serum creatinine level with anti-IL-1 treatment (anakinra in 10 patients, anakinra in 7 patients, followed by canakinumab) [15]. Ataş N et al. observed

TABLE 2 | Renal outcomes of treatment in the patients taking canakinumab + colchicine and canakinumab monotherapy.

	Canakinumab + Colchicine ($n=27$)			Canakinumab ($n=22$)		
Renal outcomes	**Pretreatment**	**Posttreatment**	***p***	**Pretreatment**	**Posttreatment**	***p***
GFR ≥ 60 (mL/min/1.73 m²)		***n* = 22**			***n* = 18**	
Proteinuria (UPCR), median–IQR	110 (60–210)	95 (70–240)	0.254	100 (60–240)	115 (90–600)	0.758
GFR < 60 (mL/min/1.73 m²)		***n* = 5**			***n* = 4**	
Proteinuria (UPCR), median–IQR	2400 (400–3000)	860 (270–1770)	0.500	410 (240–3530)	440 (200–3185)	0.285
Patients with amyloidosis		***n* = 9**			***n* = 7**	
Proteinuria (UPCR), median–IQR	900 (270–3000)	720 (270–980)	0.214	710 (570–5800)	570 (230–930)	0.600
Serum creatinine (mg/dl), median–IQR	1.2 (1.06–1.74)	1.93 (1.09–2.53)	0.051	1.33 (1.02–2.29)	1.22 (0.83–1.53)	0.249
GFR (mL/min/1.73 m²), median–IQR	69 (45–95)	44 (28–75)	0.021	65 (44–98)	55 (26–80)	0.112
Patients with renal tx		***n* = 6**			**n = 2**	
Proteinuria (UPCR), median–IQR	1.47 (1.12–1.91)	2.17 (1.3–2.7)	0.249	1.48 (1.43–1.53)	1.74 (1.19–2.29)	0.655
Serum creatinine (mg/dl), median–IQR	59 (45–86)	38 (28–60)	0.116	54.5 (44–65)	53.0 (26–80)	0.655
GFR (mL/min/1.73 m²), median–IQR	335 (130–2400)	495 (90–860)	0.917	750 (570–930)	585 (570–600)	0.317

Abbreviations: GFR, glomerular filtration rate; IQR, interquartile range; UPCR, urine protein-to-creatinine ratio.

a significant decrease in the median (IQR) proteinuria level of 22 AA amyloidosis patients after a median of 18.5 (12–37) months of anakinra treatment, but canakinumab treatment, which was started in six patients when proteinuria continued with anakinra, was ineffective on proteinuria [16]. Bektas M et al. reported that in 137 patients with FMF-related amyloidosis [92 patients used b-DMARDs and 88 (62%) used anti-IL1], 31% had a complete response to treatment in terms of renal findings, 23.6% had a stable course, and renal progression occurred in 38.5% [17]. They also stated that with biologic DMARDs (b-DMARDs), creatinine values continued to increase despite a significant decrease in proteinuria levels [17]. Yıldırım D et al. compared FMF patients who used ($n=96$) and did not ($n=29$) colchicine along with IL-1 antagonist (89 anakinra and 36 canakinumab). It was observed that proteinuria levels did not change after treatment in both groups (with or without amyloidosis), and creatinine levels decreased with IL-1 monotherapy and increased with the combination of IL-1 antagonists and colchicine. Moreover, while there was no new-onset proteinuria, amyloidosis, or progression of proteinuria in those who did not use colchicine with IL-1 antagonist, it was seen in two, one, and six patients who used colchicine, respectively [18]. In our study, after canakinumab treatment of patients with AA amyloidosis, proteinuria, and GFR levels remained stable in approximately half of the patients (9 of 18 patients who were not on HDRT), a significant increase in proteinuria in 3/18 patients and new AA amyloidosis developed in 1 patient. Among those using colchicine, excluding those who developed rejection, it is observed that there is a significant decrease in proteinuria in two, and renal stability is achieved in half of the patients. However, in those who did not use colchicine, although approximately half of them were stable in terms of renal involvement, one patient developed graft AA amyloidosis, and one patient developed ESKD after rapidly progressive disease.

Renal function at the beginning of treatment is considered important for the effect of IL-1 inhibitors on proteinuria [9, 19]. It was observed that patients whose renal functions were normal or moderately impaired (creatinine level < 1.5 mg/dL or GFR ≥ 60 mL/min/1.73 m²) at the beginning of anti-IL-1 had a decrease in proteinuria and no change in GFR after treatment. However, in one study, it was observed that anti-IL-1 had no effect on proteinuria in patients with GFR < 60, and in the other, there was an increase in proteinuria and a decrease in GFR with creatinine levels ≥ 1.5 [9, 19]. We observed that the initial GFR level was not statistically significant regarding the effect of canakinumab treatment on proteinuria and that it may also have positive effects in patients with GFR < 60 mL/min/1.73 m².

In a study evaluating 36 kidney transplant patients who received anti-IL-1 therapy and colchicine, although the number of attacks and inflammatory markers decreased, proteinuria levels remained stable, creatinine levels increased, and GFR decreased [20]. It is also reported that IL-1 inhibitors have been effective in

TABLE 3 | Characteristics of patients with AA amyloidosis treated with canakinumab + colchicine.

Patient No	Gender/ Age	Mutation	Pre-CAN period	CAN duration (months)	Colchicine dose (mg/ day)	Proteinuria (UPCR) (mg/mg)		GFR (ml/min/m²)		Renal outcome	
						Before CAN	After CAN	Before CAN	After CAN	Proteinuria	GFR
1	M/30	M694V/M694V R202Q/R202Q	Renal AA	72	1.5	4600	980	142	126	↓	↓
2	F/42	M694V/M694V/ R202Q	Renal AA and CKD	56	1	10000	1770	38	24	↓	↓
3	M/29	M694V/M680I	Renal AA, ESKD, and HDT	31	0.5	4400	4800	11	11	Exitus	Exitus
4	F/37	M694V/M680I/ R202Q	Renal AA	39	1	900	600	117	75	↓	↓
5	M/36	M694V/M694V/ R202Q/R202Q	KT due to renal AA	51	0.5	2400	860	45	19	Graft rejection	Graft rejection
6	M/38	NA	KT due to renal AA AA in allograft	24	1	3000	5270	49	44	↑	↓
7	M/40	M694V/R761H	KT due to renal AA Graft rejection and AA in allograft	12	1	400	270	28	32	—	↑
8	F/33	NA	KT due to renal AA	2	1.5	80	80	110	108	—	—
9	F/49	NA	Renal, thyroid, and GIS AA KT due to renal AA	3	0.5	80	120	106	90	—	—
10	M/47	M680I/M694V/ R202Q	KT due to renal AA	72	1	130	720	86	28	Graft rejection	Graft rejection
11	M/22	M694V/M694V	KT due to renal AA	43	1	270	90	95	96	—	—
12	M/56	M694V/V726A	KT due to renal AA	13	1.5	130	90	69	60	—	—

Abbreviations: AA, amyloidosis A; CAN, canakinumab; CKD, chronic kidney disease; ESKD, end-stage kidney disease; GFR, glomerular filtration rate; GIS, gastrointestinal system; HDT, hemodialysis treatment; KT, kidney transplantation; NA; no analysis; UPCR, urine protein-to-creatinine ratio.

TABLE 4 | Characteristics of patients with AA amyloidosis treated with canakinumab.

Patient no	Gender/ Age	Mutation	Pre-CAN period	CAN duration (months)	Proteinuria (UPCR) (mg/mg)		GFR (ml/min/m^2)		Renal outcome	
					Before CAN	After CAN	Before CAN	After CAN	Proteinuria	GFR
1	M/43	M694V/E148Q	Renal AA, ESKD, and HDT	3	5600	6400	13	13	—	—
2	M/39	M694V/M680I/ R202Q	Renal AA	12	6500	5800	55	55	—	—
3	M/43	M694V/M694V/ R202Q	Renal, cardiac, bladder, and GIS AA	24	530	6240	76	16	↑	↓
4	F/54	M694V/M694V	—	18	230	310	44	50	—	—
5	M/60	M694V/ —	Renal AA	30	710	780	98	80	—	↓
6	M/43	M680I/M680I	—	40	160	710	109	91	↑	↓
7	M/34	NA	KT due to renal AA AA in allograft	6	930	600	65	80	↓	↑
8	F/34	M694V/M694V	KT due to renal AA	36	570	570	44	26	AA in allograft	AA in allograft
9	M/33	NA	KT due to renal AA Graft rejection and HDT	6	—	—	9	7	—	—

Abbreviations: AA, amyloidosis A; CAN, canakinumab; ESKD, end-stage kidney disease; GFR, glomerular filtration rate; GIS, gastrointestinal system; HDT, hemodialysis treatment; KT, kidney transplantation; NA; no analysis; UPCR, urine protein-to-creatinine ratio.

kidney transplant patients with FMF-related AA, with lower rejection rates and more prolonged graft survival [20]. In another study conducted by Şimşek et al. on patients who underwent kidney transplantation due to end-stage renal failure caused by FMF-related amyloidosis, patients receiving colchicine were compared with patients who received IL-1 antagonist (seven canakinumab and two anakinra) therapy in addition to colchicine (at a dose of 1.0 mg/day) due to amyloid deposition or colchicine resistance in the allotransplant [21]. After treatment with IL-1 antagonists, while a significant decrease in mean SAA levels was observed, there was no difference in mean daily proteinuria and serum creatinine levels. Histopathological examinations performed in the posttransplantation period showed that approximately 60% of patients who achieved remission with the maximum tolerated dose of colchicine and 80% of patients treated with IL-1 antagonists and colchicine combination had amyloid deposition at the allotransplant, with no histological improvement in any patient [21]. In our study, similar to this study, daily proteinuria and creatinine levels in transplant patients were stable.

High-level evidence supports colchicine as an effective treatment for FMF, and adherence has been identified as a key issue in determining treatment success [22]. In particular, patients with recurrently elevated acute-phase reactants or unstable disease should be monitored more regularly for treatment adherence [22]. In studies conducted to demonstrate anti-IL-1 effectiveness in AA amyloidosis, it has been reported that colchicine continues to be used at the maximum tolerated dose in 87.7%–100% of patients [14–19]. However, data in the literature regarding colchicine compliance show that 35%–40% of FMF patients do not use colchicine regularly, meaning that compliance with colchicine treatment may be lower than expected [5–7]. In a large population-based study, more than 50% of patients in both colchicine-responsive FMF patients and colchicine-resistant patients treated with IL-1β inhibitors had poor adherence to colchicine [23]. In our patients with AA amyloidosis, while patient compliance with canakinumab treatment was 72.7%, the rate of patients using colchicine was 56.4%, which was lower than previously reported. Although the number of cases is insufficient, this study more clearly demonstrates the effect of canakinumab monotherapy on proteinuria in cases of FMF-associated amyloidosis than previous studies. According to a meta-analysis, while anti-IL-1 agents were found to be effective in reducing acute attacks and inflammatory markers in FMF, a randomized controlled trial by Ozen et al. observed that, despite low CRP levels during canakinumab treatment, SAA levels remained moderately above the normal range (exceeding 50 mg/L and 70 mg/L in 42% and 30% of patients, respectively), particularly in those requiring higher doses of canakinumab [13, 24]. Therefore, in particular, in cases with multiple organ AA amyloidosis and/or kidney transplant history, with a risky mutation profile, and especially in cases with nonattack manifestations, colchicine should be continued at the maximum tolerated dose. Considering ethical concerns, anti-IL-1 monotherapy is an alternative that should only be discussed in patients with severe colchicine intolerance or adverse effects. Protocols for reducing or discontinuing colchicine should be developed in this patient group.

The most important limitations of this study are that it is retrospective and has a low patient number. The lack of statistical significance in comparisons between groups may be due to the small sample size. The high proportion of renal transplant patients with presumably more severe disease among those using combination therapy may have influenced the results. Prospective studies involving a large population are needed to reveal the primary effect of anti-IL-1s in preventing and treating AA amyloidosis. Unfortunately, although anti-IL-1 treatments are effective and safe in reducing inflammatory activity in FMF patients, conducting a randomized controlled trial on discontinuing colchicine treatment is not ethically possible. Another limitation is that more than half of the patients with AA amyloidosis were kidney transplant recipients, and it is difficult to evaluate the effect of canakinumab on renal outcomes in the transplanted kidney.

In conclusion, with our current knowledge, it is difficult to make a recommendation regarding the discontinuation or continuation of colchicine treatment in all FMF patients who initiated anti-IL-1 treatment. However, our findings suggest that canakinumab monotherapy may be considered, particularly in patients who are unable to continue colchicine treatment due to toxicity or intolerance.

Author Contributions

F.E. and T.S.Ö. designed this study. T.S.Ö., M.D., and M.N. collected the data. T.S.Ö. and V.Y. participated in the data management and performed the statistical analyses. M.E.T. analyzed the results. T.S.Ö. and F.E. interpreted the results and wrote the article. F.E. managed and supervised the project. All authors reviewed and approved the final version of the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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ORIGINAL ARTICLE

Histopathological Evaluation of the Anterior Cruciate Ligament in Patients With Advanced Gonarthrosis

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ABSTRACT

Introduction: Knee osteoarthritis is a degenerative disease of the knee joint that leads to progressive loss of articular cartilage. There is only limited information in the literature on the degeneration of the anterior cruciate ligament (ACL) in patients with gonarthrosis. In this study, ACL samples excised during the surgery of patients undergoing total knee replacement (TKR) in 2019–2020 were evaluated histopathologically. The present study aims to investigate the relationship between the degeneration of the ACL and the stage of gonarthrosis.

Methods: Direct X-rays of 47 patients undergoing knee arthroplasty were evaluated and stratified into two groups: Stage 3 (Group 1) and Stage 4 (Group 2). ACL samples were examined histopathologically. Staining was performed with hematoxylin–eosin (H&E), Alcian blue, and Masson's trichrome. The degree of degeneration was determined according to the Movin score and Bonar score. Based on these scores, the statistical significance of the relationship between the stage of gonarthrosis and histopathological examination results was evaluated.

Results: When Group 1 patients were graded according to total Movin scores, the result was mild degeneration in 72.7% of the patients, while the corresponding rates were 76% in Group 2. None of the patients had a score reflecting severe degeneration. The total Bonar scores showed mild degeneration in 68.2% of Group 1 patients. In Group 2, the scores showed mild degeneration in 52% of the patients. No statistically significant difference was found between the groups in either scoring.

Conclusion: It was determined that comparable degeneration occurred in the ACL with the progression of the stage of gonarthrosis.

1 | Introduction

Degenerative changes in the knee joint resulting from a variety of different reasons reduce the quality of life by causing pain and restriction of movement. Knee arthroplasty is a surgical treatment option that orthopedic surgeons commonly apply in patients with advanced stages (Stages 3–4) of degenerative osteoarthritis of the knee joint (gonarthrosis) [1].

ACL has a microstructure similar to that of other soft connective tissues [2]. This ligament consists of multiple fascicles, whose basic unit is collagen and is surrounded by connective tissue

referred to as the paratenon. Histologically, three regions can be distinguished within the ACL. The proximal aspect is highly cellular, rich in round and oval cells, and contains some fusiform fibroblasts, collagen type II, and glycoproteins such as fibronectin and laminin. The middle aspect, containing fusiform and spindle-shaped fibroblasts, is a special region of high-density collagen fibers, cartilage, and fibrocartilage [3]. The distal aspect is rich in chondroblasts and oval fibroblasts and contains low-density collagen bundles. The fibroblasts on both sides of these collagen bundles are round to oval in shape [4]. Two types of fibrils were defined by Strocchi and colleagues. The first type of fibrils is secreted by fibroblasts, has irregular outlines with variable diameters, and constitutes 50.3% of the entire ACL. The second type is a uniform type with regular margins, and these fibrils, secreted by fibro-chondroblasts, constitute 43.7% of the ACL. These small homogeneous fibrils maintain the three-dimensional organization of the ligament [5]. Cells and matrix components make up the remaining 6% of the ACL tissue [6].

Across different joint tissues, the articular cartilage appears to undergo both mechanical and age-related degeneration [7]. The ACL is especially necessary for knee kinematics in rotational movements and also functions as a stabilizer in anterior and posterior gliding movements [8, 9]. The ACL, being the most commonly injured ligament of the knee, resists medial rotation, especially in the first 30° of knee flexion [10], as well as resisting varus and valgus strains.

In patients with gonarthrosis, conservative treatment methods such as activity restriction, weight loss, use of instruments such as crutches or a cane, anti-inflammatory drugs, intra-articular injections, and physical therapy are applied as the first-line approach while various surgical treatment options are applied in cases where such conservative treatment fails [11]. Total knee arthroplasty is indicated in patients with advanced osteoarthritis who do not benefit from the aforementioned conservative treatment options [11, 12]. Arthroplasty is typically applied to patients aged 60 years and over with Stage 3 and Stage 4 gonarthrosis. In total knee arthroplasty operations, while the ACL is cut routinely, the posterior cruciate ligament may be cut or preserved depending on the prosthesis design [13].

The present study aims to investigate the presence of degeneration and evaluate the relationship between the stage of gonarthrosis and degeneration in the ligament using histopathological examination of ACL samples excised from patients undergoing total knee arthroplasty for gonarthrosis.

2 | Methods

This study has been approved by the Local Ethics Committee as per the decision dated 02/22/2022 with the number 2020/143/06/05. All patients provided written informed consent before participating in the study. Radiographs taken during routine investigations of the 47 patients included in the study were evaluated, and these patients were stratified into two groups based on having Stage 3 or Stage 4 disease. Stage 3 patients constituted Group 1, and Stage 4 patients constituted Group 2. The team who performed the histological examinations performed these evaluations in a blinded fashion.

2.1 | Inclusion Criteria

Patients over 50 years of age who have advanced gonarthrosis were included in the study.

2.2 | Exclusion Criteria

Patients with chronic conditions other than hypertension, those without an anterior cruciate ligament, patients who had previously undergone surgical intervention (including arthroscopy) in the same knee, those with a history of infection in the same knee, patients with femoral or tibial fractures of the same extremity, and those with multiple ligament instability were not included in the study.

2.3 | Radiological Evaluation

Knee X-rays of the patients were evaluated and classified according to the Kellgren-Lawrence classification system [14]. Joint narrowing, osteophytic lesions, subchondral cysts, and bone contour deformities were evaluated with this classification system [14].

2.4 | Surgical Procedures and ACL Harvest

Preoperative antibiotic prophylaxis was administered to each patient. The patients were placed in the supine position, anesthesia was applied, and a tourniquet was placed in each case. After appropriate surgical site cleansing, sterile drapes were placed. A skin incision was made through the midline of the patella. The medial parapatellar approach, which is the standard procedure in total knee arthroplasty, was used for arthrotomy. After the patella was tilted laterally with the knee in extension, the joint space was exposed by positioning it in 90° flexion. At this point, the ACL was released from the femoral and tibial junctions with a scalpel and preserved as a sample for pathological examination. The subsequent stages of the surgery were applied in their respective order, and finally, the prosthesis was implanted.

2.5 | Histopathological Examinations

Tissue sections were taken from the proximal part of the ACL. All the ACL samples were placed in 20 mL of sterile 10% formalin in a plastic container to be transferred to the pathology department. The samples were then dehydrated, embedded in paraffin, and cut to obtain 4-μm sections. One section from each sample was routinely stained with hematoxylin and eosin (H&E) (Figure 1A). The two other sections were stained with Alcian blue (Figure 1A)(pH 2.5) (Rtu, Bio-Optica, Milan, Italy) and Masson's trichrome (Figure 1B) (Rtu, Ventana, Tucson, AZ, USA) on the Ventana BenchMark Special Stains automated slide stainer (Roche Diagnostics, A.S, Istanbul, Turkey). The samples were stained with Masson's trichrome to highlight fibrosis [15] and with Alcian blue to highlight glycosaminoglycan (GAG) degeneration. All histopathological examinations were performed using an Olympus CX41 (Olympus, Japan) light microscope and

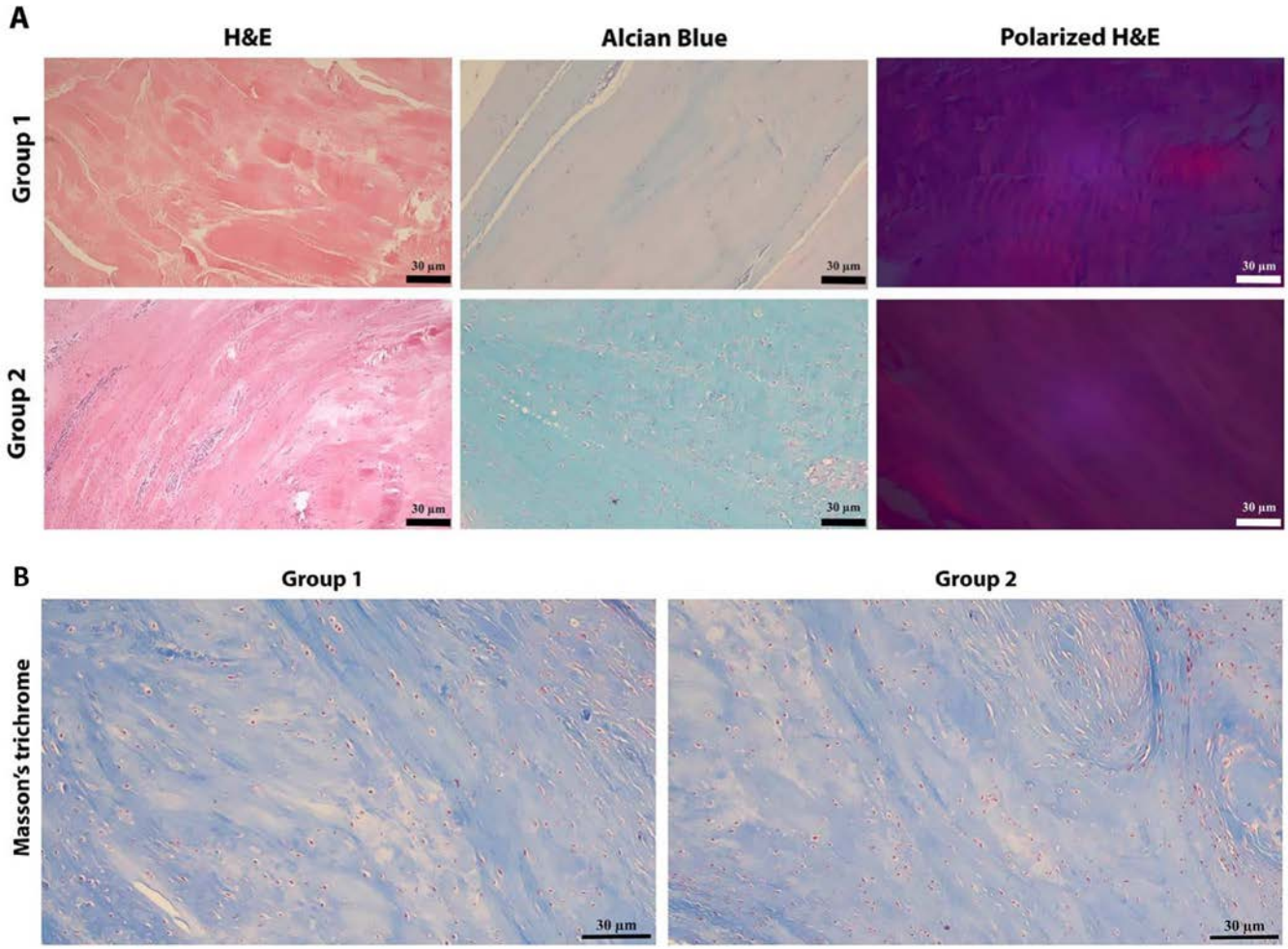

FIGURE 1 | A Histopathological images of ACL (400×). B Masson's trichrome stain (400×).

an image analysis system (Kameram Gen III Image Analysis Software, Istanbul, Turkey). The samples stained with H&E were evaluated using both polarized and nonpolarized light microscopy.

Each slide was evaluated with a semi-quantitative scale of Bonar and Movin scores modified by Maffulli as described elsewhere [16, 17]. The variables included in the Movin scale are (1) fiber structure; (2) fiber arrangement; (3) rounding of the nuclei; (4) regional variations in cellularity; (5) increased vascularity; (6) decreased collagen stainability; (7) hyalinization; and (8) GAG content [17]. Each variable was scored between 0 and 3, with 0 being normal, 1 slightly abnormal, 2 abnormal, and 3 markedly abnormal. The slides stained with H&E were used to evaluate the first seven variables, and those stained with Alcian blue were used to evaluate GAG content. The total semi-quantitative histological score for a given slide could vary from 0 (normal tendon) to 24 (the most severe abnormality detectable) [17] (Figure 1A). The variables included in the Bonar scale are (1) cell morphology; (2) ground substance; (3) collagen; and (4) vascularity. In this scale, a 4-point scoring system is used, where 0 indicates normal appearance and 3 indicates markedly abnormal appearance. Overall, the total score for a given slide may range from 0 (normal tendon) to 12 (the most severe abnormality detectable) [17] (Figure 1A).

For the evaluation of fibrosis, the thickness increase of the fibers was scored between 0 and 3 (Figure 1B).

2.6 | Statistical Analyses

The SPSS 23.0 statistical package program (version 23.0; SPSS Inc., Chicago, IL, USA) was used to transfer the data obtained in this study to the computer environment and for statistical analyses. While evaluating the data, first, a test to check normal distribution was applied to all data. The data with normal distribution were presented as mean ± standard deviation (x ± SD). The data that were not normally distributed were presented as median values. An independent sample *t*-test was applied to the data with normal distribution. The Mann–Whitney U test was used for the data that were not normally distributed. The Chi-square test was used in the comparison of categorical variables. $p < 0.05$ was considered statistically significant.

3 | Results

Twenty-two of the 47 patients included in the study had Stage 3 disease and were allocated to Group 1, while 25 had Stage

4 disease and were allocated to Group 2. The mean age was 67.13 ± 6.79 years in Group 1 and 67.8 ± 6.75 years in Group 2. Group 1 consisted of 16 female and 6 male patients. Group 2 consisted of 18 female and 7 male patients. In Group 1, the affected knee was the right knee in 13 of the patients and the left knee in 9. In Group 2, the affected knee was the right knee in 14 of the patients and the left knee in 11 (Table 1).

TABLE 1 | Demographics of patients with gonarthrosis by stage.

Stage		Group 1 ($n = 22$)	Group 2 ($n = 25$)	p
Age (year) (mean ± SD)		67.13 ± 6.79 (57–79)	67.8 ± 6.75 (52–77)	0.8
Gender	Female (%)	16 (47.1%)	18 (52.9%)	0.95
	Male (%)	6 (46.2%)	7 (53.8%)	
Side	Right (%)	13 (48.1%)	14 (51.9%)	0.83
	Left (%)	9 (45%)	11 (55%)	

The microscopic examination revealed mild degeneration in the fiber structure and fiber arrangements of patients with Stage 3 and Stage 4 disease. A mild-to-moderate loss of stability in collagen bundles was detected in polarized examinations. Mildly increased cellularity was observed homogeneously throughout the tissues. In the samples stained with Masson's trichrome, moderate fibrosis was detected in both groups. Alcian-blue staining showed mild GAG degeneration.

The mean total semi-quantitative Movin score was 4.7 ± 2.8 in Group 1 and 4.4 ± 3.5 in Group 2 (Figure 2A). The mean values of the parameters included in the semi-quantitative Movin scoring are shown in Figure 2A, and the distribution of the parameters is presented in Table 2. According to the total semi-quantitative

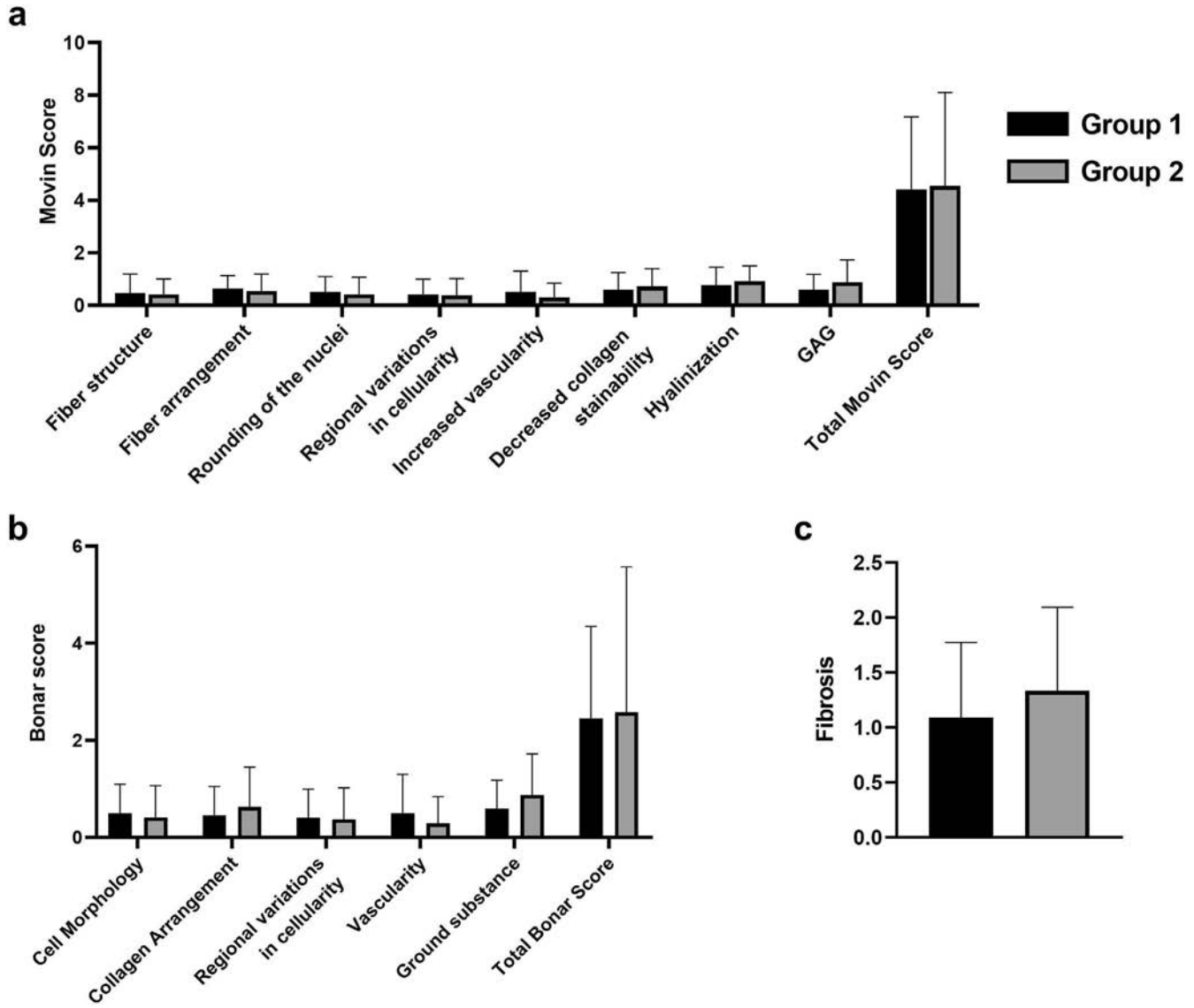

FIGURE 2 | (a) Comparative mean semi-quantitative Movin scores by group. (b) Comparative mean Bonar scores by group. (c) Comparative mean fibrosis scores by group.

Movin scores, the result was normal in 3 patients in Group 1, whereas mild degeneration was detected in 16 patients and moderate degeneration in 3. As for Group 2, the result was normal in 3 patients, while there was mild degeneration in 19 patients and moderate degeneration in 3 (Table 3). Based on these total semi-quantitative Movin scores, patients in Group 2 had more histological changes compared to Group 1, although without a statistically significant difference (p:0,96).

TABLE 2 | Distribution of ACL pathologies by Movin score.

	Group 1 ($n=22$)				Group 2 ($n=25$)				
Variable	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	***p***
Fiber structure	14	5	3	0	16	8	1	0	0.44
Fiber arrangement	8	14	0	0	13	10	2	0	0.15
Rounding of the nuclei	11	10	1	0	17	6	2	0	0.29
Regional variations in cellularity	13	8	1	0	18	5	2	0	0.43
Increased vascularity	14	5	2	1	18	6	1	0	0.62
Decreased collagen stainability	10	10	2	0	11	11	3	0	0.94
Hyalinization	7	12	3	0	6	16	3	0	0.79
GAG	9	12	1	0	10	10	4	1	0.41

TABLE 3 | Distribution of ACL pathologies by total semi-quantitative Movin score.

	Group 1 ($n=22$)	Group 2 ($n=25$)
Normal (0)	3 (13.6%)	3 (12%)
Mild (1–8)	16 (72.7%)	19 (76%)
Moderate (9–17)	3 (13.6%)	3 (12%)
Severe (18–24)	0 (%)	0 (%)

The mean total Bonar score was 2.6 ± 1.9 in Group 1 and 2.5 ± 2.9 in Group 2 (Figure 2B). The mean values of the parameters included in Bonar scoring are shown in Figure 2B, with the distribution presented in Table 4. In Group 1, the total Bonar scores showed a normal result in 3 patients, mild degeneration in 15 patients, and moderate degeneration in 4. As for Group 2, 6 patients had a normal result, while 13 had mild degeneration, 5 had moderate degeneration, and 1 patient had severe degeneration (Table 5). Based on these Bonar scores, patients in Group 2 had more histological changes compared to Group 1, although there was no statistically significant difference (p:0,55).

According to Bonar scoring, 13.6% of Group 1 patients were normal, 68.2% were mild, 18.2% were moderate, and 0% were severe, while 24% of Group 2 patients were normal, 52% were mild, 20% were moderate, and 4% were severe (Table 3).

According to total Movin scoring, 13.6% of Group 1 patients were normal, 72.7% were mild, 13.6% were moderate, and 0% were severe, while 12% of Group 2 patients were normal, 76% were mild, 12% were moderate, and 0% were severe (Table 5).

A comparison of Group 1 and Group 2 in terms of fibrosis (Figure 2C, Table 4) revealed that there were more histological changes in Group 2; however, the difference was not statistically significant (p:0,69).

4 | Discussion

There is only a limited number of histopathological studies in the literature on the degeneration of the anterior cruciate ligament in patients with advanced gonarthrosis [18, 19]. In the present study, we used the Bonar and Movin scoring systems and detected mild-to-moderate degeneration in patients with advanced gonarthrosis. We also observed that this degeneration did not increase significantly with the increasing degree of gonarthrosis.

Although aging and trauma are considered the strongest risk factors for osteoarthritis (OA) [20], studies are reporting that the absence of ACL is also an important cause of osteoarthritis

TABLE 4 | Distribution of ACL pathologies by Bonar and fibrosis scores.

		Group 1 ($n=22$)				Group 2 ($n=25$)				
Variable		**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	***p***
Bonar	Cell Morphology	11	10	1	0	17	6	2	0	0.29
	Collagen arrangement	12	9	1	0	15	5	5	0	0.13
	Regional variations in cellularity	13	8	1	0	18	5	2	0	0.43
	Vascularity	14	5	2	1	18	6	1	0	0.62
	Ground substance	9	12	1	0	10	10	4	1	0.41
Fibrosis		4	12	6	0	4	11	9	1	0.69

TABLE 5 | Distribution of ACL pathologies by total Bonar score.

	Group 1 ($n = 22$) (%)	Group 2 ($n = 25$) (%)
Normal (0)	3 (13.6%)	6 (24%)
Mild (1–4)	15 (68.2%)	13 (52%)
Moderate (5–10)	4 (18.2%)	5 (20%)
Severe (11–15)	0 (0%)	1 (4%)

[7]. Due to the absence of ACL deficiency as an exclusion criterion in our study, the impact of ACL deficiency on gonarthrosis was not evaluated in this study. A 2021 study conducted in patients with ACL damage whose knees were evaluated with magnetic resonance imaging (MRI) showed rapid progression of cartilage degeneration, especially in the medial tibia, compared to patients with a normal ACL [21]. There are conflicting publications on the reduction of gonarthrosis after reconstruction in patients with ACL rupture [22, 23]. Thomas et al. reported that OA developed in approximately 40% of patients after ACL reconstruction [24]. ACL reconstruction is performed in patients to address their existing instabilities and improve functional scores, allowing them to return to their preinjury activity levels. Patients who underwent ACL reconstruction were not included in this study.

ACL has been evaluated in several studies in patients with gonarthrosis [19]; however, degeneration by stage has not been investigated in patients with advanced gonarthrosis. In the present study, ACL samples were collected from patients with Stage 3 and Stage 4 gonarthrosis during arthroplasty and examined histopathologically. Our findings support the presence of ACL degeneration in patients with Stage 3 and Stage 4 gonarthrosis; however, no significant differences were found between these two groups.

Histological analysis is a reliable method to assess the degree of ACL degeneration [18, 19, 25]. Movin and Bonar scoring systems are commonly used for the analysis of tendon histopathology, providing a semi-quantitative measure of tissue degeneration [17, 26–28]. Both are highly correlated and assess similar characteristics [17]. Currently, Movin or Bonar systems are used as repeatable scales [27].

Although there are several scoring systems used for the evaluation of the anterior cruciate ligament, none of them appear to be a routinely used approach [28]. Different scoring systems have been used in different publications [29, 30]. Mullaji et al. in their study, correlation was made by comparing histological findings with radiological findings while examining the cruciate ligaments in knees with arthrosis [30]. They found a significant positive correlation between the radiologic grade of arthritis and the degeneration of the ACL. However, a subjective classification was used to determine the histological findings. Without scoring systems, it may be difficult to classify the findings or grade their severity. Allain et al. also perform macroscopic and histological evaluations, but the lack of a scoring system can lead to subjective interpretations [29]. Especially for histological evaluations, classifying the changes in the ligament structure to a certain degree increases the comparability of different samples and makes the results more objective. Both studies effectively examine the effects of osteoarthritis on ligament tissue, however, without a specific histological scoring system. Scoring systems help to make histological evaluations more systematic and objective. Since these scoring systems lack the sufficient level of objectivity seen in Movin and Bonar scores, there is no specific scoring system to evaluate the ACL. Because the ACL has similar histological features to the tendon, Movin and Bonar scoring systems were preferred in the present study. To the best of our knowledge, this is the first study to use Movin and Bonar scores for the histopathological evaluation of ACL.

In 2001, Allain and colleagues evaluated ligaments by grouping them into four stages [29]. In 2008, Mullaji et al. stratified the anterior and posterior cruciate ligaments into five stages, varying from no change (0) to severe changes (3) [30]. The incidence of cruciate ligament degeneration in osteoarthritic patients was higher in the study by Mullaji et al. compared to the study by Allain et al. [29, 30]. They attributed this finding to the fact that tricompartmental osteoarthritis was more common in the patients included in their study. In the present study, 25 patients had tricompartmental osteoarthritis, while the others had bicompartmental osteoarthritis. The different results may reflect this difference in patients.

When Group 1 patients were graded according to their total Movin scores, mild degeneration was achieved in 72.7% of the patients, while this rate was 76% in Group 2. None of the patients had a score reflecting severe degeneration. In total Bonar scores, mild degeneration was seen in 68.2% of patients in Group 1 and 52% of patients in Group 2. The reason why these two scoring systems yield different percentages is that the criteria and unit ranges evaluated in Movin and Bonar are different.

Some patients undergoing total knee arthroplasty exhibit intact anterior cruciate ligaments [31]. Not all patients with total gonarthrosis demonstrate equally distributed arthrosis in all three compartments; particularly, the lateral compartment may not present arthrosis at the same level. Theoretically, these patients could be adequately treated with a bicompartmental knee arthroplasty, minimizing intraoperative blood loss and preserving cruciate ligaments, thereby maintaining the natural kinematics of the knee [32, 33]. Some authors have advocated for bicompartmental knee replacement over total knee arthroplasty due to its association with reduced blood loss and faster rehabilitation [34]. Hence, bicompartmental surgery can be considered in selected cases [31, 35–38].

In this case report, since the patient's ACL was healthy, instead of a total knee prosthesis, a three-compartment unicondylar knee prosthesis was applied, preserving the ACL [39]. As seen in our study, some of our patients who underwent total knee prostheses had healthy ACLs. The tricompartmental arthroplasty applied in this case can be applied as a treatment option for patients with healthy ACLs.

In a 2003 study, only 26% of the patients with osteoarthritis were found to have normal histopathological features of the ACL. However, the control group of that study consisted of bone bank donors, patients with above-knee amputation, and ACL samples

from cadavers [19]. In a study Mont et al. conducted with 174 patients, ACL was found to be intact in 43 patients, worn in 85, and torn in 15, while ACL was absent in 31 knees [25]. In their study, 94 patients had Stage 3 disease, and 49 patients had Stage 4 disease. The study did not report in detail what percent of the patients classified as Stage 4 had ruptured ACL. We believe they obtained different results from ours due to the fact that our study did not include patients with ruptured ACL. In the same publication, it was emphasized that 85% of the cases had histological changes [25]. In the present study, histopathological changes were detected in 86.2% of Stage 3 patients and 88% of Stage 4 patients.

Our study is not without limitations. The limitations include the small sample size, that is, 47 patients included in the study, and the fact that MRI results were not available since MRI scans could not be requested in patients with advanced gonarthrosis owing to the retrospective design. The pre- and postoperative clinical examinations of the patients were not included as it was thought that they would not affect the results of this study. Furthermore, Stage 1 and Stage 2 patients could not be included since arthroplasty is not indicated in those groups. Finally, the histopathological examination of the posterior cruciate ligament was not included in our study as the prosthesis implanted during the surgical intervention in patients with gonarthrosis is often one that protects the posterior cruciate ligament.

In this study, patients with Stage 3 and Stage 4 gonarthrosis were evaluated, and their ACL samples were examined histopathologically. According to total Bonar and total Movin scores, the findings have shown that there is no statistically significant increase in ACL degeneration with increasing disease stage in patients with gonarthrosis. Further research is warranted to confirm these findings.

5 | Conclusion

The present study has shown that, according to histopathological evaluation, there is no increase in ACL degeneration similar to the increase in severe degeneration of cartilage with the increasing stage of gonarthrosis. We believe that our study will contribute to the literature since there is only a limited number of studies that include the histopathological evaluation of ACL in patients with advanced gonarthrosis.

Author Contributions

Elif Polat performed formal analysis, investigation, data curation, visualization, and contributed to conceptualization, methodology, validation, and writing, reviewing, and editing of the original draft. Burak Günaydin contributed to conceptualization, methodology, validation, supervision, reviewing, and editing of the original draft. Sevil Karabağ contributed to conceptualization, methodology, supervision, writing, reviewing, and editing of the original draft. Nurettin Heybeli contributed to methodology, validation, supervision, writing, reviewing, and editing of the original draft. All authors read and approved the final manuscript.

Acknowledgments

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Research data are not shared.

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ORIGINAL ARTICLE

Old Age, B Cell Function and Count Are the Critical Factors for Predicting Infection Risk in Patients With Autoimmune Rheumatic Diseases Undergoing Immunosuppression: A Cohort Study

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ABSTRACT

Objective: The study aims to assess baseline immune parameters that predict infection risk in autoimmune rheumatic disease (ARD) patients, with the goal of identifying high-risk individuals requiring immunosuppressive therapy escalation, based on infection rates during a one-year follow-up.

Methods: The independent cohort study was conducted at a tertiary rheumatology center in India from December 2019 to March 2022. It included adult participants with ARDs undergoing immunosuppression. Ethics approval and informed consent were obtained. Patients underwent detailed history, clinical examination, and baseline investigations, which included complete hemogram, inflammatory parameters, immunoglobulin levels, cellular levels of the immune system, complement levels, and viral markers. Descriptive statistics, ANOVA, chi-squared tests, *t*-tests, and Fisher's exact tests were used. OLS regression analyses identified significant predictors of infection risk. They were followed up for a period of 1 year for any infection episodes.

Results: Of the 106 participants recruited, 4 were excluded due to disease-related complications during the 3-month period of follow-up. The mean age of the participants was 38.21 ± 12.73 years, with an average follow-up duration of 13.1 ± 8.35 months. Among the remaining 102 participants, younger age was associated with a lower infection risk (OR 1.047). Protective factors against infection included lower levels of immunoglobulin E (IgE) (OR 0.379), methotrexate (MTX) use (OR 0.247), and biologics (OR 0.543). Conversely, lower Immunoglobulin G (IgG), elevated neutrophil counts (OR 3.588), higher neutrophil-to-lymphocyte ratios (NLR) (OR 2.577), low platelet counts (OR 0.546), and steroid use, which increased the risk fivefold (OR 5.686), were identified as risk factors. Ordinary Least Squares (OLS) regression analysis highlighted age, IgG levels, CD19 lymphocyte counts, WBC counts, and ESR as significant predictors of infection risk between the groups.

Conclusion: Older age, low IgG, low B cell count (CD19) predict susceptibility to infections; high neutrophil counts, low platelets, and elevated NLR are key predictors of developing infection in ARDs patients. Careful monitoring and tailored treatment strategies are essential to reduce infection risks. Further research is needed in this direction to develop predictive algorithms.

1 | Introduction

Autoimmune rheumatic diseases (ARDs) have seen a significant rise in incidence in recent decades, driven by factors such as improved diagnostics, increased awareness, environmental influences, genetic and epigenetic factors, aging, and lifestyle changes [1]. Globally, the prevalence of ARDs has increased from 3.2% to 19.1% since 1965, with women accounting for over 85% of cases [2, 3]. Correspondingly, global healthcare expenditure on ARDs has also risen dramatically, reaching $127 billion in 2021, up from $49 billion in 6 years [4].

Immunosuppressive therapies (IST), particularly novel biological treatments targeting specific immune pathways, have become key in managing ARDs. These therapies help reduce tissue damage, alleviate symptoms, improve quality of life, and increase survival rates [5, 6]. However, they carry significant risks, including infections, which are a leading cause of morbidity and mortality in patients. Other potential adverse effects include non-immune toxicities, neoplasms, and immune-related reactions, all of which complicate the management of these diseases [7–12].

Effective immune monitoring is crucial for tailoring patient care and assessing infection risk, especially in those undergoing immunosuppressive therapy. Routine tests, such as leukocyte counts, may be beneficial, but more specific markers, such as Immunoglobulin G (IgG) levels and lymphocyte subsets, offer better predictions of infection susceptibility [13–16]. While not all patients experience increased infection risks, those with increased susceptibility require closer monitoring. In patients with ARDs, escalation of IST may be necessary when they do not respond to lower doses. However, pre-assessment guidelines at this critical point are lacking in the literature. This study aims to develop an infection prediction index by analyzing baseline immune parameters to identify high-risk patients who may require IST escalation. The goal is to improve patient outcomes and optimize healthcare resources through early identification and targeted intervention.

2 | Materials and Methods

2.1 | Study Population

The independent cohort study was conducted at a tertiary referral center for rheumatology in India between December 2019 and March 2022. The study duration was extended due to the COVID-19 lockdown. It involved adult outpatients and inpatients diagnosed with ARDs, who required escalation in their immunosuppression. Escalation was defined as adding or escalating mycophenolate dosage, cyclophosphamide, rituximab, or increased dose of steroids. Patients with comorbidities such as diabetes mellitus, hypertension, and ischemic heart disease were included, provided these conditions were well controlled and did not significantly impact their quality of life. The study excluded patients with conditions that significantly increased infection risk, had active infections, those unable to communicate, individuals with other causes of immunodeficiency (hereditary or acquired), pregnant women, and those who refused consent or were unwilling to follow up for a year. Institutional ethics committee approval was obtained prior to the initiation of the study (IEC-CRICR/SN-122/015/2020).

2.2 | Sample Size Calculation

As a preliminary and exploratory investigation, an initial sample size of 100 was planned, aiming to create 2 groups.

2.3 | Data Collection

The study collected baseline immunological data at the time of recruitment, prior to the escalation of immunosuppressive therapy, in accordance with the inclusion criteria. This included detailed patient histories of serious infections and immunizations, as well as comprehensive physical examinations. Baseline immune parameters assessed included complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and measurements of immunoglobulin levels (IgA, IgM, IgE, and IgG). Complement levels (C3, C4) were measured using turbidometry, while cellular markers, such as CD4, CD3, CD8, B cells (CD19), and natural killer cells (CD56), were quantified via flow cytometry. Immunization history was evaluated through antibodies to hepatitis B surface antigen (anti-HBs) and Rubella IgG. Virological status for CMV, herpes simplex virus (HSV), EBV, and varicella-zoster virus (VZV) was determined by measuring specific IgM and IgG levels for each.

Patients were monitored over 1 year to track infection episodes, treatments, and hospitalizations, both at the current center and other healthcare facilities. Participants were categorized based on their diagnoses (SLE, scleroderma, or other conditions), and relevant data were recorded. During the follow-up period, participants were classified into five groups according to the Common Terminology Criteria for Adverse Events (CTCAE): those with mild, asymptomatic, or no infections requiring no intervention; those with moderate infections requiring minimal local or non-invasive treatments; those with severe infections that were medically significant but not immediately life-threatening; those requiring hospitalization or experiencing life-threatening infections; and those who died from an infectious cause. For analysis, participants were subsequently grouped into two main categories based on the above classification: Group 1 included individuals with no infections or mild infections, while Group 2 comprised individuals with moderate to severe infections [17].

Immunoglobulin levels (IgA, IgG, IgM, and IgE); T-cell markers (CD3, CD4, CD8); CD19; CD56; complements (C3, C4); hemoglobin; white blood cell count (WBC); platelet count; and percentages of neutrophils and lymphocytes were classified into normal, below range, and above range categories based on standard reference levels. The neutrophil-lymphocyte ratio (NLR), ESR, and CRP were categorized as normal, high, or significantly high [18, 19]. IgG and IgM levels for Rubella, HSV (1 and 2), CMV, and EBV were classified as positive or negative for infection, with participants grouped into four categories: not infected, infected but not active, reactivation, and acute infection.

For VZV, participants were categorized as having active or inactive infections, while anti-HBs titers were classified as

vaccine-reactive or non-reactive. Rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), antinuclear antibodies (ANA), and ANA profile antibodies were recorded as positive or negative. Previous treatments, including exposure to steroids, disease-modifying antirheumatic drugs (DMARDs), and immunosuppressants, were documented. Steroid use was then compared between the two groups.

2.4 | Statistical Analysis

Data were collected and organized using MS Excel (Version 2409 Build 16.0.18025.20030). Descriptive statistics analyzed participants by disease diagnosis and compared two groups, those with no or minimal infections and those with increased infections—with categorical variables expressed as percentages and continuous variables reported as mean ± SD. Continuous data were evaluated using analysis of variance (ANOVA), while categorical data were analyzed using chi-square tests, t-tests, and Fisher's exact tests in SPSS version 21. For predictive analysis, ordinary least squares (OLS) regression was conducted for all variables and significant variables individually. Odds ratios and relative risks for no/minimal infections versus severe infections were calculated, and a regression table was generated using Python code in Jupyter Notebook (6.5.4). The Bonferroni correction was initially considered but was not applied in this study due to its overly conservative nature. Furthermore, the Bonferroni–Hochberg correction for multiple testing corrections was performed using the "Multiple Testing Corrections" website, where all the P-values were entered to generate the Hochberg adjusted value [20].

3 | Results

Of the 141 eligible patients, 106 agreed to participate in the study. The remaining patients either refused consent or were unable to attend the outpatient department (OPD) due to the COVID-19 pandemic or because they lived far from the center. During the 3-month follow-up, four enrolled patients died and were excluded from the analysis, as their deaths were attributed to primary disease-related complications rather than infections. As a result, 102 patients were included in the final analysis, with a mean age of 38.21 ± 12.73 years and an average follow-up duration of 13.1 ± 8.35 months. At the time of recruitment, patients were on the following medications: 32 participants were on methotrexate (MTX), 30 participants on mycophenolate mofetil (MMF), 71 participants on hydroxychloroquine (HCQ), 71 participants on low to moderate-dose steroids, and 16 on biologics. Among these, 61 patients received rituximab (RTX) at a dose of 1–2 g per cycle. Additionally, 7 patients were treated with higher-dose MMF (2–3 g/day), 80 received steroids at doses greater than 1 mg/kg body weight, and 7 patients received cyclophosphamide. The classification of patients based on diagnosis revealed 58 with SLE, 4 with RA, 18 with systemic sclerosis, 7 with myositis, 6 with ANCA-associated vasculitis, 3 with Sjögren's syndrome, and 10 with other undifferentiated connective tissue diseases that do not meet any specific diagnostic criteria (Table S1).

Comparison between the two infection groups revealed that the infection risk was lower in the younger age group, with a mean age of 35.73 ± 11.77 years in the no or minimal infection category and 42.61 ± 12.85 years in the moderate to severe infection category (OR 1.04). The presence of baseline ARDs did not show a specific tendency to increase infection rates. Gender, with a predominance of females (66 in the no or minimal infection group and 29 in the moderate to severe infection group), was not a significant risk factor (OR 0.910). Lower IgG levels were associated with a higher infection risk, as individuals with below-normal IgG levels were significantly more likely to experience increased infections (OR 0.367). Lower IgE levels (< 100 IU/mL) were linked to a decreased risk of infection (OR 0.379). Higher neutrophil counts (OR 3.588) and elevated NLR (OR 2.577) were associated with a greater risk of infection, especially when these measures exceeded normal thresholds. A lower platelet count increased the risk of infection (OR 0.546). MTX demonstrated a protective effect against infection (OR 0.247). Notably, over 90% of patients with increased infection susceptibility were on steroids, compared to just 70% in the group with fewer infections (OR 5.686), indicating that steroid use increases infection risk by fivefold compared to other immunosuppressants. Although the use of biologics was not statistically significant, it showed a trend toward reduced infection risk (OR 0.543) (Table 1).

Other parameters, including diagnosis, IgM levels, T cell markers (CD3, CD3 lymphocytes, CD4, CD4%, CD8, CD8%, CD4/CD8 ratio), CD19, NK cell markers (CD56, CD56 lymphocytes), anti-HBs titers, rubella antibodies, CMV antibodies, HSV (1 + 2) IgM and IgG antibodies, EBV IgG, hemoglobin, WBC, lymphocytes, ESR, VZV IgM, C3, C4, and prior medication history were not statistically significant (Table S2).

The OLS regression analysis for parameters with a p-value threshold of < 0.25 showed that age, IgG, CD19 lymphocytes, WBC, and ESR had statistically significant effects in predicting infections between the two groups. Advanced age and leukocytosis were associated with an increased risk of infections, while higher levels of IgG and CD19 lymphocytes were linked to a lower risk (Table 2). Further details of the OLS regression analysis are available in Tables S3 and S4.

The Bonferroni–Hochberg correction yielded a threshold value of 0.008. Age, IgE, and steroid had p values below this threshold. Hence, IgE was excluded, while the other two were considered moderately significant. However, relationships between predictive factors were evaluated using odds ratios and regression analysis. The majority of the results were consistent across these methods and demonstrated statistical significance.

4 | Discussion

The present study identified age, neutrophil count, IgG levels, platelet count, and steroid use during follow-up as significant predictors of infection risk. The predictive model from the regression analysis highlighted IgG, WBC, age, NLR, CD19 lymphocytes, and ESR as key factors, underscoring their importance in monitoring immune health and infection risk.

The study found that the risk of infection is higher in elderly patients with ARDs. This aligns with findings by Rua-Figueroa et al., who developed an enhanced scoring system to predict

TABLE 1 | Comparison of factors between no/minimal infections and moderate to severe infections.

Factors	No/minimum infections ($n=71$)	Moderate to severe infections ($n=31$)	p	Odds ratio (confidence interval)
Age (years)	35.73 ± 11.77	42.61 ± 12.85	0.010[b]	1.047 (1.010–1.085)[d]
Gender M (F)	5 (66)	2 (29)	> 0.999[c]	0.910 (0.167–4.968)[e]
IgG ($n=100$)				
Below (n)	4 (5.7%)	6 (20%)	0.008[c]	0.367 (0.187–0.720)[d]
Normal (700–1600 g/L)	24 (34.3%)	15 (50%)		
Above (n)	42 (60%)	9 (30%)		
IgE ($n=99$)				
< 100 IU/mL	32 (45.7%)	20 (68.9%)	0.035[a]	0.379 (0.152–0.948)[e]
> 100 IU/mL	38 (54.3%)	9 (31%)		
Neutrophils				
Below (n)	47 (66.2%)	10 (32.3%)	0.003[c]	3.588 (1.322–9.738)[d]
Normal (45–75 cells/μL)	23 (32.4%)	21 (67.7%)		
Above (n)	1 (1.4%)	0 (0%)		
NLR				
Normal (< 2 cells/μL)	11 (15.5%)	3 (9.7%)	0.003[c]	2.577 (1.250–5.312)[d]
High (2–4 cells/μL)	35 (49.3%)	6 (19.4%)		
Significantly high (> 4 cells/μL)	25 (35.2%)	22 (70.9%)		
Platelets				
Below (n)	8 (11.3%)	11 (35.5%)	0.004[c]	0.546 (0.225–1.322)[d]
Normal (1.5–4.5 cells/μL)	59 (83.1%)	16 (51.6%)		
Above (n)	4 (5.6%)	4 (12.9%)		
Present medication history				
Methotrexate	35 (49.3%)	6 (19.35%)	0.005[a]	0.247 (0.090–0.675)[e]
Leflunomide	5 (7.04%)	0 (0%)	0.319[c]	NA
Mycophenolate mofetil	5 (7.04%)	2 (6.45%)	> 0.999[c]	0.910 (0.167–4.968)[e]
Hydroxychloroquine	60 (84.51%)	26 (83.87%)	0.935[a]	0.953 (0.301–3.020)[e]
Steroids	51 (71.83%)	29 (93.55%)	0.017[c]	5.686 (1.239–26.086)[e]
Biologics	51 (71.83%)	18 (58.06%)	0.172[a]	0.543 (0.225–1.311)[e]
Cyclophosphamide	5 (7.04%)	2 (6.45%)	> 0.999[c]	0.910 (0.167–4.968)[e]

Abbreviations: CRP, C-reactive protein; F, female; IgE, immunoglobulin E; IgG, immunoglobulin G; M, male; NA, not applicable; NLR, neutrophil-to-lymphocyte ratio.
[a]Chi-squared.
[b]t-test.
[c]Fisher's-exact test.
[d]Logistic regression.
[e]Crosstabs risk factor odds ratio.

serious infections in SLE patients, identifying age ≥ 60 years as a significant risk factor for severe infections in their adjusted multivariate model [21].

The current study demonstrated that lower IgG levels were associated with an increased risk of infection in patients with ARDs, particularly in those expected to receive immunosuppressants. This finding aligns with previous research by Li et al., who demonstrated that reduced IgG levels (≤ 12 g/L) could serve as a predictive marker for infections in patients with ARDs [22]. Additionally, the study suggests that higher neutrophil counts and elevated NLR are linked to an increased possibility of

TABLE 2 | Regression analysis of factors related to infections in ARDs.

Factors	Coef	Std err	*t*	$p > \|t\|$	[0.025	0.975]
Constant	1.0131	0.701	1.446	0.153	−0.386	2.412
Age	0.011	0.004	2.574	0.012	0.002	0.02
IgM	0.0185	0.156	0.119	0.906	−0.292	0.329
IgG	−0.24	0.084	−2.866	0.006	−0.407	−0.073
IgE	−0.0003	0.1	−0.003	0.997	−0.2	0.2
CD4	−0.1239	0.113	−1.097	0.277	−0.349	0.102
CD4/8 ratio	−0.0088	0.06	−0.148	0.883	−0.128	0.111
CD19 lymphocytes	−0.1437	0.063	−2.298	0.025	−0.269	−0.019
WBC	4.07E-05	1.66E-05	2.449	0.017	7.53E-06	7.40E-05
Neutrophils	0.1473	0.134	1.101	0.275	−0.12	0.414
Lymphocytes	0.011	0.013	0.87	0.387	−0.014	0.036
NLR	0.06	0.148	0.405	0.687	−0.235	0.355
Platelets	−0.1494	0.097	−1.547	0.126	−0.342	0.043
ESR	0.1495	0.07	2.124	0.037	0.009	0.29
CRP	0.0013	0.003	0.468	0.641	−0.004	0.007
C4	−0.0008	0.005	−0.148	0.883	−0.012	0.01

Abbreviations: C4, complement component 4; CD19, cluster of differentiation 19; CD4, cluster of differentiation 4; CD4/8, CD4 to CD8 ratio; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; NLR, neutrophil-to-lymphocyte ratio; WBC, white blood cells.

infection in patients with ARDs. Mokhtar et al. concluded that neutrophil counts can be a useful tool for the early diagnosis of infection in SLE patients [23]. Similarly, Buonacera et al. emphasized that elevated neutrophil counts and NLR are predictive factors for infections in ARDs. Elevated neutrophil levels and NLR have been observed in various conditions, including bacterial or fungal infections, acute stroke, myocardial infarction, atherosclerosis, severe trauma, cancer, post-surgical complications, and any situation involving tissue damage that triggers a systemic inflammatory response syndrome [24].

In line with the current study findings, Kim et al. found that NLR was significantly higher in SLE patients with infections compared to those experiencing disease flare-ups (14.2 ± 15.4 vs. 3.3 ± 2.2, $p < 0.001$) [25]. Moreover, D'Amico et al. reported that elevated NLR is associated with increased disease activity at the onset of relapsing–remitting multiple sclerosis [26]. Both Newman et al. and Tian et al. identified NLR as a marker of inflammation in various ARDs [27, 28]. Chandrashekara et al. also suggested that NLR may serve as a valuable and cost-effective biomarker for assessing inflammation in rheumatoid arthritis (RA). Unlike traditional markers such as CRP and ESR, NLR is less influenced by cytokines that can affect CRP and ESR levels [29]. The present study identified increased ESR as a predictive factor associated with a higher frequency of infections in patients with ARDs. Francisco et al. observed that elevated ESR is present in over 90% of patients with SLE and is associated with symptoms such as fever, fatigue, myalgias, and overall increased disease activity. Elevated ESR levels, whether mild, moderate, or significant, correlate with disease activity and damage accumulation [30]. Mokhtar et al. also highlighted that infections are common in SLE patients and that the ESR serves as a useful marker for the early diagnosis of infection [23]. Additionally, Mokhtar et al. emphasized that both ESR and neutrophil counts are critical tools in predicting the risk of infection in SLE patients [23]. Based on theoretical and clinical observations, patients with neutropenia are known to have a higher risk of infection due to lower WBC counts and altered NLR. However, the present study suggests that higher neutrophil counts and elevated NLR are linked to an increased infection risk in patients with ARDs, which may seem contradictory. This finding, however, does not disprove the inverse association; rather, it highlights that elevated WBC and NLR values may also act as predictors, emphasizing the importance of thorough assessment for possible occult infections in these patients. The current cohort was screened for the presence of any active infections, including chronic viral infections, such as CMV and EBV, and was found to have none at the time of enrollment and escalation of immunosuppression. Thus, the presence of high neutrophil counts and a high NLR may reflect asymptomatic infections. Conducting a study focused on a separate cohort of similar patients could provide valuable insights and guidelines for the diagnostic work-up of occult infections. Moreover, Lee Schwartzberg revealed that neutropenia is associated with a significantly increased risk of infections, particularly in individuals with severe neutropenia or certain underlying conditions [31].

The present study identified that a lower platelet count was associated with an increased risk of infection in patients with SLE. Ziakas et al. reported that thrombocytopenia is not directly

linked to end-organ damage or mortality; however, it identifies a subgroup of patients with increased morbidity, making it a significant complication that impacts overall prognosis [32]. Jin et al. concluded that the severity of thrombocytopenia in SLE patients may serve as a valuable independent prognostic factor for predicting survival [33].

CD19 counts are also significant predictors of infection susceptibility, with lower counts associated with an increased risk of infections. However, there are conflicting observations regarding the relationship between CD19 and infection susceptibility. Lazarou et al. observed that pre-therapy B cell counts were not reliable predictors of increased infection risk [34]. Conversely, Heusele et al. identified that SLE patients with a history of serious infections were significantly older, more likely to have diabetes mellitus, exhibited lower CD19 counts, and frequently initiated their first rituximab course while on prednisone doses greater than 15 mg/day [35]. The impact of low B cell counts is not solely dependent on their number; it has been shown to be influenced by factors such as the underlying disease, vaccine response, and coexisting immunoglobulin levels.

The present study also found that drugs, particularly steroids, appear to contribute to the risk of infection due to their immunosuppressive effects. The current study indicated that immunosuppressants, especially MTX, may have a protective effect against infection. They were moderately significant. However, a meta-analysis by Ibrahim et al. found that MTX was associated with an increased risk of infection in rheumatoid arthritis (RA) (RR: 1.25; 95% CI, 1.01–1.56; $p = 0.04$; $I^2 = 0\%$), suggesting a higher risk of infections specifically in RA patients [36]. Furthermore, Ponce et al. reported that MTX reduces the risk of severe dengue in RA patients [37]. The steroid doses varied throughout the entire treatment course (prior and current medication) hence, the study could not assess the effect of steroid dose on the risk of infection. Similarly, He and Li reported that impaired immunity, the use of immunosuppressants, and corticosteroids significantly elevate the risk of infection in SLE patients [38]. Ruiz-Irastorza et al. concluded that even moderate doses of prednisone elevate the risk of major infections in patients with SLE [39].

The strengths of this study lie in its comprehensive evaluation of infection risks in patients with ARDs undergoing therapy and requiring escalation of IST. By incorporating predictive models that encompass a wide range of immune parameters, the research enables precise stratification of patients based on infection risk. This approach facilitates the early identification of high-risk individuals, allowing for timely implementation of preventive measures or closer monitoring during therapy escalation. To address multiple testing bias, the Bonferroni–Hochberg correction was applied.

The use of ordinary least squares (OLS) regression analysis further enhances the identification of critical infection predictors. Moreover, the focus of the study on patients requiring IST escalation provides valuable insights into factors influencing infection risk during this crucial phase of treatment. By assessing a broad spectrum of immunological factors including both adaptive (B and T cells) and innate immunity (e.g., neutrophils, other immune lymphocytes)—alongside chronic viral infections (e.g., CMV, anti-HBs, rubella), the study offers a detailed and integrated view of immune status. This holistic approach not only identifies potential biomarkers and risk factors but also underscores key clinical changes, making the findings highly relevant for optimizing patient management.

However, the study has several limitations, including its reliance on cross-sectional data, which may not fully capture the evolving dynamics of infection risk over time, particularly in relation to changes in IST. The variability in participants' use of immunosuppressive medications (e.g., MTX, HCQ, MMF) could influence infection risk and complicate the interpretation of treatment-related outcomes. Some factors, such as specific immunological markers, showed no significant association with infection risk, possibly due to sample size constraints or measurement sensitivity. Moreover, the study did not extensively distinguish between subtypes of ARDs, which might have provided more detailed insights into infection risks across different disease manifestations.

Future research should focus on the long-term dynamics of infection risk concerning different immunosuppressive treatments and examine the specific effects of various ARDs subtypes. Further validation of immune parameters is crucial for developing robust and reliable predictive models.

5 | Conclusion

Advanced age, low IgG levels, low CD19 cell count, and the use of higher immunosuppressive drugs are predictors of increased susceptibility to infections. Additionally, high neutrophil counts, elevated NLR, and ESR are key predictors of infection development. Further studies are needed to explore algorithms using these parameters to identify patients who may require more intensive follow-up for infections.

Author Contributions

The design and conceptualization were undertaken by Dr. Chandrashekara S and Dr. Prakruthi Jaladhar, while the laboratory component of the study was supervised by Dr. Renuka Panchagnula. All authors contributed to data collection, patient recruitment, and manuscript writing, with overall guidance provided by Dr. Chandrashekara S. All authors had access to the data.

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Ethics Statement

It was an observational study; there were no proactive interventions in the treatment for the study purpose. The study was done in accordance with the tenets of the Declaration of Helsinki and ICMR's National Ethical Guidelines for Biochemical Research involving human participants 2017. The study was done after approvals from the Scientific review board and Institutional Ethics Committee (IEC-CRICR/SN-122/015/2020). *Research Involving Human Participants and/or Animals*: Yes. Involved human participants. All participants were enrolled after a prior written voluntary informed consent process in a language understood by the patients.

Consent

Administered to each participant by the investigator. Participation was voluntary, and no force or coercion was used to recruit participants.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Available and will be provided upon request.

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Supporting Information

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LETTER TO THE EDITOR

Acute Hemorrhagic Encephalomyelitis (AHEM) as an Initial Presentation of Neuropsychiatric Systemic Lupus Erythematosus (NPSLE)

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To the editor,

Hemorrhagic acute disseminated encephalomyelitis or acute hemorrhagic encephalomyelitis (AHEM) or Hurst's disease is a very rare and severe form of acute disseminated encephalomyelitis (ADEM) seen in young adults and children [1]. Nervous system involvement in systemic lupus erythematosus (SLE) is seen in two-thirds of patients and can occur at any stage of the disease. Neuropsychiatric systemic lupus erythematosus (NPSLE), also called neurolupus, has varied presentations including stroke, encephalopathy, cognitive impairment, cranial neuropathies, meningitis, acute transverse myelitis, and encephalitis. ADEM as a presentation of NPSLE is rare [2]. AHEM, especially as an initial presentation of neurolupus, is reported in very few cases [3]. We hereby report such a case in a 40-year-old previously healthy lady.

1 | Case

A 40-year-old female with no comorbidities presented with a history of low-grade fever for 4 days, followed by 1-day history of altered sensorium. There was a history of decreased appetite, myalgia, and joint pain for the previous 1 month. Initial evaluation for joint pain was positive for rheumatoid factor. There was no history of convulsions, vomiting, headache, or bleeding tendencies. On examination, the patient was drowsy but arousable to deep stimuli, not following commands, with left hemiparesis, no neck rigidity, and eye movements normal. MRI brain on presentation showed multifocal and confluent areas of T2/FLAIR hyperintense signals involving bilateral thalami (R>L), midbrain, and dorsal aspect of pons. There was also asymmetric involvement of bilateral middle cerebellar peduncles (L>R), external capsules, and left temporal white matter. Susceptibility-weighted imaging (SWI) showed hemorrhage within the central aspect of the thalamic signal changes (Figure 1). The remote possibility of thrombosis of the deep venous system was ruled out on CT venogram. A repeat MRI done 3 days later showed interval worsening of the vasogenic edema of the involved structures. MRI of the spine was normal. A differential diagnosis of acute viral encephalitis, acute necrotizing encephalopathy, or hemorrhagic ADEM was considered. On evaluation, Hb—12.4 g%, total count—3400, platelets—1.05 lakh/cmm. ESR—36/1st hr, CRP—36 mg/dL, urine routine showed nil protein, RBC—24-26/hpf, WBCs—8–10.hpf., and spot protein creatinine ratio was 1.6 g. Direct and indirect coomb's test was negative. Her S. creatinine was 0.7 mg/dL; liver and thyroid function tests were normal. Chest x-ray was normal. Routine microbiology evaluation for fever, including blood culture, dengue serology, malaria antigen test, Widal, and Weil–Felix test were negative. CSF study showed increased protein (323 mg/dL), without any significant cells in the CSF (5 cells/cmm). CSF meningoencephalitis panel (infection panel done by PCR) was negative. Serum NMO, MOG, NMDA, AMPA1 and 2, CASPR, LGI 1, and GABA B1 and B2 antibodies were negative. CSF Japanese encephalitis IgM antibody was negative. ANA (IF) was 3+, at 1:80 titers with a speckled pattern. ANA profile was strongly positive (3+) for RNP/Sm and RO 52 antibodies. Antiphospholipid antibody panel, C3 and C4 levels, and anti-dsDNA titers were normal. Serum and CSF autoimmune and paraneoplastic panels were negative. CSF oligoclonal bands were negative. A diagnosis of SLE was considered based on ANA and Sm/RNP positivity, with hematological, renal, and neurological manifestations. She fulfilled the newer ACR/EULAR criteria [4] Based on serological and

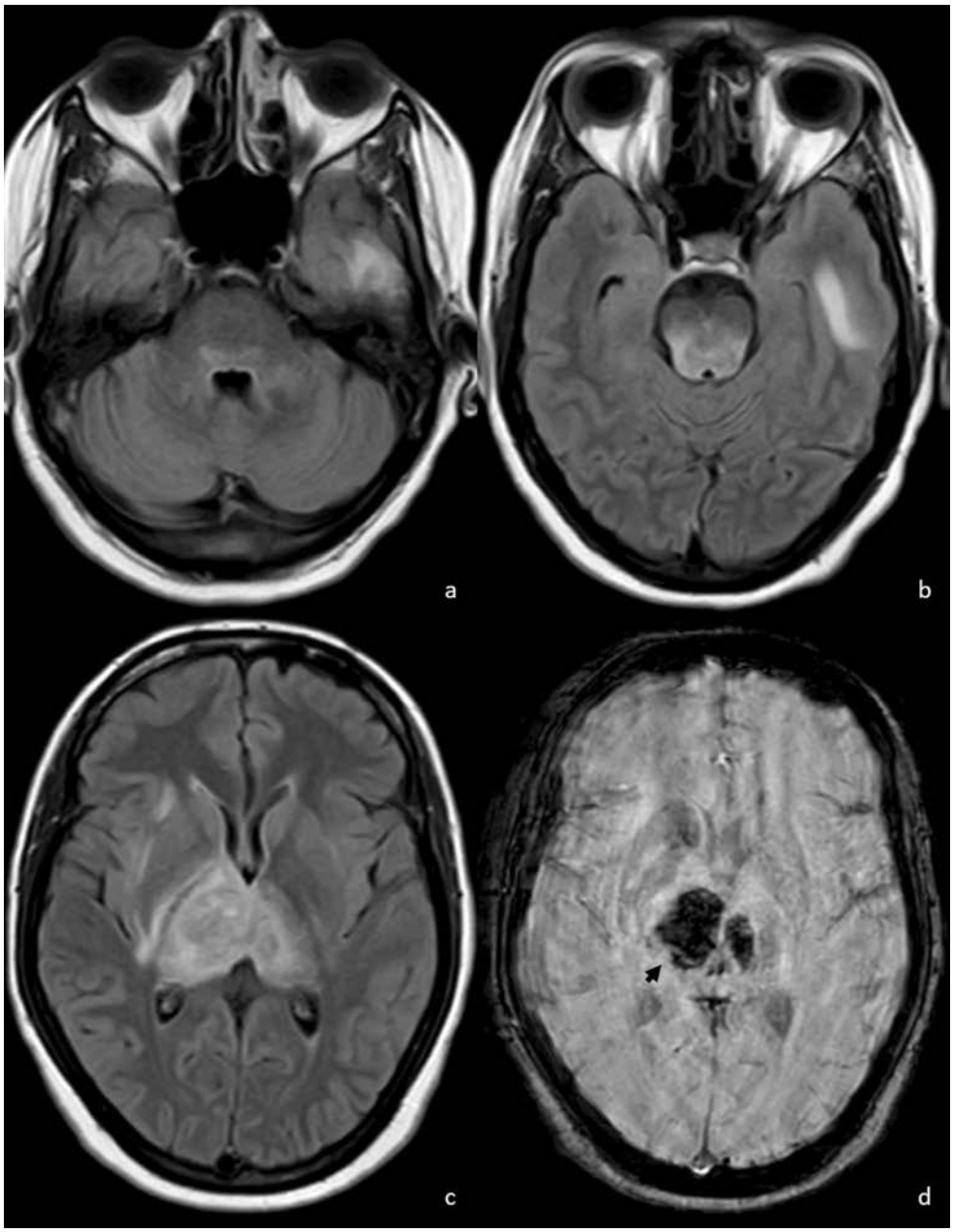

FIGURE 1 | MRI brain. FLAIR images showing asymmetric hyperintense signals involving dorsal midbrain and pons, bilateral middle cerebellar peduncles (a,b), thalami and external capsules, left temporal white matter (c). SWI (d) showing hemorrhagic changes within the thalami (arrow) bilateral (L>R).

imaging findings, a diagnosis of AHEM as a first presentation of NPSLE was considered.

She was treated with injection methylprednisolone 1 g IV for 5 days was given followed by oral steroids. She was given 1 g of rituximab, with two doses given 15 days apart. The patient had significant improvement in her neurological state, with complete improvement and no residual neurological deficits after 6 months of follow-up and is leading a normal life. A follow-up MRI 6 weeks after the initiation of immunosuppression showed complete resolution of vasogenic edema, except for signs of old hemorrhage in the thalami and dorsal midbrain (Figure 2). Her hematological and renal manifestations also recovered completely.

2 | Discussion

Acute disseminated encephalomyelitis (ADEM) is an autoimmune demyelinating disease of the central nervous system. It is usually a monophasic illness, more common in children; usually, the etiology is post-infectious or postvaccination, or idiopathic. ADEM can be a first presentation of multiple sclerosis or Neuromyelitis optica spectrum disorder [5]. Acute hemorrhagic encephalomyelitis is a rare fulminant immune-mediated inflammatory and necrotizing demyelinating disorder of the central nervous system. It was first described by Hurst [6]. It is more common in young adults than in children when compared to ADEM [1]. It is characterized by Acute to subacute multifocal encephalopathy with varied presentations, including seizures

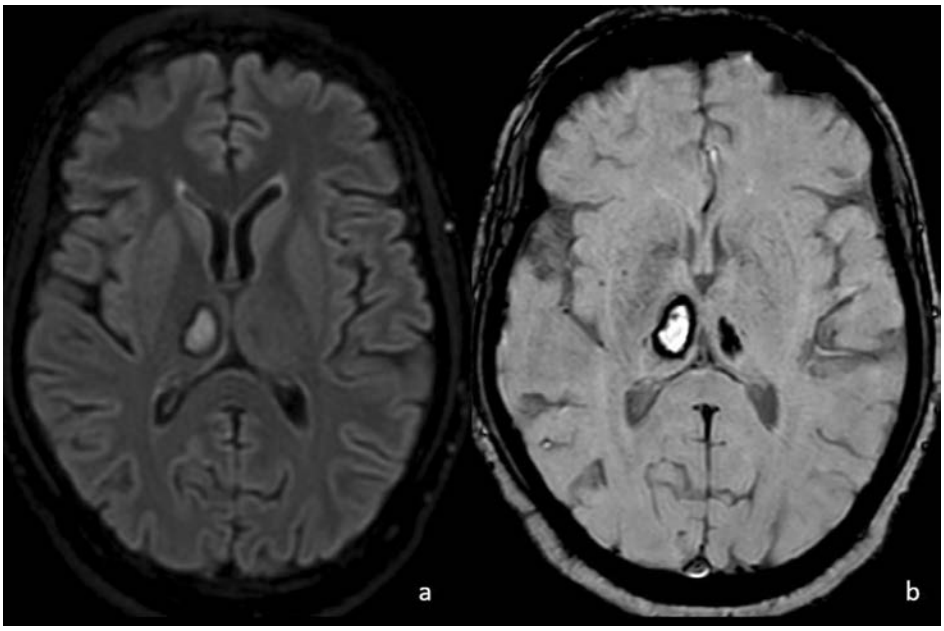

FIGURE 2 | Six weeks follow-up MRI (FLAIR, a; and SWI, b) showing chronic bleed within bilateral thalami (R>L) with resolution of thalamic swelling and edema.

or stroke-like presentations and progressive encephalopathy. Spinal cord involvement is less in AHEM when compared to ADEM [1]. Early recognition and aggressive immunotherapy have improved outcomes in AHEM.

In the literature, the occurrence of neuro-psychiatric systemic lupus erythematosus (NPSLE) varies from 21% to 95% [7–10]. The frequency and patterns of severe neurologic and psychiatric events in SLE are extremely heterogeneous and remain incompletely understood. Recently, the prevalence of NPSLE disease was determined using a standardized protocol based upon the American College of Rheumatology (ACR) nomenclature and case definitions of NPSLE [11]. The meta-analysis done revealed that the overall NPSLE prevalence when all studies were pooled together was 52.2% [12]. Demyelinating syndrome, Guillain–Barre syndrome, autonomic disorder, myasthenia gravis, and plexopathy each showed a prevalence rate of less than 0.5% in the Swiss cohort; the nervous system was involved in 193 of 688 patients (28.1%) [12]. Similarly, another study about Neuropsychiatric Events at the Time of Diagnosis of Systemic Lupus Erythematosus, An International Inception Cohort Study conducted by members of the Systemic Lupus International Collaborating Clinics (SLICC), revealed NPSLE syndromes in 28% of SLE patients [13]. There were no patients with Guillain–Barre syndrome, demyelinating syndrome, myasthenia gravis, or plexopathy.

Bermejo et al. reported AHEM as the first manifestation of SLE [3]. Acute demyelinating encephalomyelitis is very rare, with only six cases in the literature, and the hemorrhagic variant is even more infrequent, with only one case in the literature diagnosed by autopsy until he reported. Recently, there are other reports of ADEM/AHEM in SLE [14]; there is also a report of Hurst syndrome with mixed connective tissue disease [15].

3 | Conclusion

NPSLE as presenting symptoms is very rare in lupus and AHEM is the further rarest presentation. Physicians should be aware of this condition, and radiologists should be able to realize it as early as possible and to be treated as early as possible. If diagnosed early, it can be treated, and complete recovery can be achieved.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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LETTER TO THE EDITOR

Spontaneous Colovaginal Fistula-A Rare Chronic Complication in Lupus Nephritis

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Dear editor,

Gastrointestinal (GI) involvement in systemic lupus erythematosus (SLE) is a well-observed clinical entity, and its prevalence in medical literature ranges from 15% to 75% [1]. The most commonly described gastrointestinal manifestations of SLE include pancreatitis, peritonitis, and lupus enteritis [2]. Lupus enteritis complicated by duodenojejunal fistula [3] and rectovaginal fistula associated with coexisting Crohn's disease [2] have been described as rare GI complications in the clinical context of SLE. Herein, we describe an interesting case of spontaneous colovaginal fistula, it's possible etiology, and treatment strategies in a patient with biopsy-proven lupus nephritis.

A 52 year old, South Asian female, known case of biopsy-proven lupus nephritis (class 3 + 5) since 2017 on a minimal dose of prednisolone (5 mg once a day) and mycophenolate mofetil (500 mg twice a day) presented with a history of passing fecal matter per vagina for 2 days. Examination revealed that the abdomen was soft and nontender, and vaginal examination revealed no abnormalities. Her height, weight, and body mass index were 158 cm, 78 kg and 31.2 kg/m2 respectively. She had a history of abdominal hysterectomy performed 30 years prior, with a short history of constipation in the previous week.

Laboratory investigation revealed hemoglobin −11.2 g/dL, WBC-11050/mm^3, platelets-211 000/mm^3, creatinine −0.5 mg/dL, urea-30 mg/dL with normal serum electrolytes, and normal liver function tests. Urine examination revealed trace albumin levels without any sediment, and the urine spot protein-to-creatinine ratio was 0.2. Serological evaluation revealed negative viral serology for human immunodeficiency virus, hepatitis B, and hepatitis C. There was no obvious source of infection on clinical assessment. The serum complement levels were normal, antinuclear antibody (ANA) was 1:100, and dsDNA was negative. She was subjected to contrast enhanced computed tomography (CECT) of the abdomen with rectal contrast which revealed contrast extravasation from the sigmoid colon into the vaginal vault through a fistulous tract measuring 3.2 mm (Figure 1). Colonoscopy revealed a normal colon without features of inflammatory bowel disease, and a fistulous opening could not be identified. However, expulsion of gas into the vaginal vault was noticed on inflation of the colon for a better view.

It was decided to go ahead with abdominal surgical exploration which revealed fistulous opening at the level of sigmoid colon with adherent appendix communicating with the vaginal vault. (Figure 2A,B) Laparoscopic resection of the fistula, adherent appendix and proximal sigmoid colon along with primary anastomosis with distal sigmoid was performed as part of the operative management. Histopathological examination of the resected colonic specimen and appendix encircling the fistulous tract revealed viable colonic mucosa, without mucosal pathology. It also revealed a fistulous tract lined by granulation tissue composed of congested blood vessels, crypt injury (Figure 2C), lymphoplasmacytic infiltration with eosinophils in the lamina propria along with sheets of adipocytes (Figure 2D), architectural

Abbreviations: ANA, antinuclear antibodies; MMF, mycophenolate mofetil; SLE, systemic lupus erythematosus.

distortion, absence of atypical cells and granulomas suggestive of mycophenolate mofetil-induced colonic injury, and features of chronic appendicitis in the adherent appendix specimen.

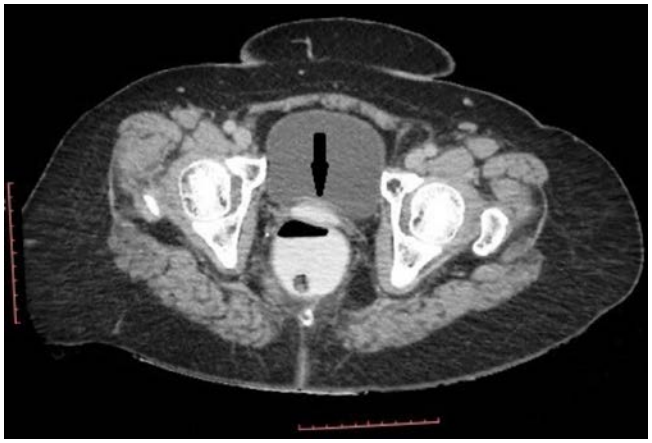

FIGURE 1 | Contrast enhanced computerized tomography of the abdomen revealing contrast extravasation noted from the sigmoid colon (black arrow) into the vaginal vault on administration of rectal contrast (Magnification: 600 DPI).

Mycophenolate mofetil was temporarily discontinued, and the patient was administered hydrocortisone intravenously during the first 3 days since the patient was kept nil per mouth. On post operative Day 4, hydrocortisone was switched to oral prednisolone (10 mg once daily), and mycophenolate was gradually introduced at reduced doses on post operative Day 6. The patient's post operative period was uneventful, and she gradually transitioned to a solid diet on post operative Day 5. She was discharged on a soft solid diet, had minimal immunosuppression with a transition to azathioprine, and was clinically stable on follow-up after 3 months.

SLE is a heterogeneous complex autoimmune disorder involving multiple organ systems, with clinical manifestations ranging from self-resolving systems to life-threatening organ afflictions [4]. SLE can be considered as the most sex-differentiated autoimmune disease process, predominantly involving women of reproductive age with non-Caucasian racial predisposition [4].

Gastrointestinal involvement of SLE is characterized by lupus mesenteric vasculitis, protein losing gastroenteropathy, intestinal pseudo obstruction, pancreatitis, peritonitis, coexistent coeliac disease, coexistent inflammatory bowel disease or

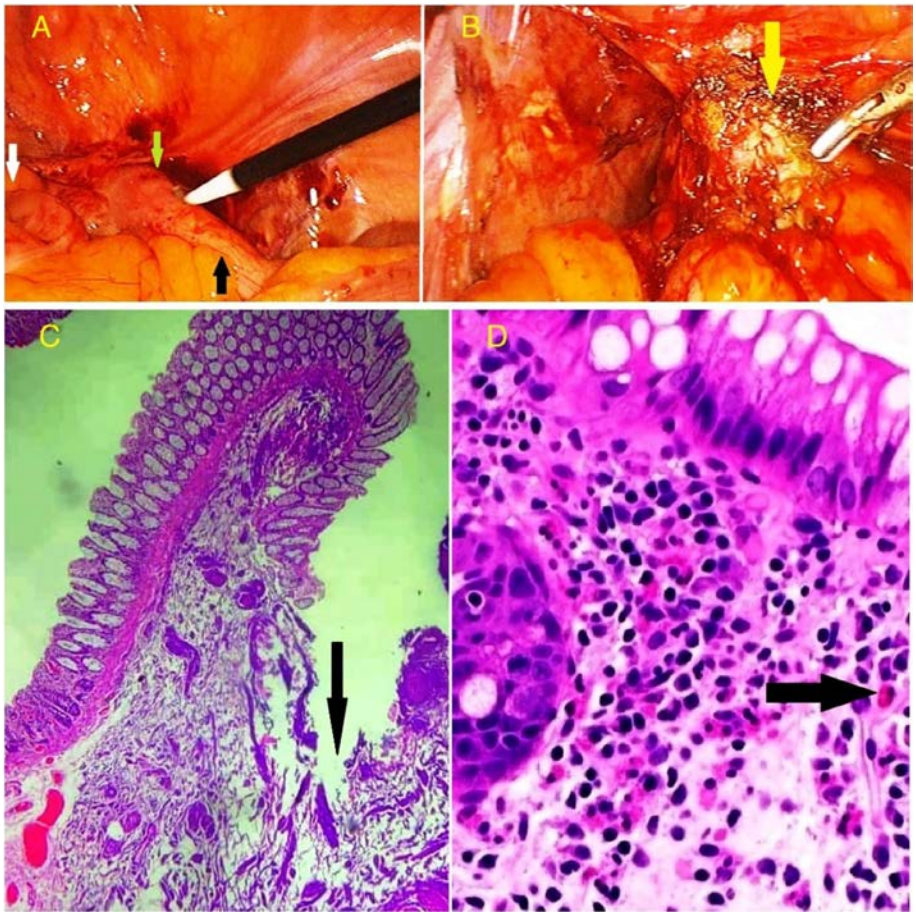

FIGURE 2 | (A) Intraoperative picture showing adherent appendix (black arrow), proximal sigmoid colon (white arrow) and fistulous communication (yellow arrow) with the vaginal vault (Magnification: 600 DPI). (B) Intraoperative picture showing prominent fistulous connection (yellow arrow) after laparoscopic resection of the adherent appendix (Magnification: 600 DPI). (C) Hematoxylin and eosin-stained colon specimen showing defect at the submucosal level in vertical plane, crypt injury, disruption of the muscularis propria and architectural distortion (black arrow) (Magnification: 100×; 600 DPI). (D) Hematoxylin and eosin-stained colon showing eosinophilic inflammatory infiltrate (black arrow) in the lamina propria of the resected colon suggestive of mycophenolate induced colonic injury (Magnification: 100×; 600 DPI).

eosinophilic enteritis [2, 5]. The colon may be involved in the form of multiple chronic ulcers or acute ischemic enteritis [5].

The pathogenesis of SLE involving the small intestine and colon includes thrombosis and immune complex deposition in the intestinal vessels due to autoantibodies such as lupus anticoagulant, anti-β2-glycoprotein antibody, anti-cardiolipin antibody, anti-endothelial antibodies, and anti-phospholipid antibodies [2, 5]. This micro vasculopathy predisposes to intestinal wall oedema, ulceration, and perforation [5]. The clinical features of colonic involvement in SLE include abdominal pain, bloating, weight loss and loose stools. They may present with abdominal distress syndrome, which is suggestive of intestinal necrosis and perforation [1, 5]. The trigger factors for immune activation in gastrointestinal SLE include nonsteroidal anti-inflammatory drugs, bacterial and viral infections resulting in changes in gut flora, herbal medicines, metallic particulates, eosinophilia, helminthic infections, and caffeine [5].

Most cases of lupus enteritis culminating in colonic perforation are vesicocolonic, complicated by multiple ulcerations in the large intestine during the active phase of the disease [3]. To our knowledge, this is the first reported case of colovaginal fistula in a patient with lupus nephritis in clinical remission. Our case is also unique in the fact that the colonoscopy was normal without any evidence of ulceration which usually is encountered in large bowel involvement of SLE [3, 5]. Rare reports of rectal ulcers and rectovaginal fistulas have also been reported in the active phase of the disease and coexistence with Crohn's disease [2, 6]. The risk factors for intestinal perforation or fistulisation in SLE include female sex, SLE duration greater than 5 years, higher SLEDAI scores, non-standard steroid use, prolonged fecal mass impaction, and increased intestinal lumen pressure [7]. Our patient had a disease duration of 7 years, prolonged steroid use, and constipation, which could have predisposed to spontaneous colovaginal fistula.

Steroids have long been implicated in colonic perforation owing to their inhibitory effect on endogenous prostaglandin production, increased collagen breakdown, decreased fibroblast perforation, immunosuppression, and alterations in gut pH [8]. Mycophenolate mofetil (MMF) is involved in colonic pathologies because deconjugation and reabsorption of glucouronidated mycophenolic acid (MPAG) occur in the proximal colon [9]. The clinical manifestations of MMF-induced colitis include diarrhea, vomiting, and abdominal pain, with a prevalence rate of 2%–9% among MMF users [9]. Histological changes associated with MMF induced colitis include crypt abscess, dilated damaged crypts, apoptotic bodies and eosinophilic epithelial changes, which were characteristically observed in our case [8–10]. Eosinophil's in the lamina propria are observed in the ischemic pattern of mycophenolate induced segmental colonic injury [11]. This differentials of mycophenolate induced colitis include inflammatory bowel disease, ischemic colitis and graft versus host disease [10, 11]. Our patient had been on long-term steroids and MMF for nearly 7 years, which would have weakened the colonic epithelium and impaired its regenerative capacity, thereby predisposing the patient to a colovaginal fistula. The histopathological changes in our patient characteristically showed architectural distortion, crypt injury, and eosinophilic inflammatory infiltrate in the lamina propria, suggesting mycophenolate-induced colonic injury. Since there is no standardized histopathological diagnostic criteria for MMF induced colitis [8–11], we scientifically implicate MMF in the causation of this spontaneous colovaginal fistula due to its long presence in the patients treatment regimen.

Colovaginal fistula is an uncommon gastrointestinal and lower reproductive tract fistula that is clinically characterized by stools per vagina, flatus per vagina, or persistent vaginal discharge [12]. Risk factors for colovaginal fistulas include abdominal hysterectomy, diverticular disease, age greater than 50 years, diabetes mellitus, smoking and obesity [12, 13]. Our patient had a history of abdominal hysterectomy, obesity (BMI 31.2 kg/m^2) and the age greater than 50 years which could have predisposed to development of colovaginal fistula. Radiological investigations of choice for evaluation of colovaginal fistula include magnetic resonance imaging or multidetector computerized tomography to accurately localize the site and course of the fistula [12]. The approach to a colovaginal fistula includes detailed physical examination, speculum examination with attention to the left vaginal apex, flexible sigmoidoscopy/colonoscopy, and barium enema study, if clinically warranted in coordination with the surgeon and gynecologist [11, 12]. Resection of involved sigmoid segment with primary anastomosis is the most favored surgical treatment, with a success rate approaching 95% [12]. Our case report is novel because the patient underwent laparoscopic colovaginal fistula with appendiceal resection, primary anastomosis with the distal sigmoid, and complete closure of the fistulous tract, which would probably be the only reported case of laparoscopic repair of a colovaginal fistula in lupus nephritis instead of the traditional transabdominal approach.

This case is a perfect testimony of rare gastrointestinal manifestations of SLE predisposed by chronic use of mycophenolate mofetil and the factors is SLE predisposing to colovaginal fistula.

Author Contributions

All authors were responsible for the conception, design, visualization, investigation, drafting of the text, sourcing, and editing of clinical images, investigation results, critical revision of important intellectual content and gave final approval of the manuscript.

Acknowledgments

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Ethics Statement

This case report was drafted after written informed consent of the patient in conformity with CARE clinical case reporting guidelines and Declaration of Helsinki. The authors are accountable for all aspects of the work (including full data access, integrity of the data, and accuracy of the data analysis) to ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Consent

The patient and the authors consent for publication of this manuscript as per the journal's editorial policies. Informed written consent for publication is obtained from the patient.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data for substantiating the findings of this manuscript is available with the corresponding author and can be provided on request.

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LETTER TO THE EDITOR

Age and Socioeconomic Deprivation as Key Predictors of Cardiovascular Risk in Patients With Autoimmune Rheumatic Diseases on JAK Inhibitors

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Dear Editor,

Cardiovascular disease (CVD) remains a leading cause of morbidity and mortality worldwide, particularly among patients with autoimmune rheumatic diseases (ARDs). This increased risk is attributed to systemic inflammation, traditional risk factors, and the effects of immunomodulatory therapies, including Janus kinase (JAK) inhibitors [1, 2]. JAK inhibitors have transformed the treatment landscape for ARDs, offering significant therapeutic benefits; however, concerns regarding their cardiovascular safety profile persist, especially in diverse patient populations facing socioeconomic disparities [3–5]. Our retrospective study evaluated cardiovascular risk predictors in 309 patients in a multi-ethnic cohort receiving JAK inhibitors at a UK teaching hospital, focusing on age and socioeconomic deprivation.

1 | Key Findings and Novel Contributions

While previous studies have investigated the cardiovascular risks associated with JAK inhibitors, our study uniquely emphasizes the interaction between age and socioeconomic deprivation as significant predictors of cardiovascular outcomes in a diverse multi-ethnic cohort. Although prior research has broadly addressed socioeconomic factors in CVD risk, our findings provide empirical evidence supporting the integration of deprivation measures into ARD-specific risk models in a multi-ethnic cohort [6]. This approach is crucial for understanding the multifaceted nature of cardiovascular risk in patients undergoing JAK inhibitor therapy.

2 | Methods and Statistical Justification

Our study included a diverse cohort of 309 ARD patients (73% White, 25% South Asian, 1% Black; mean age 59.3 years; 77% female). We analyzed cardiovascular events, including myocardial infarction, stroke, and cardiovascular-related death, in relation to age, socioeconomic deprivation, and ethnicity. Socioeconomic deprivation was assessed using the UK Index of Multiple Deprivation (IMD) deciles, encompassing various factors such as income, employment, education, health, crime, and housing quality [7, 8]. Given the small number of cardiovascular events ($n = 14$), a post hoc power calculation was performed, revealing an observed power of 0.45 for detecting an odds ratio of 1.06 for age and deprivation decile, indicating potential underpowering of the study. Sensitivity analyses, including model discrimination (AUC = 0.837) and penalized regression methods like LASSO, were employed to enhance the reliability of our findings.

3 | Results

Our analysis demonstrated that the combination of age and socioeconomic deprivation significantly predicted cardiovascular events ($p = 0.04$) in a multi-ethnic cohort (Table 1), with the model demonstrating excellent discrimination (AUC = 0.837) (Figure 1) suggesting a synergistic effect where deprivation of the patient exacerbates age-related CVD risk. In contrast, age and ethnicity alone did not emerge as independent predictors,

TABLE 1 | *p*-value, odds ratio, and 95% confidence interval of the independent variables using logistic regression analysis.

	Coefficient B	Standard error	*z*	*p*	Odds ratio	95% confidence interval
Constant	−6.89	2.24	3.08	0.002	0	0–0.08
Baricitinib	0.35	0.83	0.42	0.675	1.41	0.28–7.17
Tofacitinib	−18.93	51994.71	0	1.00	0	Unbounded
Upadacitinib	0.02	1.3	0.02	0.987	1.02	0.08–12.95
Filgotinib	0	1.00	0	0.896	1.00	Unbounded
Deprivation	0.43	0.97	0.45	0.654	1.54	0.23–10.25
Age	0	0	1.00	0.499	1.00	Unbounded
Age and Deprivation Decile	0.06	0.03	2.05	0.04	1.06	1–1.13

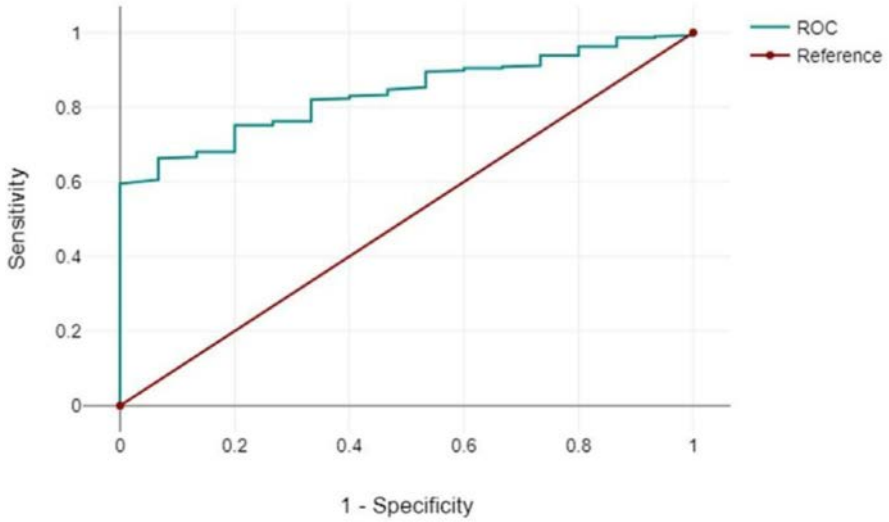

FIGURE 1 | ROC Curve of the logistic regression model. Area under the curve (AUC = 0.837).

which may reflect population-specific factors or differences in underlying health disparities.

Assessments of individual JAK inhibitors (baricitinib, tofacitinib, filgotinib, upadacitinib) did not reveal statistically significant associations with cardiovascular events in a multi-ethnic cohort, aligning with findings from recent meta-analyses [9]. However, some variables in our logistic regression model produced "unbounded" results, likely due to the small number of cardiovascular events and the sparse distribution of certain covariates. This highlights the need for larger, adequately powered studies to evaluate the cardiovascular safety profile of specific JAK inhibitors further and refine predictive models.

4 | JAK Inhibitors and Cardiovascular Mechanisms

The potential influence of JAK inhibitors on CVD risk may involve several mechanisms. For instance, alterations in lipid metabolism, specifically increases in LDL cholesterol, have been observed with JAK inhibitor therapy [10]. Additionally, there is evidence linking JAK inhibition to an elevated risk of venous thromboembolism, particularly in older patients or those with pre-existing risk factors [11]. Furthermore, JAK inhibitors may contribute to endothelial dysfunction by modulating inflammatory pathways, adversely affecting cardiovascular health [1]. Emerging data also suggest that these therapies may influence platelet activation and coagulation pathways, potentially heightening thrombotic risk in susceptible individuals [12]. Our study's inability to detect significant individual JAK inhibitor risk differences may be attributed to the limited number of events rather than an absence of risk, underscoring the need for further research into dose-dependent effects and long-term safety outcomes.

5 | Clinical Implications and Future Directions

Personalized risk stratification is vital for managing cardiovascular risk in ARD patients treated with JAK inhibitors, especially in a multi-ethnic cohort [13]. While age is a recognized risk factor, it is insufficient in isolation; socioeconomic deprivation should be integrated into cardiovascular risk models to enhance prediction accuracy [13]. Current tools, such as QRISK3, do not adequately account for these disparities, highlighting the necessity for improved risk prediction methodologies. Addressing socioeconomic barriers is equally essential, as patients in lower

IMD deciles may face delayed access to preventive care and suboptimal medication adherence. Strategies such as targeted screening, patient education, and improved access to preventive therapies are essential for mitigating these risks.

6 | Limitations and Future Research

Despite providing novel insights, our study's retrospective design and limited event numbers constrain generalizability. Future prospective, multi-centre studies are warranted to validate our findings and establish deprivation-adjusted CVD risk thresholds. Additionally, larger studies are needed to delineate the long-term cardiovascular safety of JAK inhibitors across diverse populations, employing advanced modeling techniques to refine risk estimation in underpowered analyses.

7 | Conclusion

In summary, our study underscores the significance of age and socioeconomic deprivation as critical predictors of cardiovascular risk in ARD patients receiving JAK inhibitors in a multiethnic cohort. These findings advocate for the integration of socioeconomic measures into cardiovascular risk assessment models, aiming to improve prediction accuracy and promote healthcare equity. Addressing these disparities is crucial for optimizing cardiovascular outcomes while ensuring access to JAK inhibitors for high-risk patients.

Author Contributions

All authors have reviewed the final version to be published and have agreed to be accountable for all aspects of the work. Concept and design: K.S. and B.B. Acquisition, analysis, or interpretation of data: K.S. and B.B. Drafting of the manuscript: K.S. Critical review of the manuscript for important intellectual content: K.S. Supervision: K.S.

Disclosure

Human subjects: Consent was obtained or waived by all participants in this study. Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissues.

Ethics Statement

This study adhered to ethical principles, maintaining data confidentiality and participant anonymity, as approved by the Leicestershire Research Ethics Committee.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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LETTER TO THE EDITOR

Autophagic Flux Might Be Blocked in Patients With Primary Antiphospholipid Antibody Syndrome

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Dear Editor,

Antiphospholipid antibody syndrome (APS), an autoimmune disease often associated with systemic lupus erythematosus (SLE), is characterized by the presence of β2-glycoprotein I-dependent antiphospholipid antibodies (aPL) and vascular thrombosis or obstetrical complications. In recent years, perturbations in autophagy have been implicated in the inflammatory process [1]. Pro-inflammatory cytokines can induce the formation of autophagosome [2]. Autophagy can reciprocally regulate cytokine production [2]. Furthermore, autophagy is linked to the generation of autoimmunity [3–5]. Interestingly, a mammalian target of rapamycin (mTOR) inhibitor is known to be effective in the prevention of APS nephropathy in patients receiving renal transplant [6]. A recent murine study on APS also reported that inhibiting mTOR suppresses thrombus formation by stimulating autophagy in macrophages [7]. Here, we hypothesize that autophagy is altered in primary APS (pAPS) patients and such alteration is associated with thrombosis.

From November 2019 through January 2022, we prospectively enrolled 29 consecutive pAPS patients in Taichung Veterans General Hospital. APS was diagnosed based on the revised Sapporo classification criteria [8]. All patients were free of thrombotic events within 3 months before collecting blood samples. We also recruited 45 healthy subjects without significant chronic diseases, as controls (HCs) (Table S1). The Institutional Review Board of Taichung Veterans General Hospital approved this study (IRB TCVGH NO: CF14256A and CE16246B). Written consent from all participants was obtained according to the Declaration of Helsinki. Their adjusted Global Antiphospholipid Syndrome Score (aGAPSS) score was calculated [9]. Levels of autophagy in blood lymphocytes, monocytes, or granulocytes from pAPS patients and HCs were determined using flow cytometry based on anti-microtubule-associated protein 1 light-chain 3B (LC3B) antibody (ab51520, 1: 200) (Abcam, Cambridge, UK) plus AffiniPure Goat Anti-Rabbit IgG (1: 200), and anti-p62 antibody (MABC32-AF488,

Hsin-Hua Chen and Chi-Chen Lin contributed equally to this paper.

1: 400) (Sigma-Aldrich, St. Louis, MO). Mean fluorescence intensities (MFI) were measured by flow cytometry using the FL-1 channel. Furthermore, peripheral blood mononuclear cells (PBMCs) were separated from the whole blood of 3 pAPS patients and 3 age- and sex-matched HCs using the Ficoll-paqueTM plus (Cat 17144003, Cytiva, US) and their monocytes were then isolated using the MojoSortTM Human CD14+ Monocytes isolation kit (Cat 480048, Biolegend, US), with a purity > 90%. These monocytes were seeded in 2×10^5 cells/ml and then treated with and without 2 μM rapamycin and incubated in 5% CO_2 at 37°C for 24 h. Cells were cultured with anti-LC3B antibody (1:1000) (Cat ab48394, Abcam, USA) overnight at 4°C. Secondary antibody with anti-Rabbit IgG (1:200) (Cat AB_2337972, Jackson, USA) was added and kept for 30 mins. 4′,6-diamidino-2-phenylindole (DAPI) (Abcam, Cambridge, UK) was added for 10 mins. Images were obtained with an Olympus FV1000 Laser Confocal Microscope (Tokyo, Japan) and analyzed by ImageJ (National Institutes of Health, Bethesda, MD). Mann–Whitney *U* test and Chi-Squared test were used for between-group comparisons. Correlational analyses were done with the non-parametric Spearman's correlation test. Two-tailed *p* values < 0.05, after Bonferroni's correction, were considered statistically significant.

Our results showed that levels of LC3B expression were significantly higher in lymphocytes (MFI: 2849 [IQR: 1414, 8877] vs. 729 [462, 1257]), monocytes (MFI: 6717 [IQR: 2711, 16922] vs. 1055 [731, 1898]), and granulocytes (MFI: 8925 [IQR: 3772, 16539] vs. 1584 [960, 7386]) of pAPS patients when compared with HCs (Figure 1). Levels of p62 expression were not different between pAPS patients and HCs. In addition, we examined levels of expressed LC3B and p62 during acute thrombosis and after treatment in two pAPS patients: one was a 26-year-old female with peripheral arterial disease (patient 1), and another a 33-year-old male with venous thromboembolism (patient 2); and also a SLE patient with secondary APS (a 35-year-old female with digital gangrene [patient 3]) (Figure S1). Expression levels of both LC3B and p62 in their blood lymphocytes, monocytes, and granulocytes were consistently lowered after treatment, except for the expression levels of p62 in granulocytes of patient 3. As demonstrated in Figure 2, blood monocytes isolated from pAPS patients showed more LC3B-puncta formation when compared with matched HCs ($p < 0.001$). Treatment with rapamycin, as an inducer of autophagy, reduced LC3B-puncta formation in blood monocytes derived from pAPS patients while it increased LC3B-puncta formation in blood monocytes from HCs (both $p < 0.005$). As shown in Table S2, the expression levels of both LC3B and p62 were highly correlated across blood lymphocytes, monocytes, and granulocytes. To be noted, expression levels of p62 in both blood lymphocytes and monocytes were also weakly correlated with the aGAPSS scores. In the subgroup analysis, levels of LC3B expression were significantly higher in lymphocytes, monocytes, and granulocytes of both vascular and obstetric pAPS patients when compared with HCs (Figure S2). Levels of p62 expression were, however, similar between either vascular or obstetric pAPS patients and HCs (Figure S3). Our findings remain unchanged even after excluding those pAPS patients receiving hydroxychloroquine (Figure S4).

In our pAPS patients, expression levels of LC3B were higher in blood lymphocytes, monocytes, and granulocytes when

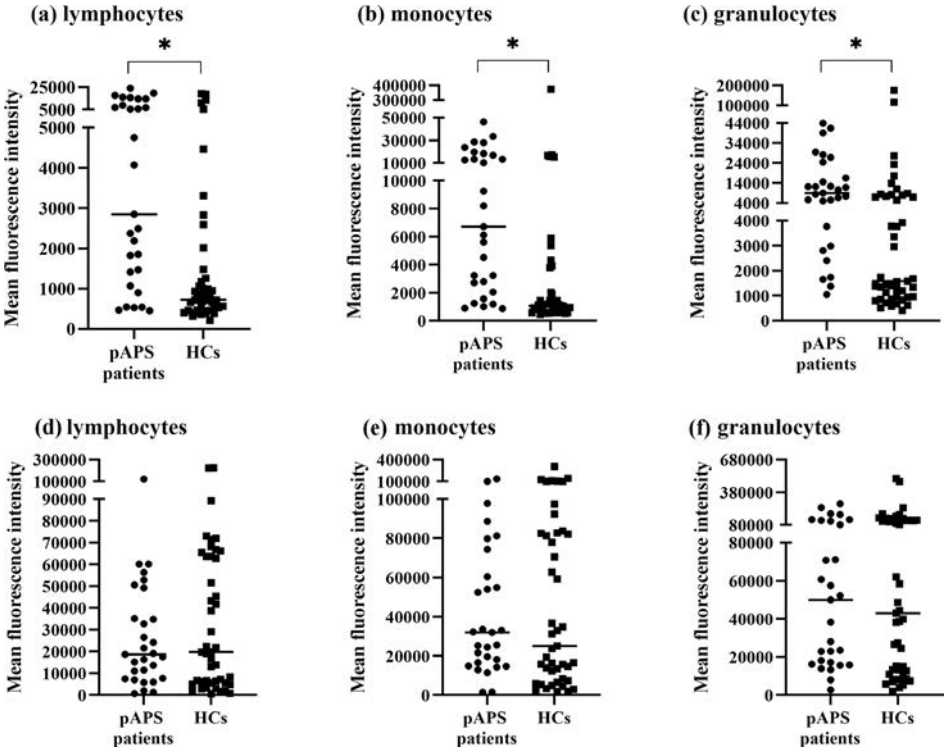

FIGURE 1 | Expression levels of LC3B in blood (a) lymphocytes, (b) monocytes, and (c) granulocytes, and expression levels of p62 in blood (d) lymphocytes, (e) monocytes, and (f) granulocytes. LC3B, microtubule-associated protein 1 light-chain 3B; pAPS, primary antiphospholipid antibody syndrome; HCs, healthy controls. *$p < 0.001$. [a]p values of < 0.008 were regarded as statistically significant for multiple comparisons.

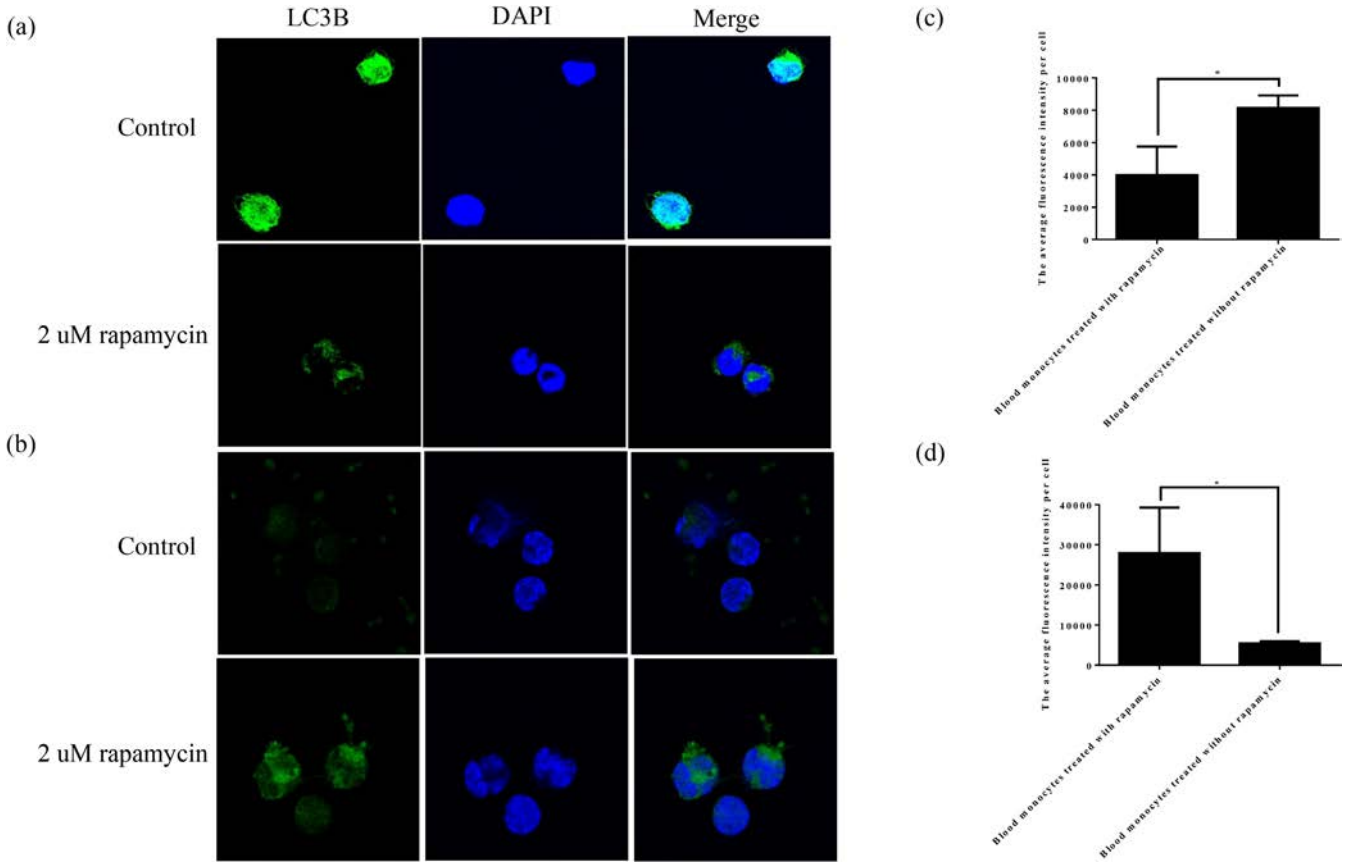

FIGURE 2 | Representative image of immunofluorescence against anti-LC3B antibody under a confocal microscope (3000×) in blood monocytes of (a) pAPS patients and (b) healthy controls (Left to right: LC3B tagged with FITC, DAPI, and both merged). Comparisons of the average fluorescence intensity of LC3B per cell in monocytes, obtained from (c) pAPS patients and (d) healthy controls, treated with and without rapamycin (determined by ImageJ). DAPI, 4′,6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; LC3B, microtubule-associated protein 1 light chain 3B; pAPS, primary antiphospholipid antibody syndrome. *$p < 0.005$, as determined by paired *t* test.

compared with HCs. There are two possible explanations for these differences in patients: (a) the formation of autophagosome has increased; and (b) the autophagic flux has been blocked. Nonetheless, expression levels of p62 in blood lymphocytes, monocytes, and granulocytes in pAPS patients were not different from those in HCs. Such a finding indicates no increase in the degradation of autophagosome; blocked autophagic flux is more likely the underlying mechanism. In addition, immunofluorescence imaging findings from blood monocytes supported this hypothesis. The formation of LC3B puncta has increased in blood monocytes isolated from pAPS patients when compared with HCs. When the addition of rapamycin, an inducer of autophagic flux [10], the formation of LC3B puncta reduced in blood monocytes isolated from pAPS patients, indicating a blocked autophagic flux.

The relationship between autophagy and thrombosis has been alluded to in platelets [11, 12], and atherosclerosis [11, 13]. Autophagy is induced after platelet activation, as reflected by the loss of LC3B [11, 14]. In platelet-specific Atg-7 knockout mice, deficits were found in platelet aggregation, and a resultant bleeding tendency was shown. Though controversial, impaired autophagy may contribute to the formation of the atherosclerotic plaque, partly through macrophage activation [13]. Furthermore, rapalink-1, a mTOR inhibitor, exerts an antithrombotic effect in APS both in vitro and in vivo, partly through enhanced autophagy in macrophages [7]. In another prior study, monocytes from healthy donors were treated with IgG from APS patients. Their mTOR pathway was activated, and lysosomal degradation during autophagy was disrupted [15].

Our study has some limitations. First, we could not totally rule out the medication effect, although the results remained after exclusion of patients who used hydroxychloroquine. Among the patients, furthermore, hydroxychloroquine use did not affect expression levels of LC3B and p62 in white blood cells (Figure S5). Second, the sample size is relatively small despite recruiting both vascular and obstetric APS patients. Third, autophagy is a dynamic process and difficult to evaluate. Per the guideline for monitoring autophagy [16], we used three methods to measure autophagy: LC3B expression levels determined by flow cytometry, p62 expression levels determined by flow cytometry, and LC3B puncta formation quantitated by confocal immunofluorescence microscopy. In the experiment by confocal immunofluorescence microscopy, additionally, we used rapamycin as a positive control to corroborate our interpretation. Nevertheless, we lacked timed experiments to directly measure the autophagic flux. Despite such limitations, autophagic flux might be blocked in blood lymphocytes, monocytes, and granulocytes in pAPS patients when compared with HCs. The blocked autophagic flux was correlated with the aGAPSS score, and the flux could possibly be reversed after treatment.

Author Contributions

Conceptualization, K.-T.T. and C.-C.L.; methodology, K.-T.T. and C.-C.L.; formal analysis, Y.-H.C. and K.-T.T.; investigation, Y.-H.C., J.-H.W., Y.-H.C., T.-Y.H., D.-Y.C., and H.-H.C.; writing – original draft preparation, K.-T.T.; writing – review and editing, C.-C.L.

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Ethics Statement

The Institutional Review Board of Taichung Veterans General Hospital approved this study (IRB TCVGH NO: CF14256A and CE16246B).

Consent

Written consents of all participants were obtained according to the Declaration of Helsinki. All the authors of the article give consent for the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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ORIGINAL ARTICLE

Relationship Between Sarcopenia, Femoral Cartilage Thickness, and Knee Osteoarthritis: Case–Control Study

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ABSTRACT

Objective: This study aims to evaluate the association between femoral cartilage thickness (FCT) and knee osteoarthritis (KO) in individuals with sarcopenia and pre-sarcopenia, highlighting the potential role of FCT in the relationship between sarcopenia and KO.

Study Design: A cross-sectional study including 80 individuals (23 pre-sarcopenia, 21 sarcopenia, and 36 healthy controls) aged 40–75 years was conducted. Using ultrasound (US), FCT was measured, and KO prevalence was compared among the three groups. Logistic regression analyses were performed to determine the predictors of KO and sarcopenia, and ROC analysis was conducted to estimate sarcopenia from FCT measurements.

Results: The mean age of the 80 participants (55 females, 25 males) was 62.22 ± 7.56 years. The sarcopenia group had significantly lower medial and lateral FCT than the control group (all $p < 0.01$). Logistic regression analysis indicated that age and sarcopenia were significant predictors of KO (all $p < 0.01$). Multinomial logistic regression showed that KO and medial FCT were significant predictors of sarcopenia (all $p < 0.05$). ROC analysis demonstrated that medial FCT effectively predicted sarcopenia ($p = 0.001$, AUC = 0.736).

Conclusions: The results of this study showed that FCT was reduced, and KO prevalence was increased in sarcopenia patients. Additionally, age and sarcopenia were predictors for KO, while KO and decreased medial FCT were predictors of sarcopenia. These findings suggest that sarcopenia may influence FCT through mechanical effects related to muscle strength loss and potentially other mechanisms, making it a potential risk factor for KO.

1 | Introduction

Sarcopenia, a condition with prevalence increasing with age, is characterized by the loss of muscle mass and function, leading to diminished physical capability and reduced quality of life [1]. All body muscles can be affected by sarcopenia, but the lower extremity muscles are particularly prone to be impacted [2, 3].

Some studies have shown a relationship between quadriceps muscle strength, thickness, and Femoral Cartilage Thickness (FCT) [4–6]. A study investigating patients with knee osteoarthritis (KO) found a positive correlation between knee extensor muscle strength and FCT, showing that decreased muscle strength is associated with reduced FCT [5]. Additionally, it has been reported that FCT decreases in various conditions that could lead to muscle weakness [7, 8]. Furthermore, an

increased prevalence of KO, a disease associated with thinning of the femoral cartilage, has been demonstrated in sarcopenia patients [9–13].

Existing studies have primarily focused on the relationship between FCT and knee extensor muscle strength or the association between sarcopenia and KO. However, as far as we can tell, no study has examined the relationship between sarcopenia, FCT, and KO. Understanding these relationships is crucial to uncover potential common mechanisms underlying these conditions and improving clinical outcomes.

This study aims to investigate FCT differences between sarcopenia patients and healthy controls and to examine the relationship between sarcopenia, FCT, and KO. Additionally, it explores the potential of FCT as a predictive marker for sarcopenia and evaluates the association between sarcopenia and KO, focusing on the role of FCT in this relationship. In this study, the aim was to contribute to understanding potential risk factors for KO in sarcopenia patients and to guide early interventions for the prevention or management of KO.

2 | Materials and Methods

2.1 | Study Population

This cross-sectional study included a total of 80 individuals.

2.1.1 | Inclusion Criteria

Individuals

- presenting to the Physical Medicine and Rehabilitation Clinic of the university hospital between February and May 2021.
- aged 40–75 years.
- 23 pre-sarcopenia, 21 sarcopenia patients, and 36 healthy controls.
- equal number of control subjects selected based on similar characteristics in age, gender, and BMI.

2.1.2 | Exclusion Criteria

Individuals

- With knee surgery, joint deformities that may affect knee function.
- With any inflammatory rheumatic diseases, malignancies, neurological diseases, and diseases that may cause muscle weakness.
- Using medications that could impact muscle strength or function (e.g., steroids).
- With cognitive impairments or conditions that affect the ability to provide informed consent.
- With uncontrolled chronic conditions, such as diabetes or cardiovascular diseases.
- Who declined to participate in the study.

2.2 | Measurements

HGS was measured using a Jamar hand dynamometer (Lafayette, IN, USA). HGS was calculated as the average of two measurements obtained from the dominant hand. Bioelectrical impedance analysis (BIA) was evaluated using the Body Composition Analyzer, model BC-418 (Tanita Corp, Tokyo, Japan). Appendicular Skeletal Muscle Mass (ASMM) is calculated as the total muscle mass of all four extremities. The diagnoses of pre-sarcopenia and sarcopenia were made according to the updated diagnostic criteria set by the European Working Group on Sarcopenia in the Elderly (EWGSOP2) [14]. Pre-sarcopenia was defined as HGS values of $< 16\,N$ for women and $< 27\,N$ for men. Sarcopenia was identified with HGS values of $< 16\,N$ and ASMM values of < 15 kg for women and HGS values of $< 27\,N$ and ASMM values of < 20 kg for men.

The same ultrasound specialist performed FCT measurements on all individuals using a B-mode ultrasound with a linear transducer at a frequency range of 6–18 MHz (e-soate, My Lab 70) while they were lying supine with their knees flexed at a 90° angle. FCT measurements were taken twice from the medial femoral condyle, intercondylar area, and lateral femoral condyle in both knees, and the average was calculated. The specialist was blinded to the groups (Figure S1).*FCT;*

- L-IFC, Left İntercondylar Femoral Cartilage thicknesses.
- L-LFC, Left Lateral Femoral Cartilage Thicknesses.
- L-MFC, Left Medial Femoral Cartilage thicknesses.
- Mean-IFC, Right and Left İntercondylar Femoral Cartilage thicknesses mean.
- Mean-LFC, Right and Left Lateral Femoral Cartilage thicknesses mean.
- Mean-MFC, Right and Left Medial Femoral Cartilage thicknesses mean.
- R-IFC, Right İntercondylar Femoral Cartilage thicknesses.
- R-LFC, Right Lateral Femoral Cartilage thicknesses.
- R-MFC, Right Medial Femoral Cartilage thicknesses.

All participants were diagnosed with clinical or radiological OA according to the ACR diagnostic criteria [15], based on history, physical examination, and knee X-ray imaging from their medical records. Knee X-rays of patients with radiological OA were staged according to the Kellgren-Lawrence (KL) grading system (0–5 grading).

2.3 | Statistical Analysis

The effect size was calculated using the G Power 3.1 [16] software program for the difference between FCT (left medial condylar

area) in the paired t test, which was 0.65 (Cohen's $d = 0.38$) [11]. Considering a margin of error of 0.05 ($\alpha = 0.05$) and a power of 0.80, the minimum required sample size was 72. A total of 80 participants were included in this study.

Statistical analyses were conducted using SPSS Statistics version 21, released in 2012 by IBM, and Jamovi version 2.2, released in 2021 by Jamovi Project. ANOVA was used for normally distributed data comparisons between the three groups, with homogeneity checked using Levene's test. In contrast, the Kruskal–Wallis test was applied for non-normally distributed values. The post hoc Tukey test was used for pairwise group comparisons.

A logistic regression model was created to predict KO using group, age, gender, BMI, and medial FCT as predictors (EPV = 60/4 = 15).

Multinomial Logistic Regression analysis was conducted to predict Pre-Sarcopenia and Sarcopenia using KO, age, gender, BMI, and FCT (EPV = 42/7 = 6). Subsequently, a second model was created by removing non-significant variables, retaining only KO and FCT (EPV = 42/4 = 10.5). These EPV values surpass the recommended minimum of 10, indicating that the sample size and model design are adequate for this logistic regression analysis.

Receiver Operating Characteristic (ROC) analysis was conducted to assess the performance of FCT models in predicting the diagnosis of pre-sarcopenia and sarcopenia.

2.4 | Ethics Statement

This study protocol was reviewed and approved by the ethics committee of Akdeniz University Faculty of Medicine (KAEK-60, 27.01.2021). It was conducted according to the ethical standards of the 2000 Declaration of Helsinki. All subjects were informed about the study and obtained their written informed consent.

We used the STROBE case–control checklist when writing our report [17].

3 | Results

The mean age of the 80 individuals (55 females and 25 males) between 40 and 75 was 62.22 ± 7.56 years (Table 1). Medial Femoral Cartilage (MFC) and Lateral Femoral Cartilage (LFC) thicknesses, KO prevalence, and KL grading values were found to be different between the groups ($p < 0.05$) (Table 2) (Figure 1).

No significant difference was found between females and males in FCT values and pre-sarcopenia and sarcopenia prevalences (all $p \geq 0.05$).

A weak positive correlation was found between MFC thickness and BMI, ASMM, and HGS (respectively, $p = 0.037$/Spearman $r = 0.233$, $p = 0.027$/Spearman $r = 0.248$, $p = 0.003$/Spearman $r = 0.333$).

TABLE 1 | Demographic and clinical characteristics of all individuals.

	N	%	Mean ± SD
Age			62.22 ± 7.56
BMI			27.29 ± 3.70
Sex *n* (%)			
Female	55	68.8%	
Male	25	31.3%	
Group			
Healty	36	45%	
Pre-sarcopenia	23	28.7%	
Sarcopenia	21	26.3%	
KO			
No	20	25%	
Present			
Clinical KO	29	36.3%	
Radiological KO	31	38.8%	
Total	60	75%	
HGS			21.62 ± 8.28
ASMM			20.19 ± 4.69
R-MFC			1.50 ± 0.23
L-MFC			1.52 ± 0.25
Mean-MFC			1.51 ± 0.22
R-IFC			2.0 ± 0.32
L-IFC			1.99 ± 0.3
Mean-IFC			2.0 ± 0.28
R-LFC			1.70 ± 0.22
L-LFC			1.71 ± 0.20
Mean-LFC			1.70 ± 0.20

Note: KO, According to the ACR diagnostic criteria, clinical or radiological knee osteoarthritis.
Abbreviations: ASMM, Appendicular Skeletal Muscle Mass; BMI, Body Mass Index; HGS, Hand Grip Strength; L-IFC, Left İntercondylar Femoral Cartilage thicknesses; L-LFC, Left Lateral Femoral Cartilage Thicknesses; L-MFC, Left Medial Femoral Cartilage thicknesses; Mean-IFC, Right and Left İntercondylar Femoral Cartilage thicknesses mean; Mean-LFC, Right and Left Lateral Femoral Cartilage thicknesses mean; Mean-MFC, Right and Left Medial Femoral Cartilage thicknesses mean; R-IFC, Right İntercondylar Femoral Cartilage thicknesses; R-LFC, Right Lateral Femoral Cartilage thicknesses; R-MFC, Right Medial Femoral Cartilage thicknesses.

In the logistic regression analysis conducted for KO prediction, age and sarcopenia were found to be significant (all $p < 0.01$, odds ratio 0.276 for age, 3.248 for sarcopenia) (Table 3) (Figure S2).

In the multinomial logistic regression analysis for predicting pre-sarcopenia and sarcopenia, KO and MFC thickness were significant for sarcopenia ($p < 0.05$). In contrast, LFC thickness was significant for pre-sarcopenia ($p < 0.05$) (Table 4).

TABLE 2 | Demographic and clinical characteristics of the pre-sarcopenia, sarcopenia, and control groups.

	Healty control (36) (Mean ± SD) (0)	Pre-sarcopenia (23) (Mean ± SD) (1)	Sarcopenia (21) (Mean ± SD) (2)	*p*	*F*	95% CI (Lower/Upper) (0–1) (0–2) (1–2)	η^2 (Effect size)
Age	60.44 ± 7.12	64.30 ± 6.62	63.00 ± 8.80	0.138	2.032	(−8619/0.9) (−7451/2340) (−4077/6686)	
BMI	27.96 ± 3.95	27.75 ± 3.02	25.56 ± 3.65	0.057	2.975	(−2091/2520) (−0.058/4685) (−0.507/4706)	
Sex *n* (%)							
Female	23 (41.8%)	16 (29.1%)	16 (29.1%)	0.624			
Male	13 (52.0%)	7 (28.0%)	5 (20.0%)				
KO							
No	14 (38.9%)a	5 (21.7%)ab	1 (4.8%)b	No	0.015*		
Present	22 (61.1%) ab	18 (78.3%) abc	20 (95.2%) bc				
KL Grading	1.42 ± 0.9 a	2.36 ± 0.92 bc	2.38 ± 1.06 bc	0.023*			
R-MFC	1.57 ± 0.24 ab	1.49 ± 0.21 abc	1.38 ± 0.19 bc	0.007**	5.352	(−0.056/0.223) (0.053/0.340) (−0.045/0.271)	0.122
L-MFC	1.61 ± 0.23 ab	1.51 ± 0.22 abc	1.38 ± 0.23 bc	0.002**	6.802	(−0.047/0.247) (0.0817/0.383) (−0.033/0.298)	0.150
Mean-MFC	1.59 ± 0.21 ab	1.50 ± 0.21 abc	1.38 ± 0.20 bc	0.002**	7.057	(−0.041/0.224) (0.078/0.3513) (−0.027/0.273)	0.155 (Large effect)
R-IFC	2.06 ± 0.31	1.95 ± 0.35	1.94 ± 0.32	0.252	1.402	(−0.090/0.321) (−0.086/0.337) (−0.222/0.242)	0.035
L-IFC	2.06 ± 0.32	1.94 ± 0.32	1.94 ± 0.26	0.217	1.558	(−0.074/0.312) (−0.078/0.318) (−0.217/0.219)	0.039
Mean-IFC	2.06 ± 0.26	1.94 ± 0.33	1.94 ± 0.26	0.165	1.845	(−0.061/0.295) (−0.060/0.306) (−0.197/0.206)	0.046 (Small effect)
R-LFC	1.77 ± 0.25 ac	1.62 ± 0.14 bc	1.67 ± 0.21 abc	0.022*	4.035	(0.019/0.291) (−0.035/0.246) (−0.203/0.105)	0.095
L-LFC	1.79 ± 0.22 a	1.66 ± 0.17 bc	1.63 ± 0.17 bc	0.005**	5.744	(0.009/0.256) (0.034/0.287) (−0.111/0.167)	0.130
Mean-LFC	1.78 ± 0.22 a	1.64 ± 0.14 bc	1.65 ± 0.16 bc	0.004**	5.516	(0.0255/0.262) (0.011/0.255) (−0.145/0.123)	0.125 (Medium-Large effect)

Note: a.b,c: Post hoc test subgroups.
Abbreviations: KL Grading, Kellgren-Lawrence Grading; KO, Knee Osteoarthritis; L-IFC, Left İntercondylar Femoral Cartilage thicknesses; L-LFC, Left Lateral Femoral Cartilage Thicknesses; L-MFC, Left Medial Femoral Cartilage thicknesses; Mean-IFC, Right and Left İntercondylar Femoral Cartilage thicknesses mean; Mean-LFC, Right and Left Lateral Femoral Cartilage thicknesses mean; Mean-MFC, Right and Left Medial Femoral Cartilage thicknesses mean; R-IFC, Right İntercondylar Femoral Cartilage thicknesses; R-LFC, Right Lateral Femoral Cartilage thicknesses; R-MFC, Right Medial Femoral Cartilage thicknesses.
*$p < 0.05$.
**$p < 0.01$.

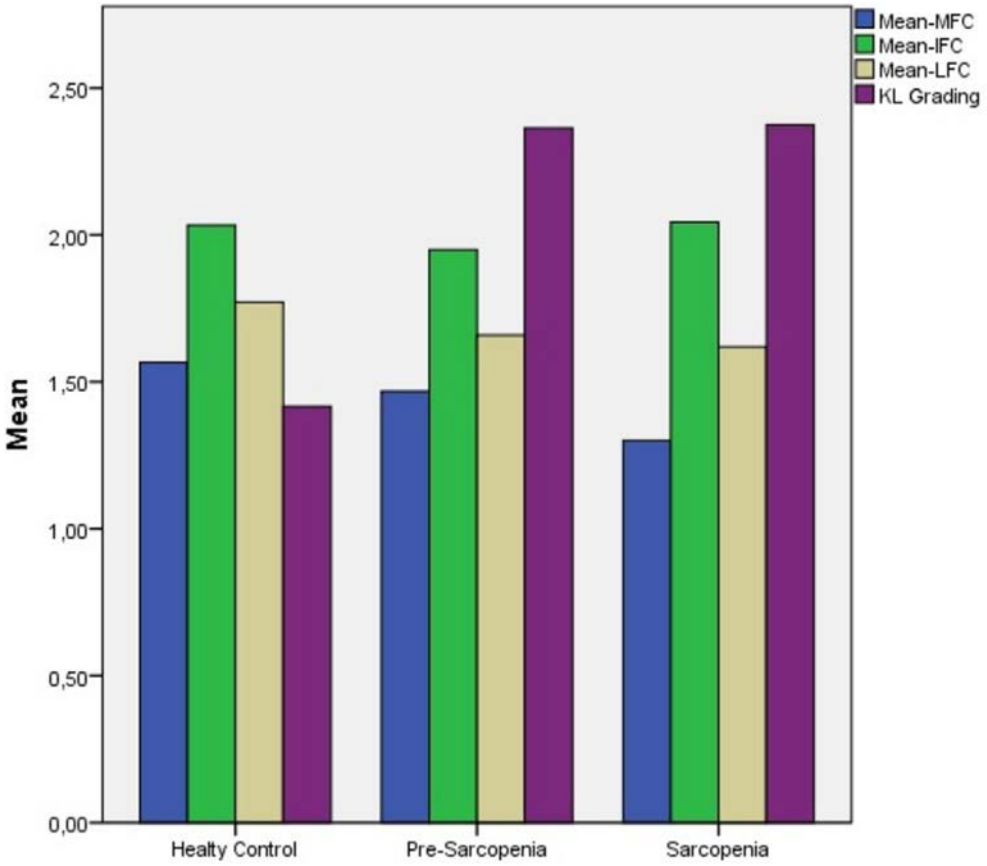

FIGURE 1 | Femoral Cartilage Thicknesses and Kellgren-Lawrence (KL) grading of the groups.

ROC analysis demonstrated the ability of FCT to discriminate between pre-sarcopenia and sarcopenia. MFC thickness was statistically significant for sarcopenia ($p = 0.001$, AUC = 0.736), while MFC, LFC, and intercondylar femoral cartilage thickness (IFC) were significant for pre-sarcopenia (all $p < 0.05$) (Figures S3 and S4) (Table 5).

For Predicting Pre-Sarcopenia;

- Mean-IFC model, Accuracy = 62.5%, F1 = 69.41%, AIC = −198.986.
- Mean-LFC model, Accuracy = 72.5%, F1 = 72.3%, AIC = −196.822.
- Mean-MFC model, Accuracy = 65%, F1 = 71.4%, AIC = −210.768.
- For predicting pre-sarcopenia, the mean LFC model is the best.

For Predicting Sarcopenia;

- Mean-IFC model, Accuracy = 48.75%, F1 = 47.6%.
- Mean-LFC model, Accuracy = 66.25%, F1 = 55.3%.
- Mean-MFC model, Accuracy = 52.5%, F1 = 51.1%.
- For predicting sarcopenia, the mean MFC model is the best.

4 | Discussion

This study's findings, which investigated the relationship between sarcopenia and KO and the role of FCT in this context, showed a decrease in medial and lateral FCT and an increase in KO prevalence in both pre-sarcopenia and sarcopenia patients compared to healthy controls. The decrease in FCT was shown to be a predictor of sarcopenia, while the presence of sarcopenia was also a predictor of KO. These findings support the idea that the reduction in FCT in sarcopenia may predispose individuals to KO and that sarcopenia may contribute to the coexistence of KO.

In the literature, studies have shown a positive correlation between quadriceps muscle strength and FCT, as well as a reduction in FCT in diseases accompanied by muscle weakness [5, 7, 8]. Based on this information, a decrease in FCT is a plausible outcome in sarcopenia, and our study results support this theory by showing a decrease in FCT in sarcopenia patients.

The literature has extensively studied the coexistence of sarcopenia and KO, and the relationship between these two conditions has been demonstrated in numerous studies [12, 13, 18–21]. Consistent with the literature, our study found an increased prevalence of KO in sarcopenia patients. Furthermore, the study demonstrated that sarcopenia may be an indicator of KO, and KO may be an indicator of sarcopenia.

TABLE 3 | Logistic regression models for predicting knee osteoarthritis (KO).

Predictor	Estimate	SE	*Z*	*p*	Odds ratio	95% confidence interval		Model fit
						Lower	Upper	
Model 1								
Intercept	−152.510	57.815	−26.379	0.008**	2.38e-7	2.85e-12	0.020	AIC = 68.5
Age	0.286	0.080	35.934	0.001**	13.310	113.881	15.557	R^2McF = 0.394
Sex (1 = Male)	−0.1693	0.755	−0.224	0.823	0.844	0.19234	37.060	R^2CS = 0.358
BMI	0.0930	0.100	0.926	0.354	10.974	0.90144	13.360	R^2N = 0.530
Mean-MFC	−0.1049	16.378	−0.064	0.949	0.900	0.03634	223.104	1.02 ≤ VİF ≤ 1.13
Group (2–0)	34.943	13.354	26.167	0.009**	32.926	24.037	451.026	AUC = 0.901
Group (1–0)	0.0267	0.8042	0.033	0.974	1.027	0.2123	496.733	
Model 2								
Intercept	−15.779	4.372	−3.609	0.001**	1.40e-7	2.66e-11	7.40e-4	AIC = 63.5
Age	0.276	0.075	3.657	0.001**	1.318	1.137	1.53	R^2McF = 0.383
Group (2–0)	3.248	1.231	2.639	0.008**	25.749	2.308	287.33	R^2CS = 0.350
Group (1–0)	−0.002	0.786	−0.003	0.998	0.998	0.214	4.65	R^2N = 0.518
								1.05 ≤ VİF ≤ 1.11
								AUC = 0.898

Note: Groupe: 0 = Control, 1 = Pre-Sarcopenia, 2 = Sarcopenia. Estimates represent the log odds of "KOA present = 1" versus "KOA no = 0".
Abbreviation: Mean-MFC, Right and Left Medial Femoral Cartilage thicknesses mean.
**$p < 0.01$.

Aging, obesity, diabetes, and vitamin D deficiency have been reported as common risk factors for both sarcopenia and KO. Reduced muscle strength, pro-inflammatory cytokines, and irisin are believed to play roles in their pathogenesis [22]. However, a definitive conclusion has not been reached regarding a cause-and-effect relationship. The notion of a vicious cycle between the two conditions is widely accepted [21, 23].

A decrease in knee extensor muscle strength and FCT in patients with KO has been demonstrated in some studies [5, 9–11]. The relationship between quadriceps muscle strength and FCT could be one of the mechanisms explaining the association between sarcopenia and KO. However, the cause-and-effect relationship between muscle strength and cartilage damage is unclear. In an animal experiment to investigate whether cartilage damage occurs due to muscle weakness or if it emerges first, rabbits' quadriceps muscles were weakened by injecting Botulinum toxin type A into the muscles. After 4 weeks, significant changes were observed in the patellofemoral region.

The results indicated that the initial signs of joint cartilage deterioration appeared with muscle weakness, suggesting that muscle weakness may be a risk factor for cartilage damage and osteoarthritis [24]. Another study investigating the relationship between knee muscle strength and cartilage thickness in individuals with KO found a positive correlation, and after a 1 month quadriceps strengthening program, an increase in quadriceps strength was correlated with an increase in cartilage thickness. This led to the interpretation that cartilage thinning might be attributed to muscle strength loss [5].

It is an accepted concept that a decrease in knee muscle strength leads to increased load on the knee and destabilization, causing cartilage damage. However, it has been reported that mechanical effects alone cannot solely explain this relationship. Muscle cells may play a significant role in cartilage homeostasis and regulating cartilage gene expression, indicating a more complex interplay beyond mechanical factors [23, 25].

The finding of reduced FCT in sarcopenia patients in this study is consistent with the literature. It indirectly supports the relationship between muscle strength and cartilage thickness and suggests that this relationship plays a role in the coexistence of sarcopenia and KO. Another finding of this study is that while a reduction in medial FCT indicates sarcopenia, it is not an indicator of KO. Instead, the significant association of sarcopenia presence with KO supports the view that sarcopenia may be a risk factor for KO.

The literature generally indicates a positive relationship between FCT and BMI [26–28]. However, one study has reported a positive correlation between FCT and muscle mass and a negative relationship with fat mass [29]. Consistent with the literature, this study found a weak positive correlation between BMI,

TABLE 4 | Multinomial logistic regression for predicting pre- sarcopenia and sarcopenia.

Predictor	*B*	SE	Wald	*p*	Exp (B)	95% CI Lower	95% CI Upper	Model fit
Model 1	Category = 2 (Sarcopenia Group), Referans = 0 (Healty Control)							Model 1
Intercept	11.0766	5.3282	2.079	**0.038***	64643.68478	1.88434	2.22e+9	AIC = 168
Age	−0.0173	0.0490	−0.353	0.724	0.98287	0.89290	1.082	R^2McF = 0.205
Sex (1 = Male)	−0.1913	0.7353	−0.260	0.795	0.82586	0.19546	3.489	R^2CS = 0.136
BMI	−0.1860	0.0993	−1.873	0.061	0.83024	0.68336	1.009	R^2N = 0.266
KOA (1–0)	2.8496	1.2804	2.226	**0.026***	17.28031	1.40496	212.539	
Mean-MFC	−4.6435	2.3418	−1.983	**0.047***	0.00962	9.77e-5	0.948	
Mean-IFC	1.4788	1.5987	0.925	0.355	4.38770	0.19116	100.711	
Mean-LFC	−2.3111	2.7845	−0.830	0.407	0.09916	4.23e-4	23.256	
	Category = 1 (Pre-Sarcopenia Group), Referans = 0 (Control Group)							
Intercept	4.5342	5.0728	0.894	0.371	93.15182	0.00448	1.94e+6	
Age	0.0378	0.0486	0.777	0.437	1.03851	0.94414	1.142	
Sex (1 = Male)	−0.1734	0.6255	−0.277	0.782	0.84081	0.24676	2.865	
BMI	−0.0131	0.0839	−0.156	0.876	0.98696	0.83735	1.163	
KOA (1–0)	0.6809	0.8172	0.833	0.405	1.97561	0.39818	9.802	
Mean-MFC	0.4558	1.9172	0.238	0.812	1.57742	0.03681	67.593	
Mean-IFC	0.7531	1.5322	0.491	0.623	2.12350	0.10540	42.782	
Mean-LFC	−5.6377	2.5910	−2.176	0.030	0.00356	2.22e-5	0.572	
Model 2.	Category = 2 (Sarcopenia Group), Referans = 0 (Control Group)							Model 2.
Intercept	8.632	3.362	2.568	**0.010***	5606.91299	7.71140	4.08e+6	AIC = 163
KOA (1–0)	−2.563	1.160	−2.210	**0.027***	0.07707	0.00794	0.748	R^2McF = 0.166
Mean-MFC	−4.558	2.248	−2.028	**0.043***	0.01048	1.28e-4	0.858	R^2CS = 0.111
Mean-IFC	0.865	1.507	0.574	0.566	2.37502	0.12394	45.513	R^2N = 0.218
Mean-LFC	−2.166	2.604	−0.832	0.405	0.11461	6.96e-4	18.869	
	Category = 1 (Pre-Sarcopenia Group), Referans = 0 (Control Group)							
Intercept	7.763	3.066	2.532	**0.011***	2351.79340	5.77862	957136.619	
KOA (1–0)	−1.040	0.670	−1.553	0.120	0.35329	0.09501	1.314	
Mean-MFC	0.518	1.901	0.272	0.785	1.67816	0.04041	69.694	
Mean-IFC	0.516	1.489	0.347	0.729	1.67591	0.09048	31.042	
Mean-LFC	−5.708	2.508	−2.276	**0.023***	0.00332	2.43e-5	0.453	

Note: KOA: 1 = Present, 2 = No.
Abbreviations: Mean-IFC, Right and Left İntercondylar Femoral Cartilage thicknesses mean; Mean-LFC, Right and Left Lateral Femoral Cartilage thicknesses mean; Mean-MFC, Right and Left Medial Femoral Cartilage thicknesses mean.
*$p < 0.05$ indicate bold values.

appendicular muscle mass, and HGS with FCT. This positive relationship suggests that muscle strength, muscle mass, and BMI influence FCT through mechanical factors and biochemical signals regulating cartilage metabolism.

Studies investigating the relationship between gender and FCT have indicated that FCT tends to be thinner in females compared to males [30]. Males' higher muscle strength can explain their thicker femoral cartilage. In this study, no difference in

TABLE 5 | ROC analysis of femoral cartilage thickness for predicting pre-sarcopenia and sarcopenia.

		AUC	SE	*p*	95% CI (Lower–Upper)	Cut off
Predicting pre-sarcopenia	Mean_MFC	0.698	0.059	0.002**	0.582-0.814	1.55
	Mean_IFC	0.636	0.062	0.037*	0.515-0.758	2.10
	Mean_LFC	0.696	0.062	0.003**	0.574-0.817	1.80
Predicting sarcopenia	Mean_MFC	0.736	0.06	0.001**	0.618-0.854	1.45
	Mean_IFC	0.566	0.071	0.370	0.427–0.705	2.03
	Mean_LFC	0.600	0.067	0.173	0.469–0.732	1.73

Abbreviations: Mean-IFC, Right and Left İntercondylar Femoral Cartilage thicknesses mean; Mean-LFC, Right and Left Lateral Femoral Cartilage thicknesses mean; Mean-MFC, Right and Left Medial Femoral Cartilage thicknesses mean.
*$p < 0.05$.
**$p < 0.01$.

FCT was found between females and males, which could be attributed to the similar rates of sarcopenia in both groups.

The limitations of this study include the small sample size for regression analyses, despite sufficient patient numbers and a large effect size (>0.14) for group comparisons, leading to wide confidence intervals in the regression models. Additionally, ethical and financial constraints prevented knee radiographs from being performed on all participants, requiring them to rely on knee radiographs retrieved from patient records instead. The study also did not evaluate factors such as obesity, exercise habits, and nutrition, which play a shared role in the pathogenesis of sarcopenia and KO. Another limitation of this study is its cross-sectional design, lack of prognostic insight, inability to establish causality, and reliance on binary outcomes, which may overlook important associations. Further prospective studies with larger patient populations and more comprehensive data are needed in this regard.

This study showed a decrease in FCT and an increase in the prevalence of KO in sarcopenia patients. It has been observed that a decrease in medial FCT may predict sarcopenia, while sarcopenia may also predict KO. Moreover, the reduction in FCT may play an important role in explaining the relationship between KO and sarcopenia.

A weak positive correlation was found between HGS and muscle mass with FCT, highlighting the importance of mechanical and biochemical factors affecting cartilage thickness. Sarcopenia may be a risk factor for KO, and these two conditions might share common pathophysiological mechanisms. Prospective studies with larger sample sizes could contribute to a better understanding of the relationship between sarcopenia, KO, and FCT.

Author Contributions

S.T., N.B., E.K. investigation, data curation, methodology, design, formal analysis, writing. S.T. performing an ultrasound. S.T., E.K., N.B. writing, supervision, review and editing. All authors have read and approved the final manuscript.

Ethics Statement

The ethics committee of Akdeniz University Faculty of Medicine (KAEK-60, 27.01.2021) reviewed and approved this study protocol. All subjects were informed about the study, and their written informed consent was obtained. Written and verbal consent was obtained from all participants.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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LETTER TO THE EDITOR

Chronic Periaortitis in Marfan Syndrome: A Case Report

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Dear Editor,

Chronic periarteritis (CP) includes idiopathic retroperitoneal fibrosis (IRF), inflammatory abdominal aortic aneurysm (IAAA), and perianeurysmal retroperitoneal fibrosis (PRF). IRF is an immune-mediated inflammatory disease characterized by persistent non-specific inflammation of the tissues around the retroperitoneal aorta with increasing fibrous tissue hyperplasia [1]. Marfan syndrome (MFS) is an autosomal dominant disorder affecting extracellular matrix formation [2]. Only four cases of IRF have been reported in patients with MFS, highlighting its rarity (summarized in Table 1). Here, we present an additional case of IRF in a middle-aged man definitively diagnosed with MFS during follow-up.

A 36-year-old man was admitted with chief complaints of intermittent claudication in both lower limbs, abdominal pain, constipation, and weariness. He had aortic root replacement surgery when he was 22 years old because of aortic insufficiency. MFS was diagnosed based on skeletal features like thoracic deformity and narrow arms, followed by genetic testing for fibrillin-1 (FBN1) gene mutations. After an 11-year follow-up post-surgery, a CT angiography (CTA) scan revealed thickening and encasement of the mid-distal abdominal aorta, iliac arteries, and adjacent soft tissues, indicating periaortitis. Upon admission, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), liver and renal function, and tumor markers were all within normal limits. Immunologic indicators such as rheumatoid factor, immunoglobulin, complements (C3 and C4), and serum IgG subclass levels were standard. A contrast-enhanced CT scan revealed a retroperitoneal soft tissue shadow around the abdominal aorta (inferior to the renal arteries) that extended to the bilateral common iliac artery, indicating retroperitoneal fibrosis. The patient was then treated with oral prednisone (40 mg/day) and cyclophosphamide (100 mg/day). His symptoms improved dramatically after using the medication. Subsequently, systemic glucocorticoids were gradually tapered over 8 months, with a daily maintenance dose of 5 mg. At the 2-year follow-up, there was a shrinkage of the maximum diameter and degree of aortic involvement in the patient's CTA image (Figure 1).

MFS is a hereditary illness caused by FBN1 gene mutations that impair connective tissue all over the body. One of its distinguishing traits is a tendency to aortic root aneurysms and dissections, which can result in structural abnormalities in various organs, including the retroperitoneum [2]. Retroperitoneal fibrosis is a rare condition that causes inflammation and progressive fibrosis masses around the aorta and other large or medium-sized arteries in the retroperitoneum. It is divided into idiopathic and secondary variants. The majority of instances of IRF have no known origin and are considered to be caused by autoimmune or inflammatory processes initiated by aortitis or aortic disorders. Evidence from case reports suggests that patients with IRF frequently present with other systemic or organ-specific autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, and vasculitis. Recent studies have shown that approximately 30% to 60% of IRF cases exhibit histological and clinical features associated with IgG4-related disease [6].

This patient, similar to four previously reported cases, shows a consistent sequence where MFS precedes IRF, suggesting a potential causal relationship or shared pathophysiological

Chengyao Wang and Huilan Liu contribute equally to this work.

TABLE 1 | Literature review of Marfan Syndrome combined with idiopathic retroperitoneal fibrosis.

Case ID	References	Age/sex	Sequence of onset	Time Interval between onsets	Clinical manifestations of IRF	Laboratory indicators	Therapeutic interventions	Follow-up assessment
1	Chong et al. [3] 1991	53/F	MFS → IRF	NA	Severe chest pain	NA	Ureterolysis with omentoplasty	Symptom-free
2		52/M	MFS → IRF	5 years	Lower abdominal and back pain	NA	Bed rest and antihypertensives	Clinically well after 1-year follow-up; CT showed spontaneous Regression of periaortic mass and normalization of left kidney function
3	Johnston et al. [4] 2007	34/M	MFS → IRF	NA	Fever, weight loss, night sweats, low-back pain, and altered bowel habit.	NA	Oral prednisolone 60 mg/day	Improved renal function; stent placed due to kidney obstruction
4	Lipscomb et al. [5] 2016	44/M	MFS → IRF	36 years	Abdominal pain, lower back pain, weight loss, altered bowel habits and general malaise	CRP: 36 mg/L ESR: 36 mm/h Serum IgG: 16.50 g/L Serum IgG4: normal	Oral prednisolone 40 mg/day, MTX 1 g twice daily; later switched to MMF due to intolerance.	Symptoms resolved, weight increased, acute phase markers normalized; CTA showed reduced peri-aortic inflammation
5	Our case	36/M	MFS → IRF	11 years	Lower limb claudication, abdominal pain, constipation, and weariness	CRP: 0.57 mg/L ESR: 4 mm/h Serum IgG2: 8.06 g/L Serum IgG4: 0.29 g/L	Oral prednisolone 40 mg/day and cyclophosphamide 100 mg/day ' gradually tapered prednisolone over 8 months to a daily maintenance dose of 5 mg.	Symptoms improved, CTA showed reduced aortic inflammation

Abbreviations: CTA, CT angiography; F, Female; IRF, idiopathic retroperitoneal fibrosis; M, Male; MFS, Marfan syndrome; MMF, mycophenolate; MTX, methotrexate; NA, not addressed.

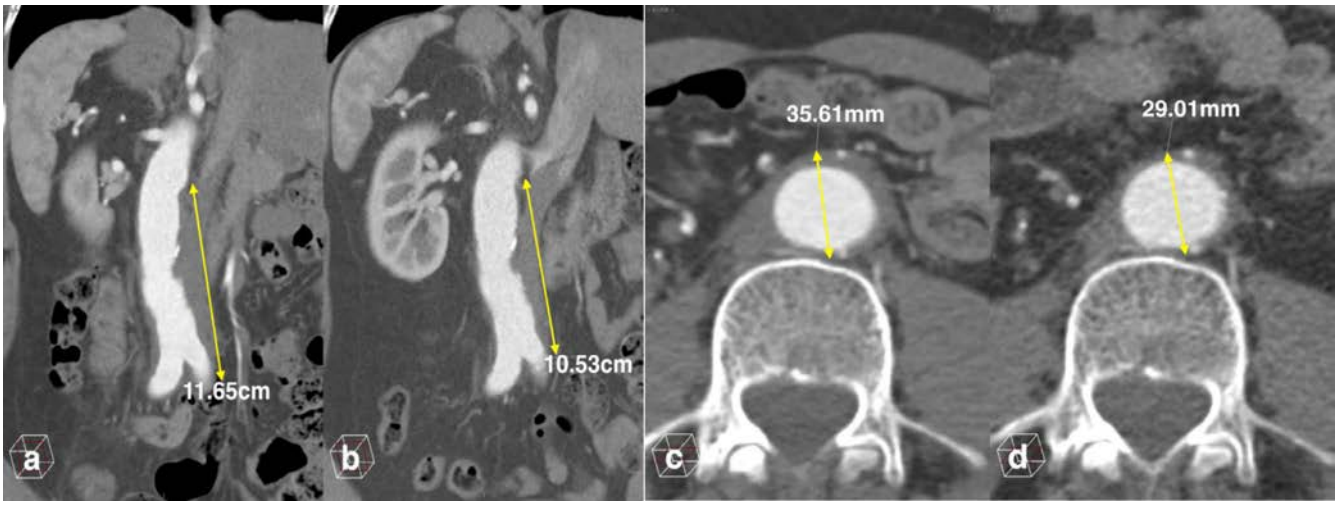

FIGURE 1 | The CTA demonstrates the positive impact of treatment. (a, b)The aortic cumulative length decreased from 11.65 cm to 10.53 cm after therapy, with fibrous thickening observed around the abdominal aorta, iliac artery, and inferior vena cava. (c, d)The maximum short-axis diameter of the aorta, including the soft tissue mass, was 35.61 mm before and 29.01 mm after treatment.

mechanisms. The overlap between these disorders may be due to common pathways involving the extracellular matrix and immunological dysregulation. FBN1 mutations in MFS alter extracellular matrix integrity, predisposing to aortic damage. Peripheral artery walls with reduced elasticity and stability in MFS may allow atherosclerotic plaque components to seep into adjacent tissues, potentially triggering the fibroinflammatory response linked to IRF [7]. These inflammatory and fibrotic alterations are probably a result of localized autoallergic reactions to particular elements found in atherosclerotic plaques. The patient's 11-year clinical history may suggest MFS's impact on the aorta, indicating early aging and degradation of the aorta, potentially linking these two rare diseases. However, the wide variation in the interval between MFS and IRF onset indicates that disease progression may be influenced by multiple factors, including genetic predisposition, environmental triggers, and the effectiveness of early interventions.

Regarding treatment, for isolated IRF and IgG4-mediated diseases, treatment options have been well documented; however, due to the limited number of cases associated with MFS, therapeutic approaches remain to be established. The consensus for the treatment of IRF supports glucocorticoids as first-line treatment and should be started promptly after diagnosis. To minimize recurrence, treatment should last at least 1 year. Immunosuppressive therapy may be used to minimize glucocorticoid reliance or to treat patients with refractory IRF [8, 9]. In the literature, only one case of spontaneous regression of periaortic soft tissue masses without steroid medication has been observed and documented via CT, adding complexity and hope to treatment strategies [10].

This case study has several important clinical implications. Firstly, it highlights the need for clinicians to be aware of the potential association between MFS and IRF, especially in patients with a history of MFS and presenting with relevant symptoms. Secondly, it emphasizes the importance of a multidisciplinary approach to diagnosis, including clinical assessment, genetic testing, imaging, and laboratory investigations. Thirdly, it provides evidence for the effectiveness of a specific treatment combination (oral prednisone and cyclophosphamide) in MFS-associated IRF, which can guide future treatment decisions. Finally, it underlines the importance of monitoring aortic damage in patients with MFS to mitigate the risk of developing complications like IRF.

Overall, this case study enhances our understanding of MFS and IRF, highlighting the need for a multidisciplinary approach to diagnosis and treatment. Understanding the impact of MFS on the aorta and its potential role in predisposing individuals to IRF could provide valuable insights into managing and treating both conditions. It underscores the importance of monitoring aortic damage in patients with MFS to mitigate the risk of developing complications like IRF.

Author Contributions

C.W. collected the data and drafted the article. H.L. and H.Z. were the physicians who diagnosed and managed the case. N.L. conducted comprehensive radiological evaluations of the case. Y.L. revised the article critically for important intellectual content.

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Ethics Statement

Beijing friendship hospital Research Ethics Committee (2022-P2-101).

Consent

The authors affirm that human research participants provided informed consent for publication of the images in Figure 1a–d.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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LETTER TO THE EDITOR

Case Report: Diagnostic Challenges of Pancreatic Cancer in a Patient With Stevens–Johnson Syndrome

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Dear Editor,

Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) is a life-threatening severe adverse drug reaction (SCAR) characterized by extensive skin detachment and multiple-organ involvement, including the liver, kidney, and gastrointestinal tract [1–3]. SJS/TEN carries significant morbidity and mortality, with reported mortality rates ranging from 10% for SJS to over 30% for TEN. Among the extracutaneous involvement, gastrointestinal involvement remains the most frequent systemic manifestation of SJS/TEN, often presenting with transient elevation of liver enzymes and necrosis of the gastrointestinal tract epithelium [4]. Up to 47% of SJS/TEN patients suffer from hepatitis, and SJS/TEN-related liver injury has a high mortality rate of 45%, particularly when accompanied by jaundice [2, 3]. It is paramount to conduct a comprehensive series of examinations in cases with intractable liver dysfunction to exclude alternative diagnoses before attributing them solely to SJS/TEN-associated liver injury. These investigations include identifying hepatotoxic medications causing drug-induced hepatitis, performing hepatobiliary laboratory tests and imaging studies, conducting autoimmune serology for autoimmune hepatitis, and performing virology tests for viral hepatitis [5–7]. Tumor assessment is also crucial, as malignancy is a significant factor in predicting the mortality risk associated with TEN [8].

Pancreatic cancer is a highly aggressive malignancy with one of the poorest prognoses among all cancers, accounting for significant global morbidity and mortality [9]. It is the seventh leading cause of cancer-related deaths worldwide, with a 5-year survival rate of less than 10%, largely due to its asymptomatic presentations, frequently remaining undetected until reaching an advanced stage [10]. However, in cases lacking viral markers, a history of hepatotoxic medication use, and inadequate response to SJS/TEN treatment, persistent liver dysfunction warrants imaging studies to investigate the possibility of underlying pancreatic cancer.

In this report, we present a case of SJS accompanied by progressive and refractory liver dysfunction and jaundice. Initially, these symptoms were attributed to complications of SJS. However, further comprehensive imaging surveys revealed the presence of pancreatic adenocarcinoma.

A 66-year-old man presented with a sudden onset of generalized, painful skin rashes that rapidly progressed from the trunk to the extremities within 1 week. The patient had no prior medical history and was not on any long-term medication regimen. Two weeks prior to the skin eruption, the patient had completed a 7-day course of levofloxacin for sinusitis. Physical examination revealed widespread erythematous maculopapular exanthemas and atypical targetoid lesions on the trunk and extremities, with erythema and skin detachment occupying 70% and 3% of total body surface area, respectively (Figure 1A–C). Mucosal involvement included the lips,

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tongue, and buccal mucosa, while ocular and urogenital mucosa were spared (Figure 1D). Histopathological examination obtained from the abdominal skin showed necrotic epidermis, vacuolar interface dermatitis, and negative results of the direct immunofluorescence study (Figure 2A). Levofloxacin-induced SJS was diagnosed based on the scoring criteria of the algorithm for drug causality in epidermal necrolysis and the Naranjo algorithm.

Laboratory examination 7 days after the index day revealed elevated liver enzymes, including aspartate aminotransferase (AST; 129 U/L) (normal, 13–40 U/L), alanine transaminase (ALT; 153 U/L) (normal, 7–40 U/L), alkaline phosphatase (ALK-P; 144 U/L) (normal, 28–94 U/L), and γ-glutamyltransferase (γ-GT; 297 U/L) (normal, 0–26 U/L). Direct and total bilirubin levels were within the normal range. The patient was treated with systemic methylprednisolone (40 mg/day), and skin lesions gradually improved.

One week later, a follow-up laboratory examination showed progressive liver dysfunction refractory to immunomodulants, as evidenced by elevated levels of AST (221 U/L), ALT (837 U/L), ALK-P (225 U/L), γ-GT (1195 U/L), direct (1.7 mg/dL) (normal, 0–0.4 mg/dL), and total bilirubin (3.8 mg/dL) (normal,

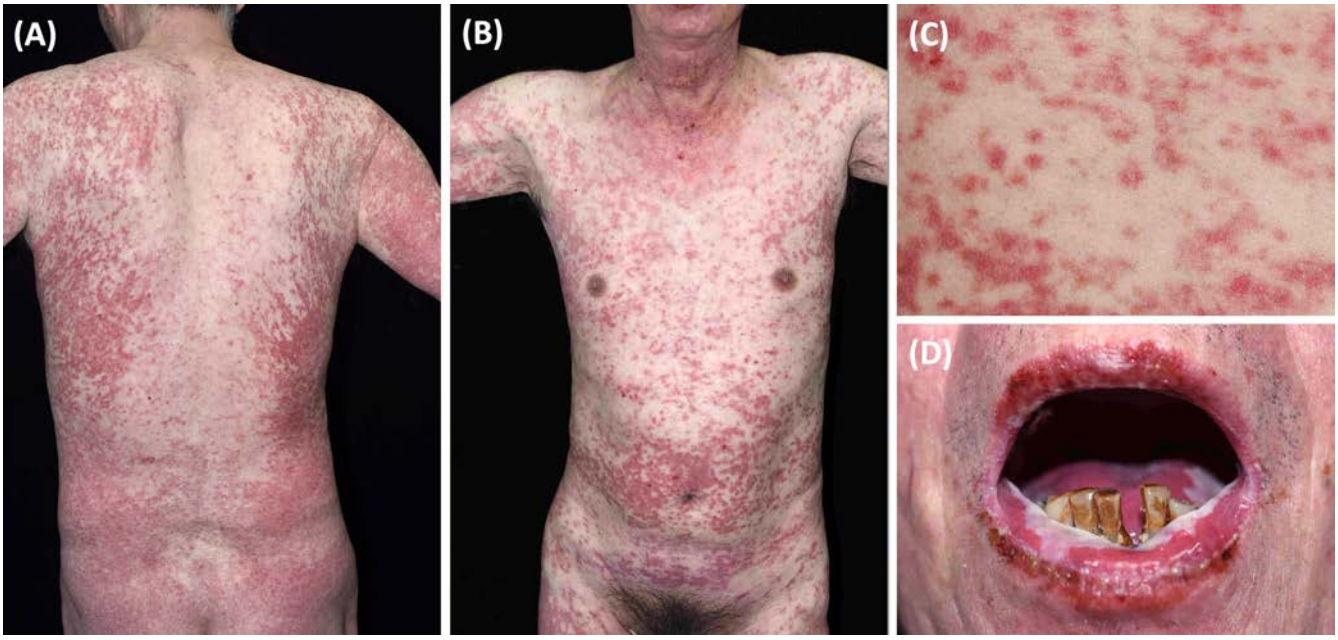

FIGURE 1 | Skin manifestations of Stevens–Johnson syndrome. (A, B) Generalized erythematous maculopapular exanthemas on the chest, abdomen, back, and buttocks. (C) Atypical targetoid lesions and skin erosions on the abdomen. (D) Mucosal breakage including lips, tongue, and buccal mucosa.

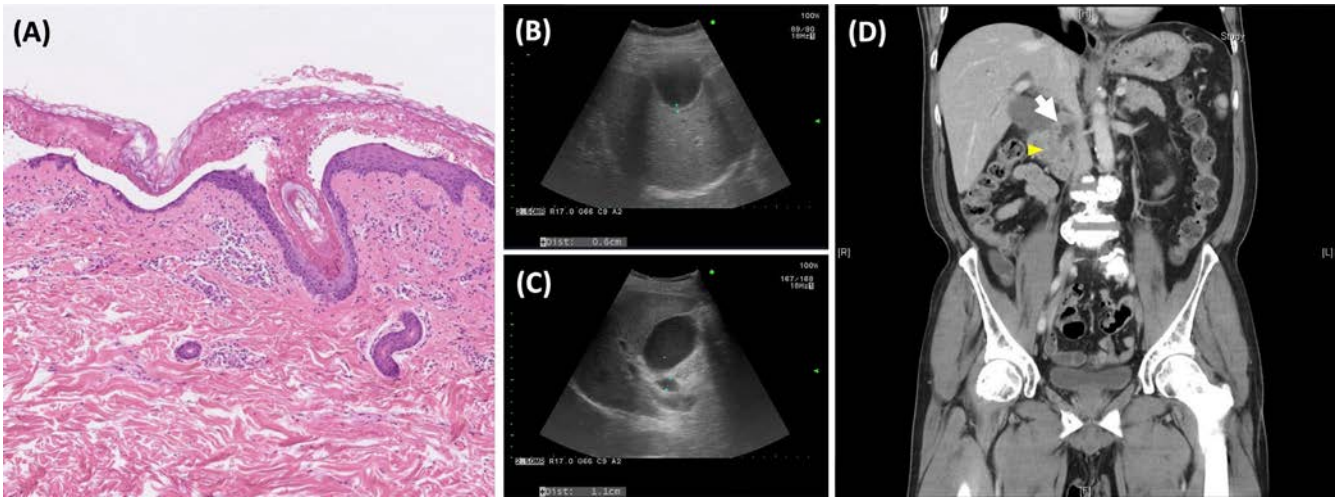

FIGURE 2 | Histopathology features and imaging surveys of the patient. (A) Skin biopsy demonstrated necrotic epidermis, vacuolar interface dermatitis with subepidermal separation, and superficial perivascular lymphocytic infiltrates (hematoxylin and eosin stain, 40×). (B) Ultrasound imaging of the gallbladder and gallbladder wall. (C) Ultrasound imaging of the common bile duct. (D) Image findings of abdominal computed tomography with contrast (arrow, dilatation of common bile duct; arrowhead, a hypodense pancreatic ductal adenocarcinoma at the uncinate process of pancreas causing distal common bile duct obstruction).

0.2–1.4 mg/dL). There was neither viral hepatitis nor autoimmune hepatitis after serial examinations, and the patient had not been exposed to any known hepatotoxic medications.

While the elevation of liver and biliary parameters is common in SJS patients, the presence of progressive liver injury and new-onset cholestasis necessitates further investigations to rule out alternative etiologies. Transabdominal ultrasound is a useful diagnostic tool for evaluating the biliary tract and establishing the level of obstruction. In our patient, transabdominal ultrasound revealed normal intrahepatic ducts and a normal visualized part of the pancreas and kidneys, but notable thickening of the gallbladder wall (Figure 2B) along with dilatation of the common bile duct (Figure 2C). Considering the sensitivity of ultrasound is dependent on the expertise of the operator and the possibility of bowel gas obscuring the pancreas, an abdominal computed tomography (CT) was arranged. The CT scan showed the presence of pancreatic uncinate process adenocarcinoma (Figure 2D, arrowhead) with distal common bile duct obstruction and biliary tree dilatation (Figure 2D, arrow). Therefore, a diagnosis of pancreatic adenocarcinoma mimicking SJS-associated liver injury was made.

After the diagnosis, our patient was referred to gastrointestinal specialists following the stabilization of his skin condition. He subsequently underwent a pancreaticoduodenectomy and received chemotherapy with tegafur/gimeracil/oteracil (TS-1). Unfortunately, the pancreatic cancer progressed with liver, lung, and abdominal lymph node metastases after 1 year.

To our knowledge, there are no existing case reports or studies in the literature documenting a similar presentation of SJS/TEN patients with incidentally identified pancreatic cancer. Written informed consent was obtained from the patient, and the relevant information has been detailed. The diagnostic process in this case posed significant challenges due to the overlapping clinical features of SJS/TEN and pancreatic cancer, particularly in the context of liver injury. SJS/TEN can present with elevated liver and biliary parameters, potentially obscuring or delaying the identification of underlying malignancies [3]. Typically, SJS/TEN-associated liver injury improves alongside the resolution of cutaneous symptoms following treatment with immunomodulators. However, in this patient, the discordance between the skin condition and persistent abnormalities in liver function tests after SJS/TEN treatment necessitated further investigation. Additionally, we thoroughly examined other potential causes of liver dysfunction and jaundice, such as viral hepatitis, autoimmune hepatitis, and medication-related hepatitis. To rule out vanishing bile duct syndrome (VBDS) or other potential hidden malignancies, abdominal imaging studies including ultrasound and CT were performed, leading to the identification of pancreatic adenocarcinoma.

The liver and skin are the most frequent organs involved in SJS/TEN [11]. Hepatitis, jaundice, and gastrointestinal symptoms are frequently documented as complications of SJS/TEN, accompanied by the elevation of liver and biliary parameters [12]. Among them, the incidence of liver injury in SJS/TEN patients ranged from 5% to 13%, and the mortality rate of SJS/TEN can reach to 36% [3, 11, 13]. Fortunately, the elevation of liver function tests mostly results from cholestatic hepatitis, which appears to be transient and typically returned to the normal range within 2 weeks. The treatment of SJS/TEN and drug-induced liver injury involves immediate cessation of the culprit drug, transfer to the intensive care unit or burn unit, and supportive care aimed at alleviating symptoms [14].

The pathophysiological mechanisms linking SJS/TEN and pancreatic cancer present significant diagnostic challenges, particularly in the context of liver dysfunction. SJS/TEN is characterized by a robust inflammatory response, mediated by proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukins, which can lead to inflammation-related cholestasis [7, 15]. This process involves the inhibition of hepatobiliary transporter gene expression and disruption of bile secretion, mimicking hepatic injury typically associated with SJS [15]. In this case, the initial biochemical evidence of liver injury prompted imaging studies to investigate potential underlying causes. Abdominal CT revealed pancreatic adenocarcinoma with distal biliary obstruction, distinguishing it from SJS-associated liver injury.

For SJS/TEN patients with intractable liver dysfunction, VBDS and bile duct obstruction should be considered in the differential diagnosis. Although VBDS is rare, it is a fatal cause of progressive cholestasis [6]. It is suggested that drugs act as haptens and produce autoantibodies against cytokeratin in the bile duct epithelium, leading to the destruction of the biliary apparatus and resultant disappearance of the intrahepatic bile duct [6]. In such cases, abnormal liver and biliary function results may persist for over 2 weeks and can be refractory to corticosteroid and ursodeoxycholic acid therapies. Imaging studies help exclude extrahepatic biliary obstruction, while liver biopsy is crucial for diagnosing VBDS. Other differential diagnoses include autoimmune and viral hepatitis, which can be easily ruled out by blood tests. Autoimmune markers, such as antinuclear antibodies and antismooth muscle antibodies, can be examined. Viral surveys, including tests for hepatotropic viruses, cytomegalovirus, and Epstein–Barr virus, can be performed. In our case, tests for viral and autoimmune hepatitis were negative, and the CT scan revealed the presence of extrahepatic biliary obstruction caused by the compression of the pancreatic uncinate process adenocarcinoma.

This case highlights the diagnostic challenge of identifying pancreatic cancer among systemic involvements of SJS/TEN. While liver dysfunction and jaundice commonly occur as complications in SJS/TEN, a significant elevation of hepatobiliary parameters may indicate cholestasis associated with pancreatic cancer. Abdominal imaging studies should be considered in SJS/TEN patients with persistent or progressive liver dysfunction that is refractory to standardized treatment before considering the enhancement of immunosuppressive therapy. Further research is needed to investigate the long-term outcomes of patients with SJS/TEN who subsequently develop malignancies. Understanding the potential mechanisms linking SJS/TEN to malignancy, such as immune dysregulation or prolonged inflammation, could provide valuable insights into disease progression. Additionally, exploring potential preventive measures, such as early screening protocols for hidden malignancies in patients with atypical presentations or refractory symptoms, may enhance early detection and treatment.

Author Contributions

All authors substantially contributed to the collection of data and drafting of this manuscript, and also approved the final version.

Consent

The authors have obtained patient consent.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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LETTER TO THE EDITOR

Case Series: Effectiveness and Safety of Telitacicept in Chinese Patients With Primary Sjögren's Disease

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Dear Editor,

Telitacicept is a novel fusion protein developed specifically for treating B cell-mediated autoimmune diseases. Limited data are available on the use of telitacicept in patients with primary Sjögren's disease (pSjD). This case series evaluated the effectiveness and safety of telitacicept in seven female patients with pSjD, suggesting it may be a promising therapeutic option.

pSjD is a complex chronic inflammatory autoimmune disease, often presenting clinically with dry mouth and dry eyes due to impaired salivary and lacrimal gland functions, respectively [1]. The pathogenesis of pSjD is intricate, characterized by chronic inflammatory cell infiltration [2]. A key feature of pSjD is the excessive activation of B cells mediated by T cells. B cells are involved in various aspects of pSjD pathogenesis, including the production of autoantibodies, antigen presentation, and cytokine production [3]. Critical players in B cell survival and activation, as well as in the prolonged lifespan of plasma cells, include B cell-activating factor (BAFF/BLyS) and a proliferation-inducing ligand (APRIL), which are produced by a range of innate immune cells [4]. Previous studies have reported elevated serum and saliva levels of BAFF/BLyS and APRIL in pSjD patients, which correlate with disease activity [5–7].

Telitacicept, a recombinant fusion protein, inhibits the maturation of immature B cells by blocking BLyS and prevents mature B cell differentiation into plasma cells by blocking APRIL, thereby reducing the secretion of autoantibodies [8]. In March 2021, telitacicept received its first approval in China for the treatment of patients with active systemic lupus erythematosus (SLE), based on its significant improvement in disease activity and acceptable safety profile in a phase IIb clinical trial [9]. Both SLE and pSjD are B cell-mediated autoimmune diseases characterized by high levels of autoantibodies [10]. The confirmed efficacy of telitacicept in SLE has further driven its expanded application in pSjD. Recently, a phase II clinical trial of telitacicept for pSjD showed promising clinical benefits and a favorable safety profile [11]. A multicenter phase III clinical trial of telitacicept is ongoing in China (NCT05673993). To date, there is limited data on the use of telitacicept in patients with pSjD, both in China and globally [11]. In this case series, the effectiveness and safety of telitacicept were assessed in seven patients with pSjD, whose baseline characteristics and treatment regimens differed from those in clinical trials. Notably, our study included patients with varying disease activity (European League Against Rheumatism Sjögren's Syndrome Disease Activity Index [ESSDAI] scores below 5 in some cases) and allowed continued use of corticosteroids and immunosuppressants, whereas clinical trials typically excluded such patients and required drug washout periods [11]. These differences provide valuable insights into the potential of telitacicept as a therapeutic option for pSjD in real-world clinical settings.

This retrospective case series included patients diagnosed with pSjD at the Department of Rheumatology and Immunology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, China, between March 2021 and December 2023. Eligible patients were aged 18 to 65 years and met the 2016 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for pSjD. All the included patients tested positive for anti-Sjögren's-syndrome-related antigen A (anti-SSA)

Jiajia Wu, You Song and Weiwei Wang contributed equally to this work.

antibodies and had been followed up for at least 8 weeks after initiating treatment with telitacicept. Exclusion criteria were use of other biological agents such as rituximab, belimumab, etanercept, or infliximab within 6 months prior to the administration of telitacicept; failure to complete at least one follow-up examination at our institution. The case series adhered to the principles outlined in the Declaration of Helsinki and received approval from the Medical Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (approve no. 2024–0077). As a retrospective study, the requirement for informed consent was waived by the Ethics Committee.

All patients received telitacicept in combination with standard treatment regimens [12, 13], including glucocorticoids and/or immunosuppressants such as mycophenolate mofetil (MMF), hydroxychloroquine (HCQ), iguratimod (IGU), and cyclosporine (CsA). Telitacicept treatment was selected through a shared decision-making process between the physician and the patient. Telitacicept was administered subcutaneously at a dose of 80 mg or 160 mg, either once a week or every two weeks. Data were collected in patients with pSjD before and after treatment with telitacicept. Recorded variables included demographic information, concurrent use of immunosuppressants or glucocorticoids, and a range of laboratory tests such as total T cells, CD4+ T cells, CD8+ T cells, CD19+ B cells, natural killer (NK) cells, IgE, IgG, IgA, IgM, complement component 3 (C3), and complement component 4 (C4). Peripheral blood samples were collected in patients for the analysis of lymphocyte subset percentages. These subsets were detected by flow cytometry. Disease activity was assessed using the ESSDAI, a 12-domain clinician-reported outcome (ClinRO) instrument evaluating systemic disease activity, and the EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI), a 3-item patient-reported outcome (PRO) instrument measuring the severity of dryness, fatigue, and joint/muscle pain over the past 2 weeks. Both ESSDAI and ESSPRI scores were recorded at baseline and the last follow-up. In addition, patients achieving minimal clinically important improvement (MCII), defined as an ESSDAI score improvement of at least three points and an ESSPRI score decrease of at least one point or 15%, were counted.

Seven cases were ultimately included in the current analysis. All seven patients were female, with ages ranging from 24 to 60 years and disease durations varying from 2 months to 7 years. Before the initiation of telitacicept treatment, all patients had been managed with glucocorticoids and/or immunosuppressants, including MMF, HCQ, IGU, and CsA. Specifically, two patients received combination therapy involving two immunosuppressants (IGU + CsA and HCQ + CsA, respectively), while the remaining five were administered monotherapy with a single immunosuppressant (MMF, HCQ, or IGU) (Table 1).

After treatment with telitacicept, six of the seven patients had decreased ESSDAI scores and achieved MCII, while one patient showed an increase in ESSDAI score compared to baseline. All patients exhibited decreased their ESSPRI scores and achieved MCII (Figure 1). Furthermore, glucocorticoid doses could be reduced in all patients, with three cases having their doses reduced to 5 mg or lower (Table S1). Immunological biomarkers were assessed in six of the seven patients before and after telitacicept treatment. Among these six patients, reductions were observed in IgG, IgA, and IgM levels compared to baseline. C3 levels were increased in three patients and decreased in the remaining three. In terms of C4 levels, four patients experienced an increase, while two had a decrease (Table 1 and Figure 1). Case 6 underwent closer monitoring of lymphocyte subset percentages during telitacicept treatment. The patient exhibited a gradual increase in the proportion of CD8+ T cells, while the proportion of CD19+ B cells progressively decreased. In addition, the levels of NK cells showed an upward trend (Figure 1). Immunological testing was performed for five patients following telitacicept treatment. Regarding antinuclear antibody (ANA) titers, two patients experienced a decrease, from 1:3200 to 1:320 in case 4 and from 1:1000 to 1:100 in case 6. Meanwhile, one patient (case 4) showed an increase in the ANA titer, from 1:320 to 1:3200; the remaining patients exhibited no significant changes. Both patients who tested positive for RF showed a marked decrease in the RF titer after using telitacicept, from 49.6 IU/mL to 27.8 IU/mL in case 2 and from 43.3 IU/mL to 14.5 IU/mL in case 5 (Table 1). During the telitacicept treatment period, no serious adverse events were reported in the seven patients.

To our knowledge, this is the first case series of pSjD patients treated with telitacicept, summarizing the characteristics and treatment outcomes of seven female patients administered telitacicept. The results demonstrated clinical improvements, with six patients showing decreased ESSDAI scores and all patients exhibiting reduced ESSPRI scores. These improvements were accompanied by reductions in glucocorticoid doses and stable immunosuppressant use. Immunologically, reductions in IgG, IgA, and IgM levels, along with specific changes in lymphocyte subsets observed in six patients, indicated immunomodulatory effects for telitacicept. Importantly, no serious adverse events were reported. These findings support the potential of telitacicept as an effective and safe treatment option for pSjD.

In a phase II clinical trial of pSjD, telitacicept administered subcutaneously at 160 mg once a week significantly reduced the ESSDAI score at week 24 compared with placebo, as well as serum IgM, IgG, and IgA levels. In addition, patients reported significant improvement in fatigue symptoms, as assessed by the Multidimensional Fatigue Inventory [11]. These findings are consistent with improved disease activity, patient-reported outcomes, and immunological markers in this case series. Due to their condition, patients in this case series received telitacicept along with glucocorticoids ± immunosuppressants, which differs from the trial. Notably, glucocorticoid doses could be reduced in all patients, maintaining or reducing the doses of immunosuppressants. This is particularly significant considering the 2019 EULAR recommendations to minimize long-term glucocorticoid use in pSjD patients due to the risks of osteoporosis, hyperglycemia, and infections [12]. The ability to taper glucocorticoid use without compromising disease control underscores the potential therapeutic advantage of telitacicept. In addition, case 6, who underwent regular laboratory tests, showed a gradual increase in the proportion of CD8+ T cells and a decrease in CD19+ B cells during treatment, providing detailed insights into the immunomodulatory effects of telitacicept. This finding is in line with the known mechanism of action of telitacicept and corroborates clinical trials involving patients with SLE or pSjD [11, 14].

TABLE 1 | Characteristics and treatment outcomes of pSS patients administered telitacicept.

Variables	Visits	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Age (years)	Baseline	31	33	45	60	28	24	49
Gender	Baseline	F	F	F	F	F	F	F
BMI (kg/m^2)	Baseline	34.89	19.84	22.03	21.48	17.3	21.09	22.19
Diseases duration	Baseline	8 month	10 month	7 year	4 month	14 year	2 month	3 year
ANA	Baseline	1:3200	1:1000	1:1000	1:3200	1:3200	1:1000	1:320
	Last visit	NA	NA	1:1000	1:320	1:3200	1:100	1:3200
Anti SSA	Baseline	+	+	+	+	+	+	+
	Last visit	NA	NA	+	+	+	+	+
Anti SSB	Baseline	+	−	−	+	+	−	−
	Last visit	NA	NA	−	+	+	−	−
Anti Ro-52	Baseline	+	+	+	+	+	−	+
	Last visit	NA	NA	+	+	+	−	+
Total T cell (%)	Baseline	71.82	64.57	69.19	80.22	82.08	63.24	76.59
	Last visit	88.87	74.7	67.67	80.2	NA	82.82	87.92
CD4+ T cell (%)	Baseline	32.56	31.87	50.63	45.51	46.17	25.41	21.69
	Last visit	44.64	36.75	53.23	41.85	NA	30.81	46.41
CD8+ T cell (%)	Baseline	34.23	23.66	14.57	31.1	33.37	33.43	6.53
	Last visit	38.26	26.72	11.74	36.23	NA	47.35	39.01
CD19+ B cell (%)	Baseline	22.68	30.05	19.05	9.83	16.61	30.94	19.59
	Last visit	7.53	15.34	19.84	15.88	NA	6.74	8.69
NK cell (%)	Baseline	4.7	5.23	9.93	8.94	1.19	4.03	2.82
	Last visit	3.2	8.73	11.77	3.62	NA	10.09	1.93
CD4+/CD8+ ratio	Baseline	0.95	1.35	3.47	1.46	1.38	0.76	3.32
	Last visit	1.17	1.38	4.53	1.16	NA	0.65	1.19
IgE (IU/ml)	Baseline	NA	20.61	16.25	38.67	60.06	33	16.8
	Last visit	15.17	16.3	8	9.83	25.3	71.95	8.93
IgG (g/L)	Baseline	NA	21.1	9.08	10.1	13.1	11.7	15.9
	Last visit	16.5	17.3	7.51	6.98	10.3	10.4	14
IgA (g/L)	Baseline	NA	5.14	2.27	2.58	3.42	1.83	1.97
	Last visit	3.51	3.8	1.28	0.85	2.09	1.36	1.75
IgM (g/L)	Baseline	NA	1.09	1.58	1.01	0.805	0.96	1.13
	Last visit	0.701	0.708	0.531	0.262	0.497	0.903	0.821
C3 (g/L)	Baseline	NA	0.878	0.704	0.74	0.692	0.803	0.74
	Last visit	1.17	0.965	0.811	0.567	0.836	0.794	0.71
C4 (g/L)	Baseline	NA	0.226	0.142	0.181	0.242	0.17	0.196
	Last visit	0.248	0.215	0.181	0.213	0.295	0.178	0.159
ESSDAI	Baseline	10	7	7	1	6	3	10
	Last visit	5	1	0	2	1	0	6
ESSPRI	Baseline	4.7	3.7	4.7	6	4.3	4.3	5.3
	Last visit	3.3	2.7	2.7	4	2.7	2.7	3.3

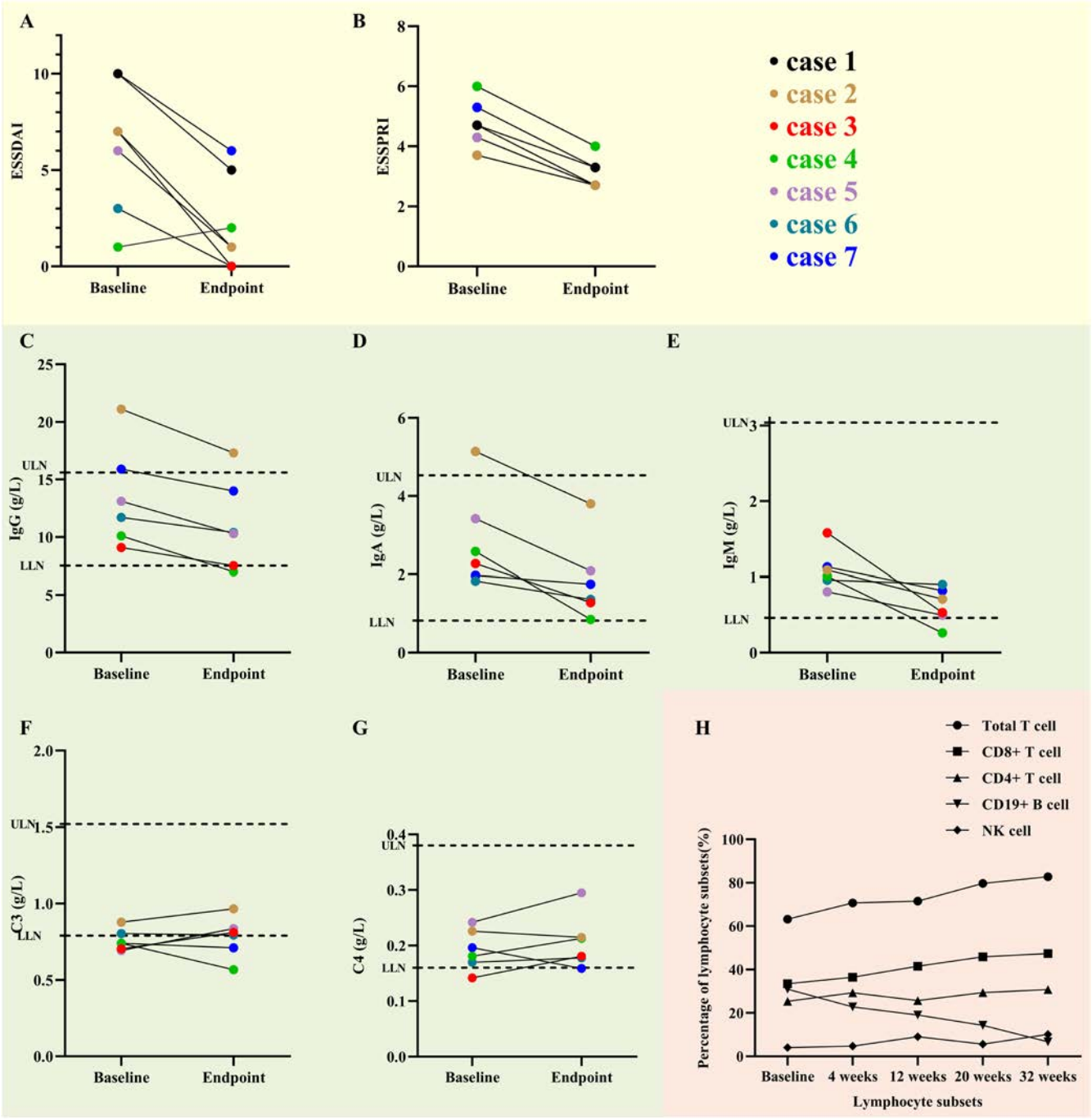

FIGURE 1 | Changes in ESSDAI scores, ESSPRI scores, immunoglobulin levels, and complement levels in patients before and after treatment with telitacicept. (A) ESSDAI scores. (B) ESSPRI scores. (C) Immunoglobulin G level changes. (D) Immunoglobulin A level changes. (E) Immunoglobulin M levels. (F) Complement component 3 levels. (G) Complement component 4 levels. (H) Lymphocyte subset level changes during telitacicept treatment in case 6. LLN, Lower Limit of Normal; ULN, Upper Limit of Normal.

The therapeutic landscape for pSjD has seen the exploration of several BAFF/BLyS and APRIL-targeted drugs, among which telitacicept, belimumab, and ianalumab are prominent. Belimumab, an anti-BAFF/BLyS monoclonal antibody, is already approved for treating active SLE and lupus nephritis (LN) [15, 16]. The open-label phase II BELISS study demonstrated that belimumab significantly decreased ESSDAI scores at week 28 and reduced serum immunoglobulin and RF levels in patients with SjD [17]. Another phase II study of belimumab combined with rituximab indicated that this combination therapy led to a consistent and significant improvement in ESSDAI scores, accompanied by a sustained reduction in peripheral CD19+ B cells [18]. Ianalumab, a monoclonal antibody targeting the BAFF receptor, exhibited a dose-related reduction in disease activity in patients with pSjD at week 24, as evidenced by ESSDAI scores [19]. Comparatively, the present case series on telitacicept, which targets both BAFF/BLyS and APRIL, also demonstrated substantial therapeutic effects in patients with pSjD. The observed immunomodulatory effects of telitacicept, particularly the reduction in CD19+ B cells and the increase in CD8+ T cells, are consistent with the mechanisms proposed by studies of belimumab and ianalumab. While telitacicept and these other agents share

similar therapeutic pathways, the dual-target mechanism of telitacicept might provide additional benefits by more comprehensively inhibiting B cell maturation and autoantibody production. Further studies with larger samples are warranted for head-to-head comparisons of these promising treatments.

The safety profile of telitacicept observed in this case series aligns with findings from previous clinical trials. No serious adverse events were reported, consistent with phase II clinical trial data, which indicated similar infection rates between the telitacicept and placebo groups of pSjD patients [11]. The main adverse effect observed in this study was local injection site reactions, indicating that telitacicept may be a safe and well-tolerated treatment option for pSjD.

This case series had several limitations. Firstly, the sample size was small, and the duration of telitacicept treatment varied among patients, with some using the drug for a short period due to financial constraints and the impact of the COVID-19 pandemic. Secondly, some patients had incomplete follow-up data, resulting in missing information. In addition, given the placebo effects on ESSDAI and ESSPRI scores in clinical trials of pSjD, it must be acknowledged that this case series lacked a control group, resulting in limited clinical insights. Another limitation is that all the patients in this study were selected from a single center in China, and the findings may not be generalizable to other ethnicities. Despite these limitations, the real-world data presented here provide valuable insights into the application of telitacicept in pSjD patients.

Our findings suggest that telitacicept may confer improvements in disease activity and symptom burden, with a favorable tolerability among patients. This case series indicated that the individualized use of telitacicept in real-world settings may achieve similar efficacy and safety as observed in strictly selected trial participants. Despite these encouraging findings, larger and controlled clinical trials are warranted to provide more robust evidence on the efficacy and safety of telitacicept in pSjD treatment, ensuring a comprehensive understanding and validation of these preliminary findings.

Author Contributions

Jiajia Wu: data curation, writing – original draft, visualization. You Song: data curation, writing – original draft, investigation. Weiwei Wang: data curation, writing – original draft, investigation. Xujing Yuan: data curation, investigation, visualization. Rong Du: project administration, supervision, validation, writing – review and editing.

Ethics Statement

The case series adhered to the principles outlined in the Declaration of Helsinki and received approval from the Medical Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (approve no. 2024–0077).

Consent

As a retrospective study, the requirement for informed consent was waived by the Ethics Committee.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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ORIGINAL ARTICLE

Clinical Features and Risk Factors for Early Bone Destruction in Enthesitis-Related Arthritis: A Cohort Study

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Keywords: bone destruction | clinical characteristics | enthesitis-related arthritis | risk factors

ABSTRACT

Objectives: To explore the clinical characteristics and identify risk factors for early bone destruction in children with enthesitis-related arthritis (ERA).

Methods: Clinical characteristics were retrospectively analyzed in 85 newly diagnosed cases with ERA at our hospital from January 2019 to December 2021. Logistic regression analyses were performed to identify risk factors for early bone destruction.

Results: In this cohort of 85 ERA patients, early bone destruction was identified in 24.7% (21/85) of cases (ERA-BD group), predominantly affecting the sacroiliac joints (66.7%), knee (14.3%), hip (14.3%), and interphalangeal joints (4.8%). The ERA-BD group exhibited significantly higher rates of polyarticular involvement (≥ 5 joints) compared to the ERA-nBD group (76.2% vs. 12.5%, $p < 0.001$), with a notably higher incidence of knee joint involvement (66.7% vs. 40.6%, $p < 0.05$) and sacroiliitis (85.7% vs. 50.0%, $p < 0.05$). Additionally, the ERA-BD group had a longer disease duration (8.0 vs. 3.5 months, $p = 0.009$) and elevated C-reactive protein levels (median: 12.3 vs. 4.4 mg/L, $p = 0.04$). However, no significant differences were observed in ESR or IL-6 levels between the two groups. Multivariate analysis confirmed that polyarticular involvement (OR = 21.39, 95% CI 5.12–89.30) and longer disease duration (OR = 4.06, 95% CI 1.33–12.39) were independent predictors of early bone destruction.

Conclusions: Our study identifies polyarticular involvement (≥ 5 joints) and longer disease duration as key independent predictors of early bone destruction in ERA, highlighting the need for a shift from joint-specific to systemic risk stratification.

1 | Introduction

Enthesitis-related arthritis (ERA), a subtype of juvenile idiopathic arthritis (JIA), is characterized by inflammation of the lower limb joints and entheses, with potential involvement of axial joints [1]. Compared to other types of JIA, ERA is associated with a poorer prognosis, manifesting as poor joint function, high joint pain scores, and prolonged disease activity. If left untreated, ERA can lead to joint destruction, exacerbation of joint pain, and an increased risk of joint deformity, significantly affecting the quality of life and long-term prognosis [2]. Imaging findings, such as bone erosion and joint space narrowing, along with physician assessments of high-risk joint damage, are recognized as key prognostic factors in JIA [3]. Studies in patients with ankylosing spondylitis (AS) and rheumatoid arthritis (RA) have shown that inflammatory activity is a key driver of bone destruction [4, 5]. This suggests that early bone destruction reflects a more active inflammatory state, and such damage can

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lead to structural deformities and ankylosis [6]. Early intervention with biologic therapies has been shown to slow structural damage, emphasizing the critical role of prompt treatment in mitigating long-term damage [7]. Therefore, identifying risk factors for early bone destruction is critical for timely intervention and improved disease management.

Research on bone destruction is more commonly conducted in RA, where risk factors such as age, gender, disease onset age, genetic factors, RA-related antibodies, and inflammatory markers have been identified [8, 9]. For ERA, existing studies have identified factors contributing to joint destruction, including persistently elevated erythrocyte sedimentation rate (ESR), delayed medical consultation (diagnostic delay), and late use of disease-modifying antirheumatic drugs (DMARDs) [10, 11]. However, research on this topic, particularly in pediatric populations in Asia, remains limited. Furthermore, most of these studies have focused on long-term bone destruction, with relatively few investigating the early stages of bone damage.

This study aims to identify clinical and laboratory predictors of early bone destruction in children with ERA, thereby enabling risk stratification and personalized intervention. The findings could offer valuable insights into optimizing disease management and improving long-term outcomes for ERA patients.

2 | Materials and Methods

2.1 | Study Population

A total of 85 newly diagnosed patients with ERA were included in this study. The diagnosis of ERA was confirmed based on the criteria established by the International League of Associations for Rheumatology (ILAR) classification [12]. Inclusion criteria were as follows: patients aged ≤16 years who met the ILAR criteria for ERA and who had not received nonsteroidal anti-inflammatory drugs, DMARDs such as methotrexate, immunosuppressive agents, or biologic therapies for at least 2 weeks before enrollment to minimize any confounding effects of these medications. Exclusion criteria included: comorbidities affecting joint or bone metabolism (e.g., osteogenesis imperfecta, significant bone metabolic disorders), a history of joint trauma or surgery within the last 6 months, chronic inflammatory diseases or autoimmune conditions that could interfere with the study's interpretation, and incomplete baseline clinical or imaging data. This study was approved by the Research Ethics Committees of the Children's Hospital Zhejiang University School of Medicine (2022-IRB-259).

2.2 | Data Collection and Definition

Data including demographic information, age of disease onset, family history, initial joint involvement, laboratory test results, and imaging findings were collected and analyzed. Baseline clinical factors were examined to identify risk factors for early bone destruction. All laboratory tests (ESR, C-reactive protein [CRP], interleukin-6 [IL-6], etc.) were performed within 24 h of admission, prior to the initiation of any treatment. Based on the presence of bone destruction within 6 months of ERA diagnosis, the patients were divided into two groups: the ERA with bone destruction group (ERA-BD group) and the ERA without bone destruction group (ERA-nBD group).

Family history, as defined by the ILAR criteria, was considered positive if there was a history of AS, ERA, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis in a first-degree relative [12]. Active joint count was defined as joint swelling or effusion, or the presence of two or more of the following signs: (1) limited range of motion, (2) joint tenderness, (3) pain on joint movement, and (4) increased skin temperature over the joint surface [12, 13]. Enthesitis was diagnosed based on clinical manifestations, including localized pain, tenderness, and swelling at the entheseal site, or through inflammatory signs detected by MRI or ultrasound examination [14, 15]. The diagnosis of sacroiliac joint arthritis was based on clinical manifestations (inflammatory back pain) and physical examination (e.g., flexion, abduction, and external rotation of the sacroiliac joints, or Gaenslen's test) [15], in addition to MRI criteria for sacroiliitis in juvenile spondyloarthritis (JSpA) [16].

Bone destruction was defined as inflammatory periarticular osteolysis, assessed through a multimodality imaging protocol integrating radiographic criteria for JSpA (joint surface irregularity, cortical defects), ASAS MRI definitions (bone marrow edema, osteolysis), and RA-derived methods (synovitis, joint space narrowing) [17–20]. This definition specifically targets early-stage disease, excluding late-phase features such as syndesmophytes or new bone formation. On x-ray, bone destruction was characterized by joint surface roughness, decreased bone density, cortical defects, or joint space narrowing. CT scans identified bone destruction through the presence of multiple bone lesions, irregular joint surfaces, or cortical defects. On MRI, bone destruction was identified by joint space changes, bone erosion, or destruction of joint surfaces, with bone marrow edema excluded as a criterion for bone destruction.

The initial evaluation for all patients involved MRI of at least one symptomatic joint to detect early bone destruction. In cases where MRI findings were equivocal, such as subtle cortical defects or indeterminate bone changes, supplementary imaging using targeted CT or high-resolution x-rays was conducted for further assessment of the affected joints. A consensus process was followed to confirm the presence of bone destruction. Two radiologists independently reviewed all imaging studies. Any discrepancies between their assessments were resolved by a third senior radiologist. The final determination of bone destruction required at least a 2/3 consensus, with findings also needing to correlate with clinical evaluations, including localized tenderness and limited joint mobility.

2.3 | Statistical Analysis

The data were analyzed using R software. Continuous variables were assessed for normality using the Kolmogorov–Smirnov test, and homogeneity of variance was tested using Levene's test. Normally distributed data were expressed as mean ± standard deviation and analyzed using *t*-tests. Non-normally distributed continuous variables were expressed as the median (interquartile range), and categorical data were expressed as percentages.

Group comparisons for continuous variables were performed using the Mann–Whitney *U* test, while binary variables were compared using the chi-square test. Logistic regression was used to identify predictors of bone destruction. Variables with a p value < 0.05 in univariate logistic regression were included in the multivariate logistic regression analysis, which was conducted using a backward conditional method and verified by a forward conditional method. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated for predictor variables. Statistical significance was set at $p < 0.05$.

3 | Results

3.1 | Patient Characteristics

In this cohort of 85 patients, there were 79 (92.9%) males and 6 females (7.1%), with a male-to-female ratio of 13.2:1. The mean age at diagnosis was 11.2 ± 2.3 years (range: 3–15), and the median disease duration (symptom-to-diagnosis interval) at hospitalization was 4.0 (1.8, 10.0) months. A family history of the disease was present in 34.1% (29/85) of cases, and 90.6% (77/85) tested positive for HLA-B27. Axial joint involvement was seen in 61.2% (52/85) of cases, primarily affecting the sacroiliac joints (96.2%, 50/52), while peripheral joints were involved in 92.9% (79/85), with the hip (79.7%), knee (50.6%), and ankle (29.1%) being the most common sites. Enthesitis was observed in 34.1% (29/85) of patients, and 28.2% (24/85) had more than four affected joints at diagnosis. Only one patient had uveitis at diagnosis (Table 1).

Laboratory tests revealed elevated ESR in 68.2% (58/85) of cases, CRP in 49.4% (42/85), and IL-6 in 37.6% (32/85). All patients had normal serum ferritin levels. Leukocytosis was observed in 20.0% (17/85), anemia in 27.1% (23/85), and thrombocytosis in 22.4% (19/85). Autoantibodies were positive in 15.3% (13/85) of patients, with antinuclear antibody (ANA) positivity in 13.3% (11/85) and other autoantibodies in 7.1% (6/85) (Table 2).

All 85 patients underwent MRI of at least one symptomatic joint, and in addition, 83 patients received x-ray examinations, while 58 patients underwent ultrasound evaluations. The radiological findings from these modalities were categorized as follows: (1) On x-ray, bone destruction (osteolysis) was identified in four cases (4.7%), characterized by joint surface irregularity or cortical defects. Inflammatory signs observed included uneven bone density in 14.5% (12/83) of cases, soft tissue swelling in 10.8% (9/83), and periosteal reaction in 7.2% (6/83). (2) Ultrasound findings primarily indicated synovial activity, with joint effusion present in 63.8% (37/58) of cases and synovial thickening in 43.1% (25/58). However, ultrasound was not used to assess bone destruction due to its limited sensitivity for detecting cortical erosions. (3) MRI revealed bone destruction in seven cases (8.2%), confirmed by joint space narrowing, cortical erosion, or osteolysis. Other inflammatory markers on MRI included bone marrow edema in 90.6% (77/85) of cases, joint effusion in 88.2% (75/85), suprapatellar bursa effusion in 18.8% (16/85), peripheral soft tissue swelling around the joint in 55.3% (47/85), and synovial thickening in 10.6% (9/85). Finally, CT scans were performed in 35 patients with ambiguous MRI or x-ray findings. Bone destruction was

TABLE 1 | Clinical characteristics of ERA, ERA-BD, and ERA-nBD.

Characteristics	ERA ($n = 85$)	ERA-BD ($n = 21$)	ERA-nBD ($n = 64$)	$p^{\text{BD vs. nBD}}$
Age, mean ± SD (years)	11.2 ± 2.3	11.7 ± 2.4	11.0 ± 2.2	0.27
Disease course, median (IQR) (months)	4 (1.8, 10.0)	8 (3.0, 12.0)	3.5 (1.5, 6.0)	0.009**
Family, no. (%)	29 (34.1)	6 (28.6)	23 (45.1)	0.54
Sex, no. (%)				
Female	6 (7.1)	0	6 (9.4)	0.15
Male	79 (92.9)	21 (100.0)	58 (90.6)	
HLA-B27 positive, no. (%)	77 (90.6)	20 (95.2)	57 (89.1)	0.40
Joint involvement, no. (%)				
Involved joints ≥ 5	24 (28.2)	16 (76.2)	8 (12.5)	< 0.01**
Axial joint	52 (61.2)	18 (85.7)	34 (53.1)	0.006**
Peripheral joint	79 (92.9)	20 (95.2)	59 (92.2)	0.64
Sacroiliac joint	50 (58.8)	18 (85.7)	32 (50.0)	0.004**
Hip	63 (74.1)	16 (76.2)	47 (73.4)	0.80
Enthesitis	29 (34.1)	10 (47.6)	19 (29.7)	0.29
Knee	40 (47.1)	14 (66.7)	26 (40.6)	0.04*
Ankle	23 (27.1)	8 (38.1)	15 (23.4)	0.19

Note: BD vs. nBD: compared between ERA-BD and ERA-nBD groups. The term "course" refers to the time from symptom onset to diagnosis. Asterisks indicate significant levels (*$p < 0.05$, **$p < 0.01$).
Abbreviations: ERA, enthesitis-related arthritis; ERA-BD, ERA with bone destruction; ERA-nBD, ERA without bone destruction; IQR, interquartile range; SD, standard deviation.

TABLE 2 | Laboratory characteristics of ERA, ERA-BD, and ERA-nBD.

Characteristics	ERA ($n=85$)	ERA-BD ($n=21$)	ERA-nBD ($n=64$)	$p^{\text{BD vs. nBD}}$
WBC, mean ± SD (10^9/L)	8.1 ± 2.2	8.2 ± 1.7	8.0 ± 2.3	0.82
HB, mean ± SD (g/L)	125.1 ± 10.1	124.4 ± 10.2	125.3 ± 10.2	0.75
PLT, mean ± SD (10^9/L)	352.8 ± 99.1	377.2 ± 86.4	344.8 ± 102.3	0.20
CRP, median (IQR) (mg/L)	7.1 (1.3, 21.6)	12.3 (5.4, 33.1)	4.4 (1.2, 18.0)	0.04*
ESR, median (IQR) (mm/h)	38.0 (14.0, 63.0)	49.0 (28.0, 69.0)	27.5 (14.0, 60.3)	0.11
Ferritin, median (IQR) (pg/L)	69.9 (42.0, 102.9)	63.0 (44.3, 112.5)	69.9 (40.8, 98.6)	0.68
ANA positive, no. (%)	13 (15.3)	1 (4.8)	12 (18.8)	0.12
TNF, median (IQR) (pg/mL)	1.2 (1.0, 1.7)	1.4 (1.0, 1.7)	1.2 (1.0, 1.6)	0.47
IL6, median (IQR) (pg/mL)	12.8 (6.5, 28.0)	13.5 (9.5, 27.5)	11.4 (6.1, 26.1)	0.39
IL2, mean ± SD (pg/mL)	2.1 ± 0.9	2.2 ± 1.1	2.0 ± 0.8	0.61
IL10, mean ± SD (pg/mL)	3.1 ± 1.2	3.2 ± 1.4	3.0 ± 1.1	0.51

Abbreviations: ANA, antinuclear antibody; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HB, hemoglobin; IL, interleukin; PLT, platelet count; TNF, tumor necrosis factor; WBC, white blood cell count.

confirmed in 14 cases (40.0%), with the majority of lesions located in the sacroiliac joints (71.4%, 10/14) and the knees (28.6%, 4/14).

3.2 | Early Bone Destruction in ERA

A total of 21 cases were identified as the ERA-BD group (24.7%, 21/85), and 64 cases as the ERA-nBD group (75.3%, 64/85). The clinical characteristics of both groups are shown in Table 1. The mean age at diagnosis was 11.7 ± 2.4 years for the ERA-BD group and 11.0 ± 2.2 years for the ERA-nBD group, and the positive family history was 28.6% (6/21) and 45.1% (23/64), respectively, with no significant differences between the two groups ($p>0.05$). The HLA-B27 positivity rate (95.2% vs. 89.1%) was also not significantly different between the two groups ($p>0.05$). The disease duration was longer in the ERA-BD group (8.0 [3.0, 12.0] months) compared to the ERA-nBD group (3.5 [1.5, 6.0] months) ($p<0.05$). At the time of initial diagnosis, there were no significant differences in overall peripheral joint involvement between the ERA-BD and ERA-nBD groups (95.2% vs. 92.2%). However, the rate of knee joint involvement was significantly higher in the ERA-BD group compared to the ERA-nBD group (66.7% vs. 40.6%, $p<0.05$), while no significant differences were observed in hip (76.2% vs. 73.4%) or ankle (38.1% vs. 23.4%) joint involvement. Axial joint involvement was more common in the ERA-BD group (85.7% vs. 53.1%, $p<0.05$), with a significantly higher incidence of sacroiliitis (85.7% vs. 50.0%, $p<0.05$) compared to the ERA-nBD group. No significant difference in the rate of enthesitis was found between the two groups (47.6% vs. 29.7%, $p>0.05$). Furthermore, polyarticular involvement (≥5 joints) was more prevalent in the ERA-BD group compared to the ERA-nBD group (76.2% vs. 12.5%, $p<0.05$). Compared to the ERA-nBD group, the ERA-BD group had higher levels of CRP (12.3 [5.4, 33.1] vs. 4.4 [1.2, 18.0] mg/L, $p<0.05$). There were no significant differences between the two groups in terms of white blood cell count, hemoglobin, platelet count, ESR, ferritin, or ANA positivity rate ($p>0.05$). The levels of cytokines tumor necrosis factor (TNF)-α, IL-6, IL-10, and IL-2 were also not statistically different between the two groups ($p>0.05$) (Table 2).

3.3 | Risk Factor Analysis

Bone destruction mainly involved the sacroiliac joints (66.7%, 14/21), followed by the knee joints (14.3%, 3/21), hip joints (14.3%, 3/21), and interphalangeal joints (4.8%, 1/21). Univariate logistic regression analysis identified several risk factors for bone destruction in the ERA-BD group, including "disease duration (OR = 3.16)," "elevated CRP (OR = 3.43)," "knee joint involvement (OR = 2.92)," "axial joint involvement (OR = 5.29)," and "number of involved joints ≥ 5 (OR = 22.40)" (Figure 1). Multivariate regression analysis, incorporating the above factors, revealed that "number of involved joints ≥ 5" (OR = 21.39) and "disease duration" (OR = 4.06) are independent risk factors for bone destruction. The analysis found no correlation between ESR, IL-6, and TNF and the occurrence of bone destruction.

4 | Discussion

This study provides the first evidence from a Chinese pediatric ERA cohort that polyarticular involvement (≥5 joints) (OR = 21.39) and disease duration (OR = 4.06) are independent risk factors for early osteolytic bone destruction, challenging the traditional focus on axial/sacroiliac damage in ERA prognosis [10, 21, 22]. Notably, knee joint involvement emerged as a novel predictor, distinct from prior studies linking hip or ankle arthritis to long-term structural damage [10]. The 24.7% prevalence of early osteolysis underscores the need for timely identification of high-risk subgroups in ERA and supports the importance of early intervention to prevent long-term structural damage.

The male predominance (13.2:1) and HLA-B27 positivity (90.6%) align with global ERA cohorts, where male sex and HLA-B27 are established genetic drivers of entheseal

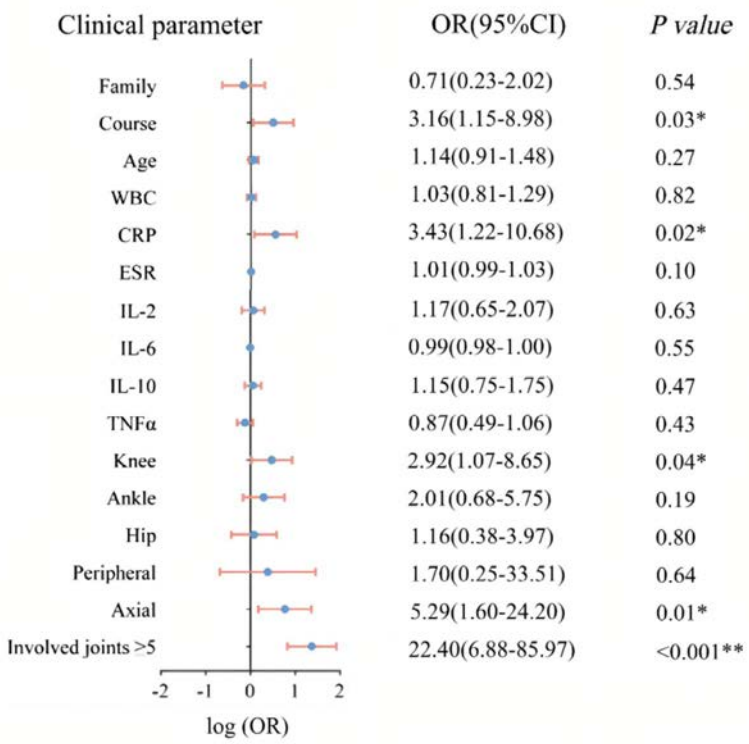

FIGURE 1 | Univariate logistic regression analysis of bone destruction. Disease duration, elevated CRP, knee joint involvement, axial joint involvement, and number of involved joints ≥ 5 were risk factors for bone destruction. Asterisks indicate significant levels (*$p < 0.05$, **$p < 0.01$).

inflammation [15, 23]. Axial involvement, particularly sacroiliitis, highlights the spondyloarthritis phenotype, while peripheral polyarthritis and enthesitis reflect ERA's characteristic dual targeting of both articular and entheseal sites [24]. Notably, elevated baseline CRP has been associated with a reduced response to initial treatment [25]. In contrast, the absence of elevated serum ferritin levels in our cohort may indicate more localized inflammation compared to systemic forms of JIA.

In our study, early bone destruction was identified as an adverse outcome of ERA. Polyarticular involvement (≥ 5 joints), particularly involving the axial/sacroiliac, hip, and knee joints, along with longer disease duration at diagnosis, were strongly associated with early bone destruction. Notably, knee involvement emerged as a specific risk factor, a finding not typically emphasized in prior studies. Multivariate analysis confirmed that polyarticular involvement (≥ 5 joints) and disease duration were independent risk factors for early bone destruction, emphasizing the need for early intervention. ERA patients often experience diagnostic delays and greater functional limitations compared to other JIA subtypes [10, 26], highlighting the importance of timely treatment to prevent long-term damage.

Our findings also contrast with long-term studies linking hip involvement, delayed diagnosis, and specific factors such as male gender and family history to poorer functional outcomes in ERA [11, 21, 22]. However, unlike previous "joint-specific" models, our data highlight polyarticular disease burden (≥ 5 joints) and disease duration as key independent risk factors for early bone destruction. This broader approach to risk stratification is crucial for optimizing early intervention strategies in ERA.

Given the persistent and active disease course often seen in ERA [27], our findings support the concept of early aggressive treatment, as previously proposed for JIA to improve long-term outcomes [28, 29]. Early use of biologics, such as TNF inhibitors, has been recommended for cases of peripheral polyarthritis with joint destruction or high-risk joint involvement [30], and our study adds to this by suggesting that patients with ≥ 5 involved joints and prolonged disease duration may benefit from early biologic treatment. This approach could help mitigate bone destruction and improve long-term functional outcomes, extending the scope of current treatment guidelines [31].

Several limitations should be considered when interpreting the findings of this study. The single-center design and retrospective data collection limit generalizability, and the small sample size may not fully capture all risk factors for early bone destruction in ERA. Future multicenter studies are needed to validate our risk model across diverse populations. Additionally, exploring IL-23/IL-17 axis biomarkers could deepen our understanding of rapid bone loss in ERA and inform targeted therapies [32]. Lastly, the lack of long-term follow-up data limits our ability to assess disease progression and the durability of treatment outcomes.

5 | Conclusions

In this first Asian pediatric ERA cohort analyzing early bone destruction, polyarticular involvement (≥ 5 joints) and prolonged disease duration were identified as independent risk factors. These findings suggest the need to revise current treatment guidelines to prioritize early biologic therapy in polyarticular ERA, particularly in patients with knee involvement. Future multicenter studies should validate these findings and explore whether early biologic intervention can effectively prevent structural damage in high-risk subgroups.

Author Contributions

J.W. and M.L. initiated the work. J.W. and X.Q. collected the data and wrote the manuscript. M.H. and Y.L. contributed to the analysis of the data. X.Y. and Y.H. assisted in evaluating and verifying the radiology reports. All authors revised and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Consent

Patient consent was waived because of the retrospective nature of the study and the minimal risk level it poses.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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LETTER TO THE EDITOR

Assessing the Effect of Avacopan on Increasing ANCA Titres During Remission Maintenance in ANCA-Associated Vasculitis

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Dear Editor,
Although advances in remission-induction therapies for anti-neutrophilic cytoplasmic antibody (ANCA)-associated vasculitis (AAV) have improved survival rates, approximately 30%–50% of AAV patients relapse [1].

ANCA titre measurements have been established as a diagnostic tool for AAV. However, their role in managing AAV during follow-up care to prevent relapses remains controversial. According to British Society for Rheumatology guidelines, elevated ANCA titres should not require adjustments to immunosuppressive treatment [2]. However, the optimal strategy for increasing ANCA levels to prevent relapse has yet to be determined.

The ADVOCATE study showed that Avacopan, an oral C5a receptor antagonist, is an effective alternative to glucocorticoid treatment for AAV [3]. However, the optimal time and duration of use of avacopan for AAV remain uncertain.

Here, we describe the clinical course of four patients with microscopic polyangiitis (MPA) who were prescribed avacopan alone when their ANCA titers increased during the period of remission maintenance.

All four patients were diagnosed with MPA based on the Watts' algorithm [4]. The patients' anti-myeloperoxidase (MPO)-ANCA titre was measured using the STACIA MEBLux MPO-ANCA chemiluminescent enzyme immunoassay (Medical & Biological Laboratories, Tokyo, Japan). All patients provided written informed consent.

Case 1: A 38-year-old Japanese man diagnosed with MPO-ANCA-positive MPA (MPO-ANCA titre: 16.8 IU/mL) during a renal biopsy due to pauci-immune necrotising glomerulonephritis on achieved remission with glucocorticoid and azathioprine, which lasted for 24 months.

His MPO-ANCA titre was negative 4 months after the start of immunosuppressive treatment. Glucocorticoid and azathioprine were tapered off again after 36 and 48 months, respectively. However, 24 months after azathioprine was discontinued, the MPO-ANCA titre reappeared and rose to 5.8 IU/mL. Avacopan (60 mg/day) was initiated without additional immunosuppressants. Four months later, the patient developed haematuria and proteinuria while the estimated glomerular filtration rate (eGFR) remained the same, and his MPO-ANCA titre increased to 24.2 IU/mL. A renal biopsy confirmed pauci-immune necrotising glomerulonephritis with cellular crescents, indicative of MPA relapse.

Case 2: A 53-year-old Japanese man who was diagnosed with rapidly progressive glomerulonephritis as a result of MPA was treated with glucocorticoid and intravenous cyclophosphamide (IVCY). Despite treatment, he needed permanent hemodialysis. The patient tested negative for MPO-ANCA, and remission was maintained with prednisolone (7.5 mg/day). Eleven months post-diagnosis, MPO-ANCA recurred (30.4 IU/mL) with fever, elevated CRP, interstitial pneumonia, and gastrointestinal bleeding, indicative of relapse. After intensifying immunosuppressive treatment by adding rituximab, the patient achieved remission, with the MPO-ANCA titre becoming negative 6 months after the

Summary

- Administration of avacopan alone may be insufficient to maintain remission in patients with increasing ANCA titers.

relapse. However, the patient tested positive for MPO-ANCA again (4.6 IU/mL) 10 months after the relapse. Despite administration of avacopan, the patient relapsed after 5 months, with alveolar hemorrhage and an MPO-ANCA titre of 44.8 IU/mL.

Case 3: An 83-year-old Japanese man diagnosed with MPO-ANCA-positive MPA (MPO-ANCA titre: 36.2 IU/mL) achieved remission with glucocorticoid and IVCY. After 5 months, he tested negative for MPO-ANCA. Remission was maintained with azathioprine (100 mg/day) and prednisolone (7.5 mg/day). Twelve months post-diagnosis, MPO-ANCA reappeared (8.4 IU/mL), and treatment with avacopan (60 mg/day) was initiated. After 4 months, the patient had a relapse with worsening haematuria, proteinuria, reduced eGFR (18.4 mL/min/1.73 m^2), and an MPO-ANCA titre of 16.6 IU/mL.

Case 4: An 86-year-old woman diagnosed with interstitial pneumonia and mononeuritis multiplex due to MPO-ANCA-positive MPA (MPO-ANCA titre: 26.8 IU/mL) achieved remission with glucocorticoid monotherapy. After 6 months, she tested negative for MPO-ANCA and remission was maintained with azathioprine (100 mg/day) and prednisolone (5 mg/day). Thirteen months post-diagnosis, MPO-ANCA reappeared (4.6 IU/mL), and treatment with avacopan (60 mg/day) was initiated. Three months later, the patient relapsed, with worsening neurological symptoms and fever.

The clinical characteristics and course of treatment of the four patients are summarized in Table 1 and in Figure S1.

The use of ANCA to evaluate disease activity in AAV remains inconclusive. While previous studies [5] have emphasized the importance of follow-up and management of patients with AAV in clinical remission who experience recurrent or increasing ANCA levels, the usefulness of ANCA levels for predicting relapses as a guide for therapeutic decisions in patients in remission remains limited [6, 7].

A previous study of four AAV cases revealed that avacopan may improve AAV symptoms without a corresponding decrease in ANCA titres [8]. Therefore, clinicians using ANCA titres as a treatment biomarker for AAV should consider this option to avoid underestimating the therapeutic effects of avacopan. However, these reports do not clarify the effect of avacopan alone, without increasing other immunosuppressive treatments, on increasing ANCA titres during remission. Although we were unable to draw any definitive conclusions from our case series, as it included only four MPA cases, our results suggest that maintaining remission solely with the addition of avacopan may be insufficient in patients with AAV who experience increasing MPO-ANCA titres. Therefore, physicians should be cautious about relapses in patients with increasing MPO-ANCA titres following avacopan initiation. Our findings should be validated in future large-scale cohort studies.

TABLE 1 | Clinical characteristics of the four patients.

Case	Age, sex	Diagnosis	ANCA seropositivity	Organ involvement	Induction treatment	Maintenance treatment
Case1	38, male	MPA	MPO-ANCA	Glomerulonephritis	GC	GC, AZA
Case2	53, male	MPA	MPO- ANCA	Glomerulonephritis, Interstitial pneumonia, Gastrointestinal bleeding, Diffuse alveolar hemorrhage	(1) GC, IVCY, (2) GC, RTX	(1) GC, (2) GC, RTX
Case3	83, male	MPA	MPO-ANCA	Sinusitis, Glomerulonephritis	GC, IVCY	GC, AZA
Case4	86, female	MPA	MPO-ANCA	Interstitial pneumonia, Mononeuritis multiplex	GC	GC, AZA

Abbreviations: ANCA, anti-neutrophil cytoplasmic autoantibody; AZA, azathioprine; GC, glucocorticoid; IVCY, intravenous cyclophosphamide; MPA, microscopic polyangiitis; MPO, myeloperoxidase; RTX, rituximab.

Author Contributions

M.Y. performed the literature search and wrote the manuscript, supported by H.S., H.K., K.I., K.K., T.K., S.B., and Y.I. All authors revised and approved the final manuscript.

Disclosure

The authors have nothing to report.

Consent

A written consent form was obtained.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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LETTER TO THE EDITOR

Rheumatoid Arthritis and Tuberculosis

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Dear Editor,

Rheumatoid arthritis (RA) is a chronic inflammatory disease that primarily affects joints, leading to progressive deterioration and potentially permanent joint damage [1]. Compared with the general population, the risk of disability and mortality is greater in the affected population [2]. Based on global epidemiological studies, the incidence of RA is expected to increase by approximately 80.2% between 2020 and 2050 [3]. Mycobacterial infections are strongly associated with the occurrence of RA, and complications of pulmonary infections are a major cause of death in patients with rheumatic disease [4]. Tuberculosis (TB) infections are the main cause of lung infections and the treatment process is complicated to cure them completely. TB is particularly dangerous for people with a weakened immune system, such as those receiving immunosuppressive therapy for RA [5, 6]. A cross-sectional study indicated that 29.5% of RA patients had latent tuberculosis infection (LTBI) and 3.4% developed active TB during treatment, underscoring the heightened risk of TB infection in RA patients due to their disease and its treatment [7]. The connection between RA and TB has been the subject of considerable research; however, the evidence base remains insufficient to demonstrate a causal relationship. The reliability of findings from observational studies is affected by a lack of consideration and control of potential confounding factors and biases, and there is a risk of reverse causality [8]. Therefore, the correlations found do not provide definitive evidence of a causal relationship between RA and TB.

This study utilized a two-sample bidirectional Mendelian Randomization analysis with Genome-Wide Association Study data to explore the potential causality between RA and TB. The primary method was the inverse variance weighted approach, supported by MR-Egger regression and the weighted median method for a thorough analysis. We also conducted reverse MR analysis to investigate any potential reverse causal effects. Our research involved 19 and 10 independent single nucleotide polymorphisms in studying the effect of RA on tuberculosis and the effect of tuberculosis on RA. There was a significant association between genetically predicted RA and TB risk in IVW (odds ratio (OR) = 1.132, 95% confidence interval (CI):1.080–1.188, $p = 3.732e\text{-}07$) and there was no significant correlation between genetic TB and RA (OR = 1.011, 95% CI: 0.966–1.058, $p = 0.635$) as shown in Table 1. No significant heterogeneity or horizontal pleiotropy was found for the instrumental variables, suggesting that these results were reliable and robust (see Table 1). The results indicate that RA is a risk factor for the development of TB, while TB does not appear to influence the onset of RA.

The World Health Organization's Global TB Report 2023 indicates that individuals with rheumatic diseases such as RA are at increased risk of TB reactivation. Compared with the general population, RA patients have been found to have a fourfold increased risk of TB infection [9]. Further confirmation was provided by a case–control study highlighting specific risk factors in RA patients that increase the risk of TB in patients who have not yet been treated with biologic. It is estimated that approximately 2 billion people worldwide have LTBI, with the majority being asymptomatic [10]. These individuals show no obvious signs or symptoms and lack clinical bacteriological or radiological evidence of active TB. Nevertheless, a significant proportion, approximately 5%–10% of LTBI patients, may progress to active TB infection. The prevalence of active TB infection is significantly higher in RA patients.

The increased risk of tuberculosis infection in RA patients is likely attributed to the perturbations in their immune system and the immunosuppressive medications used in their

TABLE 1 | The results of the MR study, heterogeneity, and pleiotropy analysis.

Exposure	Outcome	Method	SNP (*N*)	*β*	SE	*p*	OR (95% CI)	Heterogeneity				Horizontal pleiotropy	
								MR-Egger		IVW		SE	*p*
RA	TB	IVW	19	0.125	0.025	3.732e-07	1.132 (1.080–1.188)	17.110	0.447	17.381	0.497	0.007	0.607
		MR-Egger	19	0.140	0.039	2.160e-03	1.151 (1.066–1.024)						
		Weighted median	19	0.128	0.030	1.940e-05	1.137 (1.072–1.206)						
TB	RA	IVW	10	0.011	0.023	0.635	1.011 (0.966,1.058)	9.450	0.306	9.922	0.357	0.012	0.543
		MR-Egger	10	−0.004	0.033	0.910	0.996 (0.933,1.064)						
		Weighted median	10	−0.008	0.030	0.780	0.992 (0.934,1.052)						

Abbreviations: CI, confidence interval; OR, odds ratio.

treatment regimen. While immunological drugs have demonstrated remarkable efficacy in managing RA, they significantly dampen the immune response, thereby substantially elevating the susceptibility to TB in individuals with RA. This underscores the importance of vigilant monitoring and preventive measures to mitigate the risk of TB in this patient population [11]. Furthermore, the risk of reactivation of LTBI to active TB infection is increased in RA patients treated with biologics, particularly TNF inhibitors [12]. Research data suggest that the incidence rate of active TB in RA patients receiving anti-TNF therapy is five to seven times higher than that of the general population. In addition to TNF inhibitors, the treatment of RA also includes a range of immunosuppressive therapies, including various disease-modifying anti-rheumatic drugs and biological agents [13]. These drugs work through specific mechanisms to modulate the immune system to slow the progression of RA and relieve its symptoms [14]. For instance, DMARDs, such as methotrexate, can inhibit cell proliferation and reduce inflammatory reactions. Additionally, biological agents, including IL-6 receptor antagonists or anti-CD20 monoclonal antibodies, can precisely target specific immune cells or cytokines, offering a more focused approach to managing RA [15].

Our findings suggest that RA is a risk factor for the development of TB, but TB does not appear to influence the onset of RA. This study highlights the importance of timely TB prevention and screening for RA patients, with significant implications for public health strategies aimed at reducing the TB burden.

Author Contributions

Qianshi Zhang, Yifang Mei, and Dongyan Wang contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Qianshi Zhang, Xu Dong, and Pengqi Zhang. The first draft of the manuscript was written by Qianshi Zhang, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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