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EDITORIAL

Cardiovascular comorbidity in rheumatic and musculoskeletal diseases: Where we are and how can we move forward?

The outcome of rheumatic and musculoskeletal diseases (RMDs) has improved markedly over the last 2-3 decades. This is to a certain extent the result of earlier recognition, improved access to specialist care and better diagnostics but mostly due to the availability of new treatments (eg, biologic and targeted synthetic disease-modifying anti-rheumatic drugs [DMARDs]) and strategies (eg, early treatment, treat-to-target and tight-control approaches). As traditional "rheumatological" outcomes have been improving, attention has shifted toward other targets, notably prevention and control of comorbidities. A prime position among them, is occupied by cardiovascular disease (CVD), which accounts for a significant part of the still increased morbidity and mortality in RMDs compared to the general population.[1]

Papers published recently in this journal reflect some of the avenues of inquiry that have been pursued for over 25 years, since it was first suggested that rheumatoid inflammation may be linked to CVD.[2] By far the largest body of evidence about this association comes from work in the prototypic chronic inflammatory rheumatic disease, rheumatoid arthritis (RA) and the association between high-grade inflammation and accelerated atherosclerosis. In their work, Cakmak et al.[3] suggest that disease severity in treatment-naïve patients with early RA associated with lower left ventricular function and higher arrhythmia parameters, supporting the notion that more work is needed with regard to heart failure and to sudden death in patients with RA. In their meta-analysis of population-based studies, Kim and Choi[4] demonstrated that patients with spondyloarthropathies (SpA) have a significantly higher risk of myocardial infarction (MI) and stroke compared to the general population; this is not as well-documented in SpA as it is in RA but this work concurs with the continuously enlarging body of evidence that CVD is a core comorbidity across the whole spectrum of systemic inflammatory RMDs. Finally, and more "surprisingly", Sciara et al.[5] showed that subclinical atherosclerosis (ie, macrovascular disease) was more prevalent in patients with systemic sclerosis (SSc) than in controls; SSc is traditionally considered a disease primarily of the small vessels not associated with high-grade systemic inflammation, thus other, new mechanisms need to be sought.

In order to disentangle the masses of available information, understand what we already know and what we don't and move the field forward, we need to take an ordered, systematic approach to the literature. The ultimate aim is clear: to prevent or to manage effectively CVD risk in patients with RMDs. For this, we need a clear understanding of the underlying mechanisms, based on which we can then design and test the efficacy of specific interventions.

When we look at the "rheumatological" literature in CVD with a critical eye, we see that it may fall under several conceptually distinct but intricately linked categories. We may be talking about the potential causes of CVD, ranging from the classical cardiovascular (CV) risk factors (such as hypertension,[6] dyslipidemia[7] and other metabolic abnormalities – many of which are diseases in their own right) to inflammation and its direct or indirect effects on these risk factors and on the vasculature.[1] We may be talking about processes, such as atherosclerosis, but also vasculitis, thrombosis, microvascular dysfunction, myocarditis, myocardial fibrosis and others. We may be talking about the pathophysiological effects of these processes, mostly myocardial ischemia and/or myocardial dysfunction. We may be talking about the clinical expression of these processes, ranging from apparent normality to angina, MI, strokes, heart failure, arrhythmias. Or we may be talking about their ultimate outcomes, ranging again from apparent normality to (cardiac) disability and death. None of these are interchangeable, there are limits to how much we can extrapolate from one RMD to another, and there are significant implications on strategies for prevention and management and thus our patients' eventual outcomes.

There are several aspects of the problem at hand (or heart) that we need to address. The evidence for accelerated atherosclerosis in inflammatory RMDs is substantial and comes from many different sources. There is sound theoretical underpinning about the involvement of inflammatory mechanisms in the generation and evolution of atherosclerosis and "proof of concept" has recently been provided by the CANTOS trial.[8] There are many cross-sectional and some longitudinal studies of vascular function and morphology, mainly in RA but now also in SpA, lupus and other syndromes, which demonstrate an association between inflammation and extent of atherosclerosis, and more importantly that good RMD control leads to deceleration of the atherosclerotic process as assessed by such surrogates.[9] There is epidemiological evidence showing that in the context of CVD, RA behaves like diabetes mellitus,[10] which is firmly established and managed as a coronary heart disease equivalent. And there is evidence for an abundance of classical risk factors in patients with RMDs, including hypertension,[6] dyslipidemia,[7] obesity,[11] cachexia, insulin

resistance, smoking and physical inactivity.[12] Similarly, there is strong evidence for increased atheromatous plaque instability in RMD patients compared to controls. This includes basic laboratory studies showing augmented responses to stress and derangement of hemostatic mechanisms, imaging studies demonstrating an unstable coronary plaque phenotype,[13] longitudinal observational studies showing a higher re-infarction rate[14] and even autopsy studies confirming the presence of more unstable plaques in these patients compared to controls.[15] The value of more and more "me too" studies, particularly small cross-sectional studies, in this line of enquiry is now rather limited, although there may still be some questions regarding ethnic and disease subset variabilities that are worth addressing. Instead, attention needs to be diverted to a new set of questions.

1. How can we mitigate CVD risk in patients with RMDs?

The central role of systemic inflammation, "classical" CVD risk factors and their interplay in the cardiovascular morbidity and mortality in RMDs is evident and provides a framework for designing and testing interventions, implementing them in routine clinical practice and assessing their effectiveness. Logically, such interventions reside in 2 main areas: (a) effective control of inflammation, and (b) meticulous control of classical risk factors. This is where the evidence, in the context of CVD in RMDs, remains thin and needs to be strengthened both in terms of quantity and quality. Effective control of inflammation, with (almost) any means, is the main target of rheumatologists and there is abundant top quality evidence that it leads to a great improvement in "rheumatological" outcomes, such as clinical disease activity, progression of damage, physical function and even death. However, the evidence that it associates with reduced cardiovascular morbidity and mortality, using hard end-points, arises almost exclusively from long-term observational studies, including registries, rather than from randomized controlled trials.[16] Considering that most DMARDs have significant and differential effects in terms of nature, incidence and magnitude, on classical CVD risk factors (eg, lipids) and possibly type of events (eg, ischemic vs thromboembolic) this is an area that requires further investigation. Similar issues exist with the strategies required to pharmacologically control classical CV risk factors in patients with RMDs. Should thresholds for intervention be the same as in the general population and by using what risk algorithm? Recommendations for this are based on expert opinion rather than hard evidence.[17] Are all cardiovascular drugs[18] equally effective and safe in RMD populations, which are characterized by multimorbidity and polypharmacy? There have been no hard end-point trials of antihypertensives in patients with RMDs. There has been a single hard end-point randomized placebo-controlled trial of a statin (atorvastatin 40 mg) in patients with RA.[19] This demonstrated safety but was stopped early due to a lower than anticipated event rate thus failed to demonstrate its primary efficacy end-point (although the effect size appeared to be very similar to that observed in other statin trials). It also demonstrated the magnitude of the challenge of performing hard CV end-point trials in patients with RMDs, which require very large numbers, long-term follow up, guaranteed financial back-up and international participation and collaboration.

Much more attention and research also needs to be conducted on the role of non-pharmacological interventions, particularly lifestyle interventions to enhance physical activity and/or exercise levels, improve diet and reduce obesity in people with RMDs. There is an enlarging body of evidence that such interventions provide substantial improvements both in "rheumatological" outcomes (such as disease activity control, physical function and quality of life) and "cardiovascular" outcomes (such as reduction of classical risk factors and improvements in cardiorespiratory fitness).[20] The role of patient (and health professional) education,[21] the necessary infrastructure and other types of support (eg, psychological interventions)[22] to achieve and maintain good lifestyle habits also need to be evaluated.

Finally a great amount of implementation research is needed to assess what are the most effective ways of bypassing the practical difficulties in addressing this, by definition cross-disciplinary, problem. Whose responsibility is it to perform risk assessment, commence and monitor the relevant pharmacological and lifestyle interventions in people with RMDs? The rheumatologist's (who may not have the cardiovascular expertise)? The cardiologist's (who may not have the rheumatological expertise)? The primary health physician's (who may be overwhelmed by all their other tasks)? How can allied health professionals and services be best integrated in the management of cardiovascular (and other) comorbidities in patients with RMDs in each health system?[23]

2. Are there processes other than atherosclerosis that need to be investigated?

Clinical observation and evidence from newer diagnostic modalities indicate that there are mechanisms other than atherosclerosis that may be involved in CVD morbidity and mortality in RMDs. Small vessel disease / dysfunction is clinically evident for example in patients with some vasculitides or Raynaud's phenomenon. Evidence from myocardial perfusion scans, stress contrast echocardiography and most notably cardiac magnetic resonance (CMR) imaging[24] combined with coronary angiography, demonstrate that in patients with many RMDs, cardiac ischemia can occur independently of macrovascular coronary artery disease and can be reversible with immunomodulatory therapies.[25] Other pathologies, for example myocarditis and cardiac fibrosis can also be seen in specific settings. The natural history / evolution and possible role of such pathologies in myocardial dysfunction (non-ischemic heart failure), arrythmogenicity and eventual cardiovascular outcome, as well as the effect of therapeutic interventions need to be evaluated in studies designed specifically for the purpose.

Similarly, evidence is emerging for cardiac autonomic dysfunction in people with RMDs and a role of inflammatory mechanisms in this.[26] The potential association with sudden cardiac death, which

also occurs more frequently in some RMDs than in the general population, needs to be further investigated.

3. Research into biomarkers, type and timing of cardiac investigations in patients with RMDs.

A major problem with CVD in RMDs is that it is frequently clinically silent. Also that potentially important abnormalities (eg, myocarditis or fibrosis) may not be evident in widely available routine tests, such as electrocardiograms and cardiac ultrasound but be uncovered utilizing more modern technologies, such as CMR or single-photon emission computed tomography imaging, which are very difficult to integrate into routine care due to issues of access, availability of expertise and cost. There is a need to identify clinical characteristics of RMD patients that may be particularly at risk of overt or silent cardiac complications, to assess established and novel biomarkers that point to this, and to develop cardiac investigation algorithms that can realistically be integrated into routine clinical practice. Cross-disciplinary collaboration of interested experts and societies, and utilization of novel technologies, such as artificial intelligence, would facilitate these objectives.

In summary, much has been learned about the major problem of CVD in patients with RMDs through an intensive research effort over the last 25 years or so. However, much remains to be established with good quality evidence, particularly in the field of pharmacological and non-pharmacological interventions; a departure from the now traditional and accepted paradigm of accelerated atherosclerosis and renewed focus on other mechanisms may advance our understanding further; and intensive research into clinical predictors and biomarkers of RMD patients particularly at risk is urgently needed. As recent events in the era of coronavirus have clearly demonstrated, there is no better way to go about it than a clear focus and cross-disciplinary international collaboration.

KEYWORDS

atherosclerosis, cardiovascular risk, rheumatoid arthritis, systemic rheumatic diseases

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REVIEW

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Cardiovascular morbidity and mortality in patients with spondyloarthritis: A meta-analysis

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Abstract

Aim: Cardiovascular (CV) risk and mortality associated with spondyloarthritis (SpA) remain controversial. Herein, we performed a meta-analysis of the latest large-scale population-based studies to demonstrate the elevated risk of CV disease and mortality in patients with SpA than in the general population.

Methods: MEDLINE and EMBASE databases were searched systematically for population-based studies published between January 1997 and September 2019. Additional manual literature searches were also performed. All searches and data collection were performed independently by 2 reviewers. We calculated the risks of myocardial infarction (MI), stroke, and all-cause mortality in a meta-analysis and determined the risk ratios (RR) using the Mantel–Haenszel method.

Results: Among the 641 identified articles, 16 articles involving 18 cases met the inclusion criteria for our meta-analysis; these included 12 cases of ankylosing spondylitis, five cases of psoriatic arthritis, and 1 case of undifferentiated SpA. Our meta-analysis revealed a significantly high risk of MI (RR: 1.52; 95% CI: 1.29-1.80) and stroke (RR: 1.21; 95% CI: 1.0-1.47) in patients with SpA than in the general population. However, this increased risk was not significant in terms of all-cause mortality (RR: 1.23; 95% CI: 0.96-1.57).

Conclusions: Our meta-analysis demonstrated that patients with SpA have a significantly increased risks of MI and stroke, but without a significant increase in the all-cause mortality, than that in the general population. The higher risk of CV in patients with SpA than that in the general population indicates the need for strict risk factor correction and disease management.

KEYWORDS

ankylosing spondylitis, mortality, myocardial infarction, psoriatic arthritis, spondyloarthritis, stroke

1 | INTRODUCTION

Spondyloarthritis (SpA) is a group of chronic inflammatory diseases that share certain clinical features and genetic associations, such as human leukocyte antigen B27, tendency for new bone formation, enthesitis, and frequent axial skeleton involvement, as well as the sacroiliac and peripheral joints. SpA can be divided into predominantly axial and peripheral subtypes. Ankylosing spondylitis (AS) is defined as an axial case of SpA accompanied by radiological change in sacroiliac joints.[1,2] Peripheral SpA includes psoriatic arthritis

(PsA), inflammatory bowel disease-related arthritis, and undifferentiated arthritis.

The pathogenesis of SpA is associated with the production of pro-inflammatory cytokines, such as tumor necrosis factor, interleukin (IL)-17, and IL-23. Therefore, agents targeting such cytokines are used for the treatment of SpA. In this disease, chronic inflammation can exacerbate atherosclerosis of the blood vessels. Studies have explored the cardiovascular (CV) risk associated with AS[3,4] and PsA,[5] although the intensity of this risk appears to have gradually decreased in recent reports. However, the CV risk and mortality associated with SpA remain a controversial topic.

We therefore aimed to perform an updated meta-analysis using newly reported evidence and compared the risks of CV disease, including myocardial infarction (MI) and stroke associated with SpA. Also, this is the first meta-analysis to evaluate all-cause mortality in patients with SpA.

2 | PATIENTS AND METHODS

2.1 | Data sources and searches

Electronic databases, including MEDLINE and EMBASE, were subjected to a systematic search for studies written in English published between January 1997 and September 2019. Additional manual searches for relevant literature were also performed. The search strategy was designed to search MEDLINE through the PubMed interface, using the following keywords: "spondyloarthropathy," "ankylosing spondylitis," "psoriatic arthritis" AND "mortality," "coronary disease," "myocardial ischemia," "myocardial infarction," AND "stroke." Electronic database searches used both free-text words and Medical Subject Headings (Mesh). The search strategy was adapted as appropriate for all other database searches after considering differences in the indexing terms and search syntax of each database. We identified additional relevant studies that were suitable for inclusion through a manual review of the reference lists of studies identified using our initial search strategies. A PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses)[6] flow diagram of the study selection is shown in Figure 1 and the PRISMA checklist is presented in Table S1.

2.2 | Study selection and data extraction

All search screenings and data collection were performed independently by 2 reviewers (JHK and IAC) based on predefined inclusion and exclusion criteria. Methodological quality assessment of the included articles was performed according to the Newcastle–Ottawa Scale (NOS). This tool was developed to evaluate the quality of non-randomized studies. Longitudinal studies were evaluated for each aspect of quality, including selection (representativeness, selection of controls, ascertainment of exposure, and demonstration that

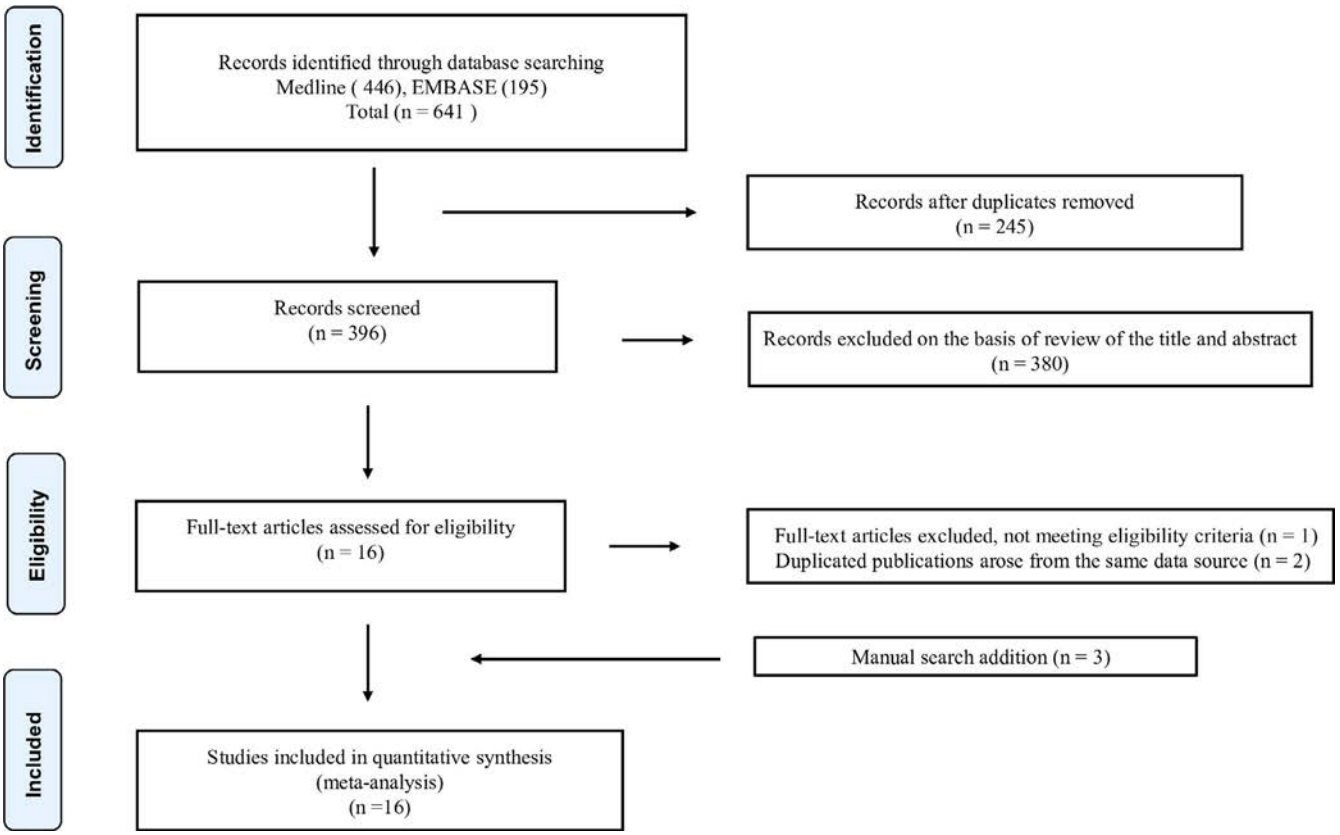

FIGURE 1 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart of study selection

outcome of interest was not presented at start of study), comparability, and outcomes (assessment of the outcome, length, and adequacy of the follow-up). If studies scored more than 5 out of the 9 points, they were considered to be of good quality, that is above moderate.[7]

We included population-based cohort studies that reported measures of association, including the odds ratio (OR), risk ratio (RR), or hazard ratio (HR), and variability, such as standard errors or 95% confidence intervals (CI), to evaluate the effects of SpA on the incidence of MI, stroke, and mortality. Disease was defined based on the clinical criteria, physicians' diagnosis, insurance or billing codes. At minimum, the data were adjusted for age and gender. The inclusion criteria were as follows: the enrollment of patients older than 15 years in a study group, who met the definition of SpA (including AS, PsA, and undifferentiated SpA). We limited the cardiac outcome to MI and excluded studies that described vague and broad concepts of cardiac problems, such as coronary artery disease, stable angina, and atherosclerosis. If multiple publications arose from the same data source, we included studies with extractable estimates reported for different types of arthritis. If estimates were reported for the same type of arthritis, we only included the most recent publication. The following variables were extracted from the studies: demographic characteristics, including country, follow-up period, age, gender, sample size, and data source (Table 1). The OR, RR, and HRs of CV events and mortality during the follow-up period were also collected.

2.3 | Data synthesis and statistical analysis

A meta-analysis was performed using Review Manager software (RevMan 5.3; The Cochrane Collaboration, Copenhagen, 2014), which was supplied by the Cochrane Collaboration. We calculated the risks of MI, stroke, and mortality in a proportional meta-analysis and determined the RRs using the Mantel–Haenszel method.

Heterogeneity among the studies was assessed using Cochran's Q statistic and I^2 statistic, which represented the percentage of heterogeneity across studies that was attributable to between-study differences. Furthermore, publication bias was tested using funnel plots. This study was exempted from an ethical review by the institutional review board of Chungbuk National University Hospital (CBNUH IRB No. 2019-01-004).

3 | RESULTS

3.1 | Identification and characteristics of included studies

We identified 641 articles for possible inclusion. Of these, 625 were excluded during the initial screening of titles and abstracts because they did not meet the inclusion criteria. We then screened the full manuscripts of the remaining 16 articles. Of these, we excluded three articles that did not meet the inclusion criteria regarding the study population or were duplicated publications. Three studies were added after the initial manual search. Finally, a total of 16 articles comprising 18 cases were included in this meta-analysis (Figure 1). Table 1 summarizes the selected studies.

We excluded case-control studies, whereas longitudinal, population-based studies that used large national patient registers or national insurance claims data were included. The study populations in the articles were derived from the following locations: 5 from Asian countries, including Taiwan and South Korea; 8 from European countries, including Sweden, Denmark, and England; 2 from Canada; and 1 from the United States. The median follow-up period was 9.0 (range, 6.0-12.5) years. Of the 18 cases included, 12, 5, and 1 involved AS, PsA, and undifferentiated SpA, respectively.

All 16 articles were assessed using the NOS and scored 5 points or more, indicating a moderate-to-good study quality (Table S2). A random-effect model was used to estimate the overall effect instead of a fixed-effect model because of the heterogeneity among the included studies. Funnel plots of the evaluation of publication bias are also shown in Figures S1, S2, and S3.

3.2 | Relationship of SpA with MI, stroke, and mortality

Six cases were included in the analysis of MI risk. The risk of MI increased significantly in patients with SpA compared to the general population, with an RR of 1.52 (95% CI: 1.29-1.80; Figure 2A). Seven cases were included in the analysis of stroke risk and the result showed an increased risk of stroke in patients with SpA (RR: 1.21; 95% CI: 1.0-1.47; Figure 2B). Five studies were selected for all-cause mortality analysis. In this analysis, the patients with SpA tended to have an increase in the all-cause mortality, but this difference was not significant (RR = 1.23; 95% CI: 0.96-1.57, Figure 2C). Funnel plots for MI, stroke, and all-cause mortality are shown in Figure S1.

3.3 | Subgroup analysis

To complement the results of each heterogeneous study, subgroup analysis was performed according to the type of arthritis disease, such as AS and PsA. The risks of MI (RR: 1.49; 95% CI: 1.34-1.66) and mortality (RR: 1.46; 95% CI: 1.15-1.86) were increased in the AS group. However, the increase was not significant for stroke (RR: 1.20; 95% CI: 0.98-1.46; Figure 3). Moreover, patients with PsA had no significant difference in their risk of stroke (RR: 1.09; 95% CI: 0.85-1.39) or mortality (RR: 0.98; 95% CI: 0.71-1.36; Figure 4). A meta-analysis to evaluate the risk of MI was not possible in patients with PsA because of the lack of cases (only 1 case was included to evaluate the risk of MI in PsA). Egeberg et al[8] have previously reported the risk of MI in patients with PsA (RR: 1.22; 95% CI: 1.05-1.43). Funnel plots for MI, stroke, and mortality in patients with AS and stroke, and those for mortality in patients with PsA are presented in Figures S2 and S3 respectively.

TABLE 1 Characteristics of the included studies

First author/ y	Country/data source	Participant/Age at screening, y	Follow-up period, y	Arthritis female (%)	Arthritis ascertainment	Incidental outcome ascertainment
Ahlehoff et al[18] /2011	Denmark/Danish nationwide clinical register	Incidental AS/ ≥18	1997-2006	None	ICD codes + medication	National cause death register, ICD-10 codes
Bengtsson et al[19]/ 2017	Sweden/National patient register	Incidental AS/ 18-99	2006-2012	31.9	ICD codes	ICD codes + medication
		Incidental uSpA/ 18-99		55.2		
		Incidental PsA/18-99		55.1		
Brophy et al[20] /2012	UK/Electronic health record	Prevalent AS/ ≥20	1999-2010	24.1	EHR READ codes	ICD codes
Chou et al[21] /2014	Taiwan/NHIRD	Incidental AS/ ≥18	2000-2009	52	ICD-9 codes	ICD-9 codes
Dai et al[22] /2018	Taiwan/NHIRD	Incidental PsA/ ≥18	2000-2012	40.5	ICD-9 codes	ICD-9 codes
Egeberg et al[8] /2017	Denmark/ Danish Data Protection Agency	Prevalent PsA/ ≥18	2008-2012	55.8	ICD codes + medication	ICD codes
Eriksson et al[23] /2016	Sweden/National patient register	Prevalent AS/ ≥18	2007-2012	32	ICD-10 codes	ICD-10 codes
Essers et al[17] /2016	UK/ CPRD	Incidental AS/ ≥16	1987-2012	29.5	EHR READ codes	EHR READ codes
Exarchou et al[24] /2016	Sweden/ NPR and census register	Incidental AS/ ≥18	2006-2012	34.5	ICD codes	ICD codes + medication
Haroon et al[25] /2015	Canada/Administrative health data	Incidental AS/ ≥15	1995-2011	46.9	ICD-9 codes	ICD-9 codes
Hung et al[26] /2016	Taiwan/NHIRD	Incidental AS/ 18-45	2000-2005	51.8	ICD-9 codes	ICD-9 codes
Kaine et al[5] /2019	USA/Claims data	Incidental PsA/ ≥18	2008-2015	55.4	ICD-9 codes	ICD-9 codes
Lee et al[27] /2018	Korea/NHIS	Incidental AS/ ≥20	2010-2014	27.46	ICD-10 codes	ICD-10 codes
Ogdie et al[28] /2014	UK/THIN	Prevalent PsA/ 18-89	1994-2010	49	EHR READ codes	EHR READ codes

Patients (n)	Controls (n)	Covariates	HR or RR (95% CI) for MI	HR or RR (95% CI) for stroke	HR or RR (95% CI) for mortality
607	4 003 265	Age, gender, concomitant medication, CHF, COPD, cardiac dysrhythmia, renal disease, cancer, rheumatologic disease and SES data			RR: 1.74 (1.32-2.30)
6448	266 435	Age, gender, venous thromboembolism, diabetes, COPD, atrial fibrillation or flutter, other atherosclerotic disease		HR: 0.76 (0.64-0.89) Adjusted HR: 1.25 (1.06-1.48)	
5190				Adjusted HR: 1.16 (0.91-1.47)	
16 063				HR: 0.96 (0.87-1.05) HR: 1.34 (1.22-1.48) for stroke	
1686	1 206 621	Age, gender, diabetes, hypertension, hyperlipidemia	HR: 1.94 (1.43-2.64) Adjusted HR: 1.28 (0.93-1.74)	HR: 1.32 (0.95-1.80) Adjusted HR: 1.0 (0.73-1.39)	
6262	25 048	Age, gender, hypertension, diabetes, hyperlipidemia, stroke, cancer	HR: 1.51 (1.30-1.76) Adjusted HR: 1.36 (1.16-1.59)		
8795	106 701	Adjusted for age, gender, hypertension, diabetes, coronary artery disease, stroke, connective tissue disease, renal disease, chronic liver disease, COPD, cancer			HR: 1.16 (1.07-1.26) Adjusted HR: 1.52 (1.39-1.66)
8149	4 300 085	Adjusted for age, gender, SES, smoking history, alcohol abuse, CVD, diabetes, hypertension, statin use, and healthcare consumption	HR: 1.57 (1.34-1.83) Adjusted HR: 1.22 (1.05-1.43)		
5358	25 006	Age, gender, venous thromboembolism, COPD, diabetes, malignancy	RR: 1.42 (1.08-1.86) Adjusted RR: 1.3 (1.0-1.7)	RR: 1.62 (1.22-2.15) Adjusted RR: 1.5 (1.1-2.0)	
3809	26 197	Adjusted for age, gender, CVD, hypertension, renal failure, BMI, smoking history, alcohol use, NSAIDs, antihypertensives, antiplatelets, antidiabetics, statin use	HR: 0.90 (0.64-1.26) Adjusted HR: 0.76 (0.53-1.09) for MI		
8600	40 460	Age, gender, education, CVD, diabetes, infection, malignancy, chronic pulmonary disease, joint surgery, NSAIDs, glucocorticoids, sDMARD and TNF inhibitor use			HR: 1.52 (1.38-1.68) Adjusted HR: 1.6 (1.44-1.77)
21 473	86 606	Adjusted for age, gender, region of residence, peripheral vascular disease, hypertension, chronic kidney disease, dementia, diabetes, inflammatory bowel disease, cancer			HR: 1.15 (0.97-1.37) Adjusted HR: 1.36 (1.13-1.65)
537	2685	Adjusted for age, gender, occupation, urbanization level, geographic region, income level, hypertension, hyperlipidemia and diabetes		HR: 1.13 (0.99-1.29) Adjusted HR: 1.2 (1.02-1.42)	
14 898	35 037	Age, gender, region, health plan type, urbanicity, CVD, autoimmune disease, cancer, diabetes, anxiety, depression, osteoporosis, uveitis, liver disease, eczema, gout, fatigue, smoking history, alcohol use, obesity		HR: 1.23 (1.11-1.38) Adjusted HR: 1.46 (1.37-1.56)	
12 988	64 940	Adjusted for age, gender, diabetes, hypertension, dyslipidemia		HR: 1.46 (1.13-1.89) Adjusted HR: 1.35 (1.04-1.75)	
8706	81 573	Age, gender, SES, urbanization, chronic kidney disease, heart disease, atrial fibrillation, diabetes, hypertension, cancer, asthma, COPD, liver disease, smoking history, BMI			HR: 0.83 (0.76-0.91) Adjusted HR: 1.02 (0.92-1.12)

(Continues)

TABLE 1 (Continued)

First author/ y	Country/data source	Participant/Age at screening, y	Follow-up period, y	Arthritis female (%)	Arthritis ascertainment	Incidental outcome ascertainment
Park et al[13] /2018	Korea/NHIS	Incidental AS/ ≥20	2010-2015	27.46	ICD-10 codes	ICD-10 codes
Szabo et al[29] /2011	Canada/RAMQ administrative data	Prevalent AS/ ≥19	1996-2006	44	ICD-9 codes	ICD-9 codes

Abbreviations: AS, ankylosing spondylitis; BMI, body mass index; CHF, congestive heart failure; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CRPD, Clinical Practice Research Datalink; CVD, cardiovascular disease; HER, electronic health record; HR, hazards ratio; ICD, International Classification of Diseases; MI, myocardial infarction; NHIRD, National Health Insurance Research Database; NHIS, National Health Insurance Service; NSAIDs, non-steroidal anti-inflammatory drugs; PsA, psoriatic arthritis; RAMQ, Régie de l'assurance maladie du Québec; RR, relative risk; sDMARDs, synthetic disease-modifying antirheumatic drugs; uSpA, undifferentiated spondyloarthritis; THIN, The Health Improvement Network; TNF inhibitor, tumor necrosis factor inhibitor; SES, social economic status.

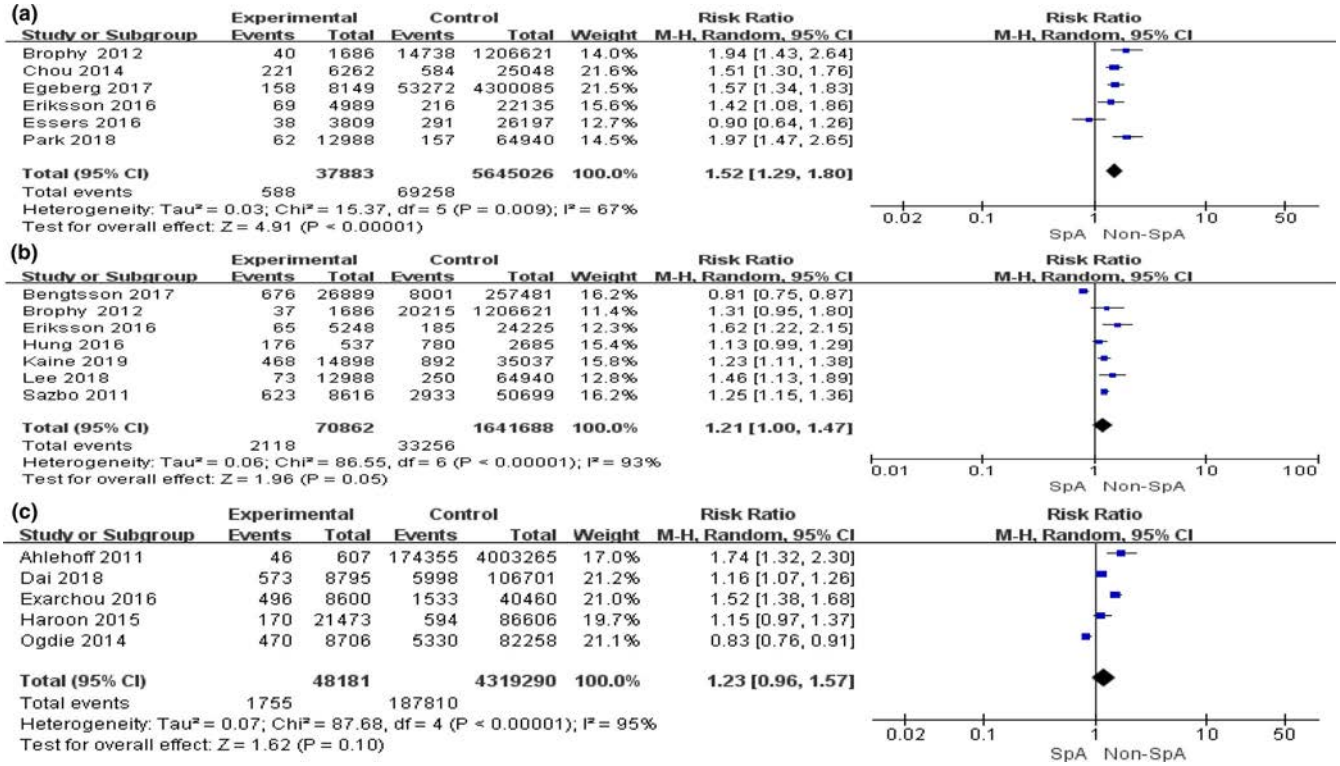

FIGURE 2 Forest plot for the comparison of cardiovascular outcomes and mortality between patients with spondyloarthritis (SpA) and controls. (A) Risk ratio of myocardial infarction (MI), (B) risk ratio of stroke, and (C) all-cause mortality

4 | DISCUSSION

This meta-analysis demonstrated that patients with SpA faced an increased risk of CV events, such as MI and stroke compared to the general population. However, the increase in all-cause mortality was not significant. On subgroup analysis, the risks of MI and mortality were significantly increased in the AS group, while the increase was not significant for stroke. In the PsA group, the risk of stroke and mortality was not significantly different from that in the general population.

Several meta-analyses have attempted to identify the risk of CV in patients with SpA. A meta-analysis of patients with AS by Mathieu et al[9] in 2011 reported an RR of 1.88 (95% CI: 0.83-4.28) for incident MI, while an updated result[10] in 2015 yielded an OR of 1.60 (95% CI: 1.32-1.93) for MI in patients with AS. Another meta-analysis by Schieir et al in 2017[11] revealed a RR of 1.24 (95% CI: 0.93-1.65) for MI in patients with AS. Although the risk of MI in patients with AS seemed to have decreased by this time, a recent update in 2019 by Mathieu et al[12] reported the risk of MI in patients with AS (RR: 1.44; 95% CI: 1.25-1.67). However, our study reported a slightly higher RR

Patients (n)	Controls (n)	Covariates	HR or RR (95% CI) for MI	HR or RR (95% CI) for stroke	HR or RR (95% CI) for mortality
12 988	64 940	Adjusted for age, gender, income, hypertension, diabetes, dyslipidemia	HR: 1.97 (1.47-2.65) Adjusted HR: 1.81 (1.34-2.34)		
8616	50 699	Age, gender, ulcerative colitis, Crohn's disease, psoriasis		RR: 1.25 (1.15-1.36) Adjusted RR: 1.25 (1.15-1.35)	

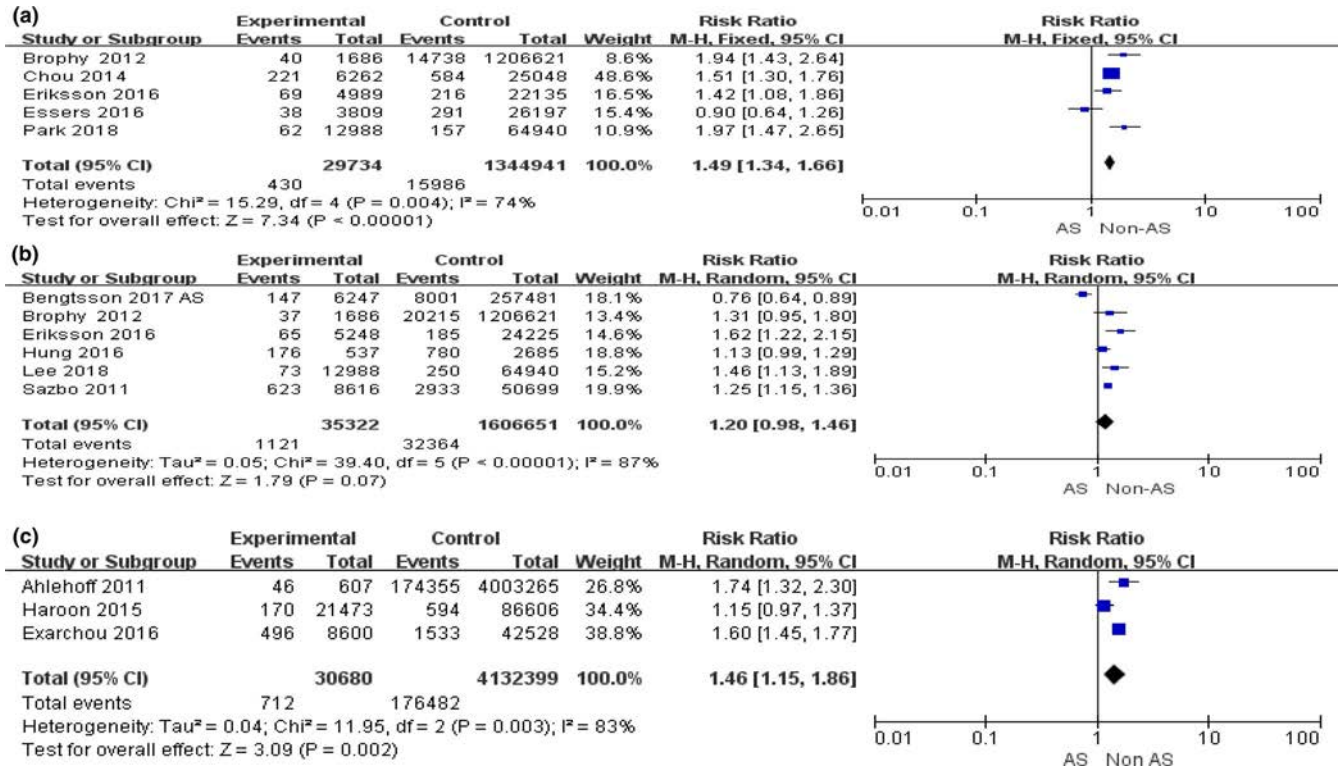

FIGURE 3 Cardiovascular risks in patients with ankylosing spondylitis (AS) in a subgroup analysis. (A) Risk ratio of myocardial infarction (MI), (B) risk ratio of stroke, and (C) all-cause mortality

of 1.49 (95% CI: 1.34-1.66). The increased risk of MI in patients with AS could be attributed to an RR of 1.97 (95% CI: 1.47-2.65) which was reported in a nationwide study in Korea.[13] In contrast, the results of other meta-analyses have shown that the ORs for stroke in patients with AS were 1.50 (95% CI: 1.39-1.62) in 2015[10] and 1.37 (95% CI: 1.08-1.73) in 2019.[12] Our study reported a much lower RR of 1.20 (95% CI: 0.98-1.46) than that reported by two previous studies. In the present study, the RR of mortality in patients with AS was 1.46 (95% CI: 1.15-1.86), and this is the first meta-analysis to evaluate all-cause mortality in patients with AS.

In PsA, the RR of MI was 1.41 (95% CI: 1.17-1.69) in a previous 2017 meta-analysis.[11] Another meta-analysis on PsA reported an OR for MI of 1.68 (95% CI: 1.31-2.15), while the OR for stroke was 1.22 (95% CI: 1.05-1.41) in 2017.[14] The cases analyzed in the previous 2 meta-analyses were case-control studies or did not meet the inclusion criteria of this study and were not included in the analysis of this study. In our meta-analysis, one study[8] presented the MI risk for the PsA group (HR: 1.22; 95% CI: 1.05-1.43), which is consistent with previous study results. The 2017 meta-analysis[14] mostly included case-control studies, which were excluded from our analysis. The mortality (RR: 0.98; 95% CI: 0.71-1.36) in the PsA group was not significantly different from that in the general population. In PsA, the risk of stroke and mortality failed to show a significant difference from that of the general population because of the clinical

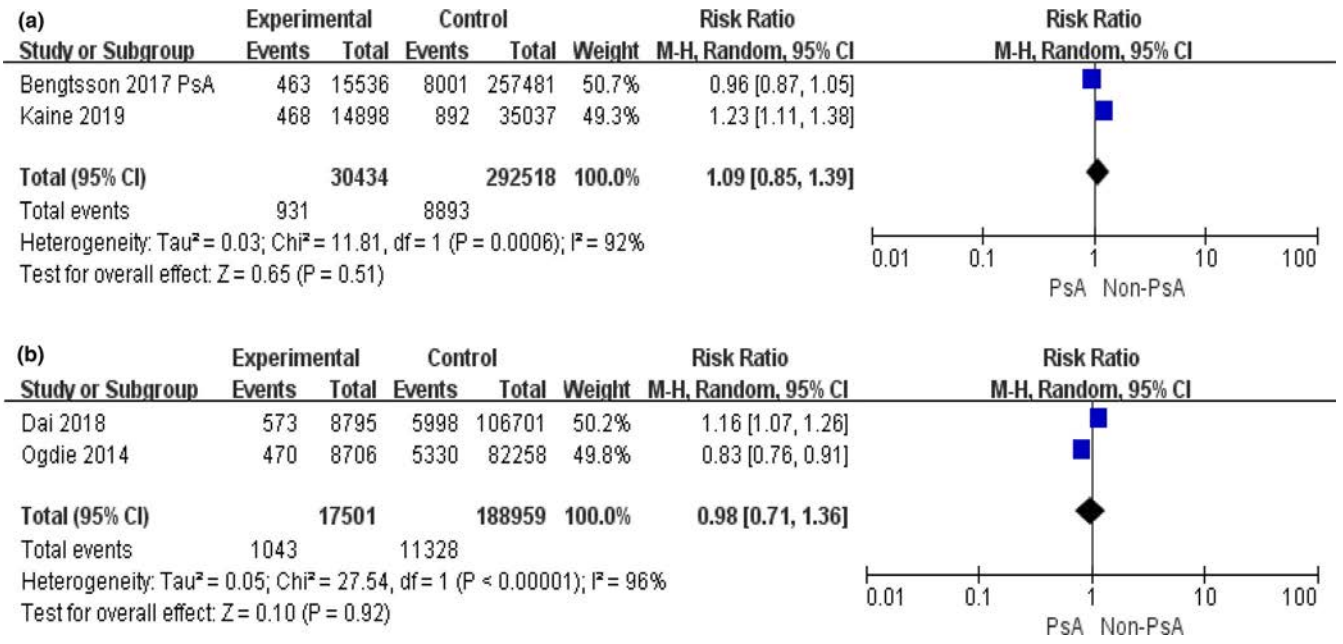

FIGURE 4 Cardiovascular risks in patients with psoriatic arthritis (PsA) in a subgroup analysis. (A) Risk ratio of stroke, and (B) all-cause mortality

diversity in psoriatic arthritis; therefore, the risk of stroke and mortality varied between patients. Although not included in the data of this study, in a recent large-scale prospective study that investigated the risk of overall CV events (including ischemic heart disease, cerebrovascular disease, peripheral arterial disease, heart failure, and mortality), the results of multivariate analysis showed no significant difference for CV risk in patients with PsA than that in the general population (HR: 0.96; 95% CI: 0.53-1.76).[15] The authors explained that this result could be attributable to the fact that almost half of the patients were receiving biologic therapy and most patients had low disease activity at the time of assessment.[15]

The major strengths of the present meta-analysis include the systematic search protocol and the identification of highly reliable evidence by large-scaled population-based studies. Patients with pre-existing CV diseases were excluded from the overall studies, and by excluding the case-control studies, this study only had longitudinal cohort studies, which helped to confirm the incidental outcome. Second, the outcomes were limited to those that were well-defined, such as MI, stroke, or death and we did not include mixed outcomes, such as atherosclerosis, peripheral artery disease, and heart failure. Third, it is also meaningful that this study is the first meta-analysis to evaluate mortality as an outcome in patients with SpA. In addition, this study also has the advantage of using the most up-to-date published studies based on the insurance claims data of 2019.

To date, it remains important to maintain strict control of CV risk factors, including hypertension, diabetes, dyslipidemia, smoking, diet, and management of disease inflammation in patients with SpA. The European League Against Rheumatism task force has provided an updated recommendation for the management of patients with inflammatory joint disorders.[16] According to the recommendations of this task force, disease activity should be controlled optimally to reduce the risk of CV disease in patients with AS and PsA. The task force also discussed the additional evidence demonstrating a reduced risk of CV disease in patients treated with disease-modifying antirheumatic drugs (DMARDs) and biologic DMARDs. Notably, a reduction in the burden of inflammation in patients with AS and PsA might have a favorable effect on CV outcomes. On the other hand, treatments with non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are associated with an increased risk of CV disease. Therefore, prescribed medicines should be used cautiously and in accordance with a specific treatment plan. Moreover, recently available treatment options, such as agents inhibiting tumor necrosis factor alpha, IL-17, and IL-23, can have different effects on CV outcomes; this clear differences between the past and present drug use patterns underscore the importance of updating CV outcomes or analyses of mortality related to inflammatory disease.

Although SpA is known to increase the risk of CV events, related assessments are challenging because of the heterogeneity of the disease or potential confounding factors, such as the continued use of NSAIDs. Eleven articles (68.8%) provided information on treatment agents for SpA and medication for comorbidities, such as hypertension, diabetes, and dyslipidemia. However, only one case[17] provided the results on the adjusted effect of medications, including NSAIDs; the remaining studies did not provide any drug-related adjusted results, which is a major limitation of this study. Second, most studies included in this analysis were conducted using registered International Classification of Diseases (ICD) codes, and the disease severity or activity in individual patient groups was not considered. Third, SpA was diagnosed using different parameters in different countries, which may have led to the slight diagnostic differences. Lastly, only 1 undifferentiated case was identified in all the included SpA cases.

5 | CONCLUSIONS

We conducted an updated meta-analysis that included new evidence and confirmed the increased risks of MI and stroke in patients with SpA. The overall CV risk and all-cause mortality remains higher in patients with SpA than in the general population. Strict disease control to reduce the inflammatory burden and efforts to modify other CV risk factors (eg, lifestyle modification, appropriate antihypertensive agents, and statin use) are required. Further studies are warranted to evaluate the association between CV risk and disease activity, as well as to identify the role of NSAIDs in the management of CV risk in patients with SpA.

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CONFLICT OF INTERESTS

None.

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SUPPORTING INFORMATION

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

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REVIEW

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Epigenetics of ankylosing spondylitis: Recent developments

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Abstract

Ankylosing spondylitis (AS) is a chronic inflammatory autoimmune disease which mainly affects the spine, sacroiliac joint and peripheral joints. To date, the exact causes and pathogenesis of AS still remain unknown. It is considered that the pathogenesis of AS is associated with genetic, infection, environment, immunity and other factors. Among them, the role of genetic factors in the pathogenesis of AS has been studied most deeply. However, over the past few years, the function of environmental predisposition and epigenetic modification in the pathogenesis of AS has received extensive attention. This paper summarizes the recent progress in the epigenetics of AS, including abnormal epigenetic modifications at AS-associated genomic loci, such as DNA methylation, histone modification, microRNA, and so on. In summary, the findings of this review attempt to explain the role of epigenetic modification in the occurrence and development of AS. Nevertheless, there are still unknown and complicated aspects worth exploring to deepen our understanding of the pathogenesis of AS.

KEYWORDS

ankylosing spondylitis, epigenetic, histone, methylation, microRNA

1 | INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory autoimmune disease which mainly affects the spine, sacroiliac joint and peripheral joints, which can lead to significant pain, limited mobility and spinal deformity.[1] However, the pathogenesis of AS has not been fully elucidated due to its complexity. Previous studies have shown that AS is a hereditary disease with familial aggregation, and over 90% of the risk of AS is determined by genetic factors.[2] It is generally known that human leucocyte antigen (HLA)-B27 encoded by the class I major histocompatibility complex (MHC-I) allele, which is positive in approximately 95% of patients with AS, is the main genetic factor predisposing to AS.[3] But only about 5% of the general population with positive HLA-B27 will develop AS. According to the twins study, HLA-B27 accounts for less than 50% of the total risk of AS.[4] It means there are other genes other than HLA-B27 playing a role in the pathogenesis and susceptibility of AS. Moreover, genome-wide association studies have identified that other non-HLA susceptibility genes play a critical role in the development of AS, including *IL6R*, *NOTCH1*, *IL10*, *CXCR2* and *TNFRSF1A*, but cannot fully explain their estimated heritability.[5] This incomplete heritability can be explained by the influence of the epigenetic modifications associated with AS.

Epigenetics was first defined by Conrad Waddington to study the mechanisms by which the genotype produces the phenotypes during development.[6] Epigenetics has been redefined since the word was first created in 1942.[7-9] The current accepted definition is the study of molecules and mechanisms that can perpetuate alternative gene activity states in the context of the same DNA

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sequence. That is to say, epigenetics studies the heritable changes in gene expression without changing the nucleotide sequence of a gene.[10] Epigenetics plays an important role in the differentiation and development of different cells by promoting or inhibiting the expression of genes. More and more studies have shown that epigenetic mechanisms can regulate the differentiation, proliferation and function of immune cells, including natural killer (NK) cells,[11] macrophages,[12] T cells,[13] and so on, thus regulating gene expression and playing a crucial role in immune response. Epigenetic modification mechanisms mainly consist of DNA methylation, histone modification, chromatin remodeling, and microRNA regulation,[14] which reportedly are critical players in autoimmune disease. A number of studies have suggested genetic overlap among autoimmune diseases,[15] and the role of epigenetics in different autoimmune diseases has also been extensively studied. For example, changes in DNA methylation levels in peripheral blood cells have been found in a variety of autoimmune diseases, including AS,[16] systemic lupus erythematosus[17] and multiple sclerosis.[18] This review will summarize the major epigenetic modifications and then focus on the latest findings from genetic and epigenetic studies to provide a novel, more efficacious management of AS.

2 | DNA METHYLATION AND AS

DNA methylation is one of the first epigenetic regulation mechanisms that has been discovered and studied most deeply. In the broad sense, DNA methylation refers to the chemical modification process in which specific bases on the DNA sequence are catalyzed by DNA methyltransferase (DNMT), and s-adenosyl methionine (SAM) is used as the methyl donor to obtain a methyl group through covalent bond binding. Such DNA methylation can occur at the c-5 sites of cytosine, the n-6 sites of adenine, and the n-7 sites of guanine.[19] In general studies, DNA methylation mainly refers to the methylation process of the 5th carbon atom on cytosine in CpG dinucleotide, which is usually concentrated in a specific region of the genome, called CpG island, located on the promoter of nearly 60% of the genes.[20] The product is called 5-methylcytosine (5-mc), which is the main form of DNA methylation in plants, animals and other eukaryotes, and the only form of DNA methylation in mammals.[21] DNA methylation is a stable modification state, which can be passed on to the new progeny DNA along with the replication process of DNA under the action of DNA methyltransferase, which is an important epigenetic mechanism. There are 2 types of DNA methylation. One involves DNA that is not methylated in either strand, called de novo methylation. The other is when one strand of double-stranded DNA is already methylated and the other unmethylated strand is methylated, a type called maintenance methylation.[22] In general, DNA methylation mainly inhibits the expression of genes, especially when they are located in gene promoter and enhancer regions.[20] A large number of studies have shown that DNA methylation can regulate gene expression by causing changes in chromatin structure, DNA conformation, DNA stability and the way DNA interacts with proteins.[23]

At present, the role of DNA methylation in autoimmune diseases has been paid more and more attention. Nevertheless, the research on AS is still in its infancy. Karami et al. found that, compared with the healthy control group, the transcription level of B-cell chronic lymphocytic leukemia/lymphoma 11B (*BCL11B*) gene was decreased and the promoter methylation level was increased in AS patients. The research has shown that promoter hypermethylation was correlated with messenger RNA (mRNA) overexpression, but not with clinical manifestations.[24] The proof-of-principle study in 2014 suggested that methylation of the suppression of cytokine signaling 1 (*SOCS-1*) can be detected in serum of HLA-B27 positive AS patients but not in HLA-B27 positive controls. Moreover, they also observed that the methylation of SOCS-1 is significantly associated with severity of a patient's spondylopathy, sacroiliitis and acute phase reactant C-reactive protein (CRP).[25] Another study in the Iranian population found that *DNMT1* expression level of AS patients decreased obviously, and the methylation level of DNMT1 promoter was higher than the control group; it can be seen that the decreased expression level of *DNMT1* is related to high expression levels of DNMT1 promoter methylation, so the research has shown that *DNMT1* disorders can regulate the development of the AS by changing the other target gene methylation levels.[26] A recent analysis of genome-wide DNA methylation profiles from 5 patients and 5 healthy subjects detected 1915 differentially methylated CpGs in peripheral blood mononuclear cells (PBMCs) of AS patients. These methylated loci were mapped to 1214 genes. Among them, the *HLA-DQB1* gene achieved the most significant signal for AS. Although it is a groundbreaking study, there are some limitations to our understanding of the exact effect of methylation. First, they only used 5 AS patients and 5 healthy controls to perform methylation analysis. It is a quite small sample that may lead to selection bias in the course of the study. Second, it used another separate 5 AS patients and 5 healthy controls to verify the relationship between hypermethylation of *HLA-DBQ1* gene and AS. Third, at present, most patients with AS are treated with nonsteroidal anti-inflammatory drugs (NSAIDs), and the effect of NSAIDs on methylation cannot be ruled out here. Therefore, more in-depth biological studies are needed to verify the conclusions of this study and further clarify the functions of *HLA-DBQ1* in the occurrence and development of AS.[27] There is increasing evidence that *HLA-B27* is closely related to the progression of AS; however, the mechanism by which this allele increases the risk of AS is unclear.[28] A recent study shows that *HLA-B27* may have specific pathophysiological effects by affecting the DNA methylation at CpG sites around HLA-B sites.[29] The preliminary study of our group also showed that the hypermethylation of I*L12B* and the abnormal methylation of the promoter of *IRF8* gene may be involved in the occurrence, development and pathogenesis of AS.[30,31] In particular, we think it is worth noting that profiling CpG methylation in specific cell types will be a useful avenue for future research, since these studies were all carried out in whole blood or PBMC. Currently, the study of DNA methylation on the immune-mediated disease is limited by study design, methylation typing methods and statistical tissue, which deserves further investigation.

3 | HISTONE MODIFICATION AND AS

Histone is the basic protein in eukaryotic somatic chromatin and prokaryotic cells, which together with DNA constitute the nucleosome structure. They are the main protein components of chromatin, which act as spools for DNA entanglement and play a fundamental role in gene regulation. These proteins are divided into 2 major classes of core (H2A, H2B, H3 and H4) and connective protein (H1 and H5) histones. Histones can be modified in a number of ways, including methylation, acetylation, phosphorylation, ubiquitylation, deimination, sumoylation, AD-P ribosylation, b-N acetylglucosamine, to influence chromatin compaction and accessibility in many different ways.[32,33] The modified histones can regulate DNA transcription through 2 major ways, 1 is to change the chromatin conformation to expose gene promoters to transcription factors, and the other is to modify histone proteins that may affect the binding of chromatin-related factors. At present, the most widely studied types of histone modifications are acetylation and methylation of lysine or arginine residues at the end of the N-terminal of histones, which can depolymerize or fold chromosome structures and thus regulate gene transcriptional activity. Histone acetylation is regulated by histone acetyltransferase (HATs) and histone deacetylase (HDACs), respectively. Histone acetylation is conducive to the dissociation of DNA and histone octamers and the relaxation of nucleosome structure, so that various transcription factors and co-transcription factors can specifically bind to DNA binding sites and activate gene transcription.[34] In contrast to HATs, HDAC enzyme can reverse the acetylation of lysine, which is a function of restoring the positive charge of lysine. This may stabilize the local chromatin structure and is primarily consistent with HDACs' transcriptional inhibition.[35] Histone methylation is another important form of histone modification and plays an important role in the establishment and maintenance of DNA methylation. Histone methylation is catalyzed by histone methyltransferases and occurs mainly on lysine and arginine residues of H3 and H4 histones. There are 2 main types of histone methyltransferases, 1 is lysine-specific histone methyltransferase, the other is arginine-specific histone methyltransferase. Lysine can be monomethylated, dimethylated and trimethylated, while arginine is mainly monomethylated and dimethylated.[36] Correspondingly, histone demethylation is catalyzed by histone demethyltransferases. The results of histone methylation depend on the specific bases involved, for example, histone H3K4 methylation can activate gene transcription,[37] while histone H3K9 methylation is associated with gene silencing.[38]

The balance between histone acetyltransferases and histone deacetylases determines the overall level of acetylated histones in cells.[39] The study found a significant increase of HAT/HDAC ratio in patients with AS treated with tumor necrosis factor (TNF) inhibitors. HDAC inhibitors have been proven to limit the production of pro-inflammatory cytokines including TNF,[40] and the results of the study also showed a significant increase in HAT/HDAC ratio during anti-TNF therapy, suggesting that TNF inhibitors and HDAC inhibitors are consistent in their anti-inflammatory effects.[41] Ya Jiang et al. have investigated that the relationship between HDAC3 and microRNA-130a (miRNA-130a) and its target TNF-1α in PBMCs of AS patients. They observed increased level of HDACs and reduced level of miRNA-130a in the PBMCs of AS patients compared with healthy controls. HDACs can be recruited to the promoter region of the gene encoding miRNA-130a and then bind to the promoter of miRNA-130a, leading to the reduced level of miRNA-130a. When HDAC3 was knocked out or inhibited, they observed the increased level of miRNA-130a and the decreased expression of the mRNA and protein of TNF-1α in PBMCs. The inspiration from this article is that HDAC3 inhibitors can limit the production of pro-inflammatory cytokines like TNF-α to inhibit the inflammatory response of AS. This study suggests that HDAC3 may be involved in regulating the underlying pathogenesis of AS through negative feedback regulation with miRNA-130a and enhanced TNF expression.[42] Another study found that histone H3K27me3 demethylases (KDM6A and KDM6B) regulate human Th17 cell development. Furthermore, GSK-J4 (KDM6 inhibitor) could suppress the role of the key transcription factor RORγt during Th17 differentiation. As is known to all, Th17 cells play a critical role in the pathogenesis of AS by secreting the inflammatory cytokine interleukin-17. Therefore, the results of this study suggest that the levels of inflammatory cytokines can be inhibited by inhibiting histone demethylase, which may be a target for AS treatment.[43]As opposed to methylation, there are few studies on the role of histone modification in the pathogenesis of AS and none of them have been performed at the genome-wide level; therefore, further studies are needed in the future.

4 | miRNA AND AS

At present, most studies on AS genetics mainly focus on genes that can encode proteins in the genome (but such genes only account for about 2% of the whole genome), while less attention is paid to genes that cannot encode proteins in the genome (about 98%) - the role of non-coding RNA (ncRNA) in the pathogenesis of AS. According to its own length, ncRNAs can be classified as long ncRNA (>200 nt in length) and short ncRNA (mainly miRNAs, less than 200 nt in length). miRNAs are small non-coding, single-stranded, endogenous expressed RNA molecules that exist in human plasma in a stable form and regulate gene transcription by causing translation inhibition and affecting the stability of mRNA, which can be used as biomarkers for disease activity, pathogenesis and prognosis.[44,45] miRNAs regulate gene expression mainly through the following 2 pathways. On the one hand, the miRNA induces degradation of the target mRNA, while the miRNA sequence matches the 3' untranslated region (UTR) of the target mRNA exactly. On the other hand, translation disorders occur when miRNA chains do not exactly match the 3' UTR of target mRNA. The second way that miRNAs affect gene expression is by regulating DNA methylation at promoter sites and histone modification.[46] miRNAs have the following special characteristics that make them reliable biomarkers. These include: (1) stability; (2) tissue

specificity; (3) miRNA expression is related to specific physiological and pathological states; and (4) the ability of miRNA to differentiate, which can improve the accuracy of diagnosis.[47]miRNAs play an important role in many biological processes, such as cell proliferation,[48] apoptosis,[49] differentiation[50] and development.[51] In recent years, with the deepening of research, the mechanism of ncRNA in complex diseases has become a research hotspot. The relationship between miRNAs and autoimmune diseases (including AS) has been preliminarily reported.

Compared with studies of DNA methylation and histone modification, the research on miRNA has received more attention. A large number of studies have shown that miR-29a plays a key role in osteogenic differentiation and is closely related to AS. Huang et al. reported the increased miR-29a expression in PBMCs of patients with AS for the first time, but they also found that miR-29a cannot reflect disease activity.[52] Downregulated serum Dickkopf homolog 1 (DKK-1) in Wnt signaling may contribute to new bone formation in AS.[53] The recent research found that miR-29a promotes osteoblast proliferation by downregulating DKK-1 expression and activating the Wnt/β-catenin signaling pathway,[54] but no correlation was observed between miR-29a and erythrocyte sedimentation rate, CRP, Bath Ankylosing Spondylitis Disease Activity Index, and Bath Ankylosing Spondylitis Functional Index.[55] The above studies suggest that miR-29a maybe a useful diagnostic marker in new bone formation. There are other biomarkers that play a role in bone remodeling in the pathogenesis of AS. Qin et al. found that the level of miR-17-5p was significantly higher in fibroblasts and ligament tissues from AS patients as compared to the non-AS individuals. The study showed that miR-17-5p can regulate osteogenic differentiation by targeting the 3' UTR of ankylosis protein homolog (ANKH). Also, downregulation of miR-17-5p slowed AS progression through regulation of cytokines, such as DKK1 and vascular endothelial growth factor (VEGF).[56] miRNAs associated with bone remodeling in AS are shown in Table 1.

miRNA can also regulate T cell survival to participate in the pathogenesis of AS. Fengju Li et al. revealed that compared with the healthy HLA-B27-negative control group, the expression of miR-130a-3p in the T cells of HLA-B27-positive patients of AS was downregulated. miR-130a-3p significantly promoted proliferation ability and inhibited cell apoptosis of Jurkat T cells by targeting HOXB1.[57] Chunfeng Hou et al. found overexpression of let-7i and downregulation of insulin-like growth factor-1 receptor (IGF1R) in T cells with AS. Based on Target scan analysis, the target of let-7i was predicted to be IGF1R. IGF1R sits on the surface of T cells and regulates T cell activation. They found overexpression of let-7i induced autophagy

TABLE 1 MicroRNAs associated with bone remodeling in ankylosing spondylitis

Author	Study year	microRNA	Targets	Signaling pathway	Function
Zhang et al.	2019	miR-29a	DKK-1	Wnt /β-catenin	Promotes osteoblast proliferation, new bone formation
Qin et al.	2019	miR-17-5p	ANKH		Regulates heterotopic ossification
Li et al.	2015	miRNA-29a	DKK1, GSK3β	Wnt /β-catenin	Regulates tumor necrosis factor-α mediated bone loss
Huang et al.	2014	miRNA-21	PDCD4		Induces the activation of osteoclasts
Ma et al.	2019	miRNA-96	SOST	Wnt	Promotes osteoblast differentiation and bone formation
Di et al.	2017	miRNA-146a	Dickkopf1		Enhanced proliferation and osteogenic potential of ankylosing spondylitis fibroblasts
Du et al.	2019	miR-495	DVL-2	wnt/β-catenin/ Runx-2	Depressed inflammatory response
Tang et al.	2018	miR-124	GSK-3β	Wnt /β-catenin	Regulates osteoblast differentiation
Zhao et al.	2020	miR-204-5p	Notch2		Inhibits the osteogenic differentiation

TABLE 2 MicroRNAs associated with regulating T cell survival in ankylosing spondylitis

Author	Study year	MicroRNA	Targets	Function
Li et al.	2019	miR-130a-3p	HOXB1	Regulates T-cell survival
Chen et al.	2017	miR-10b-5p	MAP3K7	Suppresses pathogenic Th17 cell function
Zhang et al.	2018	miRNA-16a		Reflects disease activity via regulating Th1/Th2 balance
Wang et al.	2017	miRNA-199a-5p	Rheb	Induces autophagy; inhibits the pathogenesis of ankylosing spondylitis
Xia et al.	2015	miRNA-124	ANTXR2	Induces autophagy
Hou et al.	2014	Let-7i	IGF1R	Induces autophagy to protect T cell from apoptosis

by targeting IGF1R, thereby protecting T cells from apoptosis.[58] miR-199a was also proven to modulate the mechanistic target of rapamycin (mTOR) signaling via direct targeting of Rheb to induce autophagy and slow down the onset of AS.[59] It was found that the level of miR-10b was increased in Th17 cells of patients with AS, and the expression of miR-10b was negatively correlated with the frequency of Th17 cell enrichment in vitro. miR-10b inhibited the Th17 response and was induced transiently during Th17 differentiation. Researchers further identified mitogen activated protein kinase 7 (MPK3K7), the target of miR-10b, and found that miR-10b inhibited MAP3K7 expression by binding to 3' UTR. After MAP3K7 was silenced, the production of IL-17A was inhibited, which was consistent with the effect of miR-10b overexpression.[60] There are other miRNAs involved in the pathogenesis of AS by regulating the survival of T cells, which are summarized in Table 2.

The above studies suggest that miRNA can participate in the pathogenesis of AS in different ways, but the specific molecular mechanism is still unclear, which is worthy of further discussion.

5 | CONCLUSION

Although in the past few years, genome-wide association studies have enabled us to discover new pathogenic pathways and a large number of AS susceptibility sites have been identified, it is still impossible to accurately predict the genetic risk of AS. Genetic factors cannot fully explain the pathogenesis of AS, while the expression of gene functions depends on epigenetic regulation, which varies in different tissues and cells as well as in different environments and lifestyles. Furthermore, twin studies have shown significant inconsistencies in the prevalence of AS among identical twins, suggesting that environmental factors play a significant role in the risk of AS.[4]So far, there is little information about epigenetic modifications in the pathogenesis of AS. Although the epigenetic study of AS is not very in-depth, related studies have begun to develop, and the data have started to accumulate. At present, the detection of DNA methylation, histone modification and miRNA in AS is limited by methods, and most of the current studies have problems such as small sample size, difficult control of potential confounding factors, and lack of studies in the most relevant tissues and cells. Solving these problems can improve the reliability of the study and further explain the role of epigenetics in the pathogenesis of AS. Understanding the epigenetic mechanism will better explain the pathogenesis of AS and further determine the therapeutic targets of drugs. With the development of epigenetics in the future, epigenetic studies will be able to better explain the pathogenesis of diseases and treat diseases.

CONFLICT OF INTERESTS

The authors declare they have no competing interests.

AUTHORS' CONTRIBUTIONS

Hui Yang and Yuting Chen contributed equally to this work and should be considered co-first authors. Jixiang Deng, Shanshan Xu, Xing Gao and Shiyang Guan participated in the idea of the article; Hui Yang, Yuting Chen, Wei Xu, Ming Shao collected the related papers and drafted the manuscript. Jinian Wang, Shengqian Xu, Zongwen Shuai and Faming Pan were responsible for the final review of the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL

Not applicable.

CONSENT FOR PARTICIPATION

All authors agreed to publish in the magazine. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Not applicable.

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ORIGINAL ARTICLE

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Disease severity affects myocardial functions in patients with treatment-naive early rheumatoid arthritis

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Abstract

Objectives: The cross-sectional study aimed to assess myocardial functions using global longitudinal strain (GLS) echocardiography and arrhythmia parameters with treatment naive newly diagnosed rheumatoid arthritis (RA) and no clinical evidence of cardiovascular disease (CVD).

Methods: Seventy seven newly diagnosed treatment-naive RA patients were enrolled. Disease severity was evaluated according to rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) positivity, and Disease Activity Score 28 C-reactive protein (DAS28 CRP). Myocardial functions were assessed using conventional echocardiography and GLS technique and electrocardiogram parameters cQT and Tp-e/cQT.

Results: Twenty three patients had severe disease while 54 patients were non-severe. The Left Ventricle GLS (17.98 ± 1.24 vs 21.29 ± 1.03, $P < .001$), cQT (428.71 ± 9.05 vs 394.61 ± 17.83, $P < .001$), Tp-e/cQT (0.19 ± 0.02 vs 0.16 ± 0.01, $P < .001$) for severe RA patients was reduced compared to RA non-severe patients. Penalized maximum likelihood estimation logistic regression analysis revealed LVGLS as the only significantly independent predictor of severe RA disease (OR 0.70, CI 95% 0.52-0.92, $P = .001$). Receiver operating characteristic (ROC) curves of the LVGLS was revealed 19.9 as GLS discriminative value with 88.8% positive predictive value for predicting severity. Severe RA risk increases when log-odds value was over 0, corresponds to LVGLS value less than 18 by partial effect plots.

Conclusion: RA severity was associated with lower LV systolic myocardial function and increased arrhythmia parameters. Only LVGLS was significantly independent predictor of RA disease severity.

KEYWORDS

cQT, disease severity, global longitudinal strain, rheumatoid arthritis, Tp-e/cQT

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic joint damage and physical disability. Early diagnosis and proper treatment are the key factors for treatment success.[1] Compared with the general population, RA patients' life expectancy is 8-15 years lower, due to mainly accompanying cardiovascular diseases (CVD).[2] The risk of CVD is doubled in RA patients. Furthermore, CVD is the most commonly identified cause of death in RA.[3] Conventional cardiovascular risk factors have not

been reported to contribute to the development of cardiovascular morbidity in RA.[4] Herein, chronic and occasionally severe inflammatory activity would be the primary reason for the development of CVD even after adjusting for traditional cardiovascular risk actors.[5] Consequently, RA is recognized as an independent risk factor for CVD, and therefore the European League Against Rheumatism (EULAR) task force cares about effective and appropriate treatment of CVD risk factors as much as conventional treatment of RA.[6]

CVD risk classification of RA patients is the first step for implementing preventive measures against CVD-related mortality and morbidity. Although the pathophysiological mechanism has not yet been clearly defined, the chronic inflammatory state in RA would be the main causative factor for the changes in vascular system and dysfunction in myocardia.[7,8] Recent research using strain imaging technique, which is a sensitive tool for detecting early stages of impairment of systolic function, showed impairment in global longitudinal strain (GLS) in RA patients without deteriorated ejection fractions.[9,10]

Increased inflammatory and sympathetic activity may cause electrical disturbances during ventricular repolarization. This would be the reason for arrhythmias and cardiovascular death by causing electrical disturbances during ventricular repolarization in RA patients. Recently, it was suggested that the Tp-e/cQT ratio may be a direct indicator for the dispersion of ventricular repolarization, which is independent from the variations in heart rate.[11,12] Furthermore, it has also been suggested that the Tp-e/cQT ratio is a more precise predictor of ventricular arrhythmogenesis than the QT, cQT and Tp-e intervals.[11]

Thus, we aimed to determine the state of the left ventricle function and indicators of ventricular arrhythmogenesis at time of the first diagnosis of the disease. While doing this, we also aimed to establish the strength of our findings for prediction of RA disease severity by the use of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) positivity and Disease Activity Score of 28 joints – C-reactive protein (DAS28-CRP) value.

2 | MATERIALS AND METHODS

The study was performed at Kartal Kosuyolu High Training and Research Hospital between January 2017 to December 2019. We enrolled patients with RA who fulfilled the 2010 American College of Rheumatology/EULAR RA Classification Criteria.[13] All patients were diagnosed with RA for the first time in a rheumatology outpatient clinic and referred for cardiovascular investigation to a cardiology clinic. All patients were treatment-naive, including disease-modifying antirheumatic drugs and glucocorticoids for their symptoms. First, all consecutive new diagnosed RA patients were evaluated for ventricular functions with 2-dimensional echocardiography. The patients who had impaired right and left ventricular functions were excluded. Other exclusion criteria of the study were as follows: evidence of coronary artery disease (detected by clinical history, electrocardiogram, treadmill stress test and echocardiographic proof of wall motion abnormalities), congenital or valvular heart disease, systemic arterial hypertension (hypertension was confirmed by 24-hour ambulatory blood pressure monitoring), diabetes mellitus, pulmonary arterial hypertension, arrhythmia, advanced liver and renal diseases, and inadequate image quality. Therewithal, we enrolled 90 age- and gender-matched healthy subjects as a control group. All controls were recruited from the check-up outpatient clinic of the same medical center. None of these were formerly diagnosed for rheumatological diseases. Also none of the controls had any increased acute phase reactants or positive serologic tests ahead of the study. The same exclusion criteria were applied for control subjects. This study was approved by the Local Ethics Committee and carried out in compliance with the Helsinki Declaration. All patients gave written informed consent.

RA patients were evaluated for the duration between onset to diagnosis of RA-related symptoms, visual analog scale (patient global health), number of swollen and tender joints, ACPA, RF, CRP, erythrocyte sedimentation rate (ESR), and disease activity. We measured disease activity with DAS28-CRP.[14] Herein, DAS28-CRP ≥ 5.1 was accepted as severe disease activity.[15]

Patient's data were recorded onto cohort report forms: age, gender, smoking status, body mass index, systolic and diastolic blood pressure and heart rate. After overnight fasting, blood was drawn to determine the following laboratory investigations: lipid profile (triglyceride, total cholesterol, low-density lipoprotein, and high-density lipoprotein), fasting blood glucose, serum creatinine, ESR, CRP, ACPA, and RF. Serum ACPA was measured by immunofluorescence (Thermo Fisher Scientific EliA, Germany); according to the manufacturer's instructions an ACPA level ≥5.6 IU/mL was positive, and RF was detected by latex agglutination (Plasmatec Laboratory Co, Cambridge, UK).

Echocardiograms were performed with GE Vivid 7 ultrasound system (GE Medical System, Horten, Norway) with a standard 3.5 MHz ultrasound probe to all participants according to standard guidelines[16] by 2 experienced cardiologists. Left ventricle (LV) ejection fractions (EF), LV end diastolic volume (LVEDV), LV end systolic volume (LVESV) were interpreted using biplane method of discs.[16] The E and A wave peak velocities from the trans-mitral flow profile were calculated, and E/A was achieved. Mitral annulus tissue Doppler imaging (TDI) was applied in the apical 4 chamber view utilizing 1- to 2-mm sample volume placed through the septal mitral valve annulus and the value of S' was measured.[16]

Estimated pulmonary arterial pressure (ESPAP) was measured in the apical 4-chamber view with the cursor placed through the tricuspid valve.[17] In the apical 4-chamber view by M-mode echocardiography with the cursor placed into the tricuspid lateral annulus, tricuspid annular plane systolic excursion (TAPSE) was measured.[17] Conventional 2-D grayscale imaging was used for evaluation of the global LV longitudinal strain (LVGLS).[18] For analyses of LV strain, apical views containing apical long axis, apical 4 and 2 chamber views were achieved while holding breath with a fixed electrocardiograph (ECG) recording and sufficient grayscale displays to allow optimal specification of extra-cardiac structures and myocardial tissue.

Three consecutive heart cycles equated at frame rate of at least 50 frames/s were recorded. LV endocardial surface at the end systolic frame was manually traced and the shape and width of the region of interest was manually adjusted. Offline analysis was applied using EchoPAC version 1.13.0 (GE Vingmed) blinded for all clinical and rheumatological data.

The 12-lead ECG was recorded in the supine position at a paper speed of 50 mm/s and 1 mV/cm standardization (Hewlett Packard, Page-writer, USA). cQT and Tp-e intervals were evaluated manually with calibers and magnifying glass to decrease the error measurements. Subjects with U waves were excluded from the study. An average value of 5 readings was calculated for each lead. The QT interval was evaluated from the beginning of the QRS complex to the end of the T wave, and was corrected for heart rate using the Bazett formula: cQT = QTd√ (R-R interval). The Tp-e interval was measured from the peak of T wave to the end of T wave on lead V4, V5 and V6. T waves with amplitude less than 1.5 mm or T waves with distorted morphology were excluded from analysis. Measurements of the Tp-e interval were applied from precordial leads. From these measurements the Tp-e/cQT ratio was calculated.

Two experienced cardiologists performed all the cardiological assessments. All echocardiographic and ECG examinations were analyzed and calculated in a blinded fashion according to clinical assessments.

First of all, RA patients and the control cohort were compared for the study parameters. Then the RA patients were dichotomized respectively based upon positivity of ACPA, RF and presence of severe disease activity. Patients within in those groups were compared for the study variables. After that, patients having positivity in both serologic tests and severe disease activity classified the outcome group and were compared with the rest. Predictors chosen from the latter test were analyzed by unique multivariate model.[15,19]

Statistical analyses were performed using "rms", "hmisc", "logistf" and "pROC" packages with R software v. 4.0 (R Corporation, Vienna, Austria). Statistical tests were 2-sided, and a P value <.05 was considered statistically significant. Continuous variables with normal and non-normal distributions were expressed as mean ± SD and median (interquartile range), respectively. Categorical variables were expressed as numbers and percentages of patients. Group means for continuous variables with normal and non-normal distributions were compared using Student's t tests and Mann-Whitney U tests, respectively. Categorical variables were compared using Chi-square tests or Fischer's exact test, as appropriate. Correlation analysis was calculated using Pearson's correlation coefficient. Non-normally distributed variables were log-transformed for correlation and multivariable regression analyses. Traditionally, it should be at least 10 patients with outcome in relation to the degrees of freedom (df) of the predictors included in the model (outcome/df should be 10). In this study, outcome was the patients having ACPA and RF positivity with severe disease activity. In our binary regression model, 7 candidate predictors were chosen while outcomes were present in 23 patients (23/7 = 3.3). As a result we preferred to use the penalized maximum likelihood estimation (PMLE) binary logistic regression method to reduce the overfitting risk.[20] Because the number of outcomes were low (n = 23), logistic regression using Firth's penalized maximum likelihood method was conducted. Unlike maximizing the log likelihood in traditional multivariable logistic regression, maximizing the penalized log likelihood was applied in PMLE logistic regression. Therefore the maximizing log likelihood of the model was adjusted with penalty factor. In our analysis model predictor variables LVGLS, age, VAS, number of tender joints, number of swollen joints, cQT, Tp-e/cQT were used to predict outcome variables of patients having ACPA, RF positivity and severe disease activity. The results of the penalized logistic regression analyses were given as odds ratio (OR) with 95% confidence intervals (CI). Independent contribution of the predictors to the variance of outcome was estimated. Receiver operating characteristic (ROC) curve analysis was performed for discriminative value of significantly independent predictor for establishing positive predictive value (PPV) and area under the curve (AUC). While adjusting for interference from other explanatory variables (same as PMLE), for relationship between plotted variables of outcome (severe RA) and explanatory variable (LVGLS) the partial effect plots for fitted curve as linear predictor were performed.

3 | RESULTS

Eighty-six patients with newly diagnosed RA were initially included but 9 were later excluded from the analysis because coronary artery disease was diagnosed in 2 patients, diabetes mellitus in 3 patients and 4 patients were diagnosed with hypertension during the study. At the end, 77 patients were enrolled in this study.

Demographic and clinical characteristics of the RA patients and control cohort are shown in Table 1. Mean age of the study population was 48.96 ± 5.76 years and 87% were female. There were no significant differences among patient and control groups in terms of age, gender, body mass index, systolic-diastolic blood pressure and heart rate (P > .05). The fasting glucose, and serum creatinine levels of the groups were also similar (P > .05). The RA group had ACPA positivity in 36 (46.8%) and RF positivity in 37 (48%) patients. The median DAS28-CRP score of the patients was 5.3 (4.0-6.2).

The echocardiographic and ECG parameters between patients and control cohort are also shown in Table 1. Using both the conventional evaluation of LV functions and more sensitive estimation by LVGLS we documented no significant difference between patient and control groups (P > .05). Also, ECG parameters revealed no significance between groups (P > .05).

Table 2 shows the results of comparison between the groups with or without ACPA positivity, RF positivity and presence of severe disease activity regarding rheumatological, echocardiographic and ECG parameters. All parameters including LVGLS, cQT and Tp-e/cQT were insignificant between groups (P > .05).

Twenty-three patients (29.9%), aged 46.07 ± 3.95, were ACPA and RF positive and had severe disease activity. These patients formed the severe RA group. Fifty-four (70.1%, age 50.18 ± 6.01) patients

TABLE 1 Clinical characteristics of patients with newly diagnosed rheumatoid arthritis and healthy controls

	Patients, N = 77	Controls, N = 90	*P* value
Female, no. (%)	67 (87)	79 (88)	.92
Age, y	48.96 ± 5.76	48.22 ± 4.87	.45
Smoking, n (%)	15 (19)	16 (18)	.94
Body mass index, kg/m^2	23.66 ± 2.74	24.48 ± 2.31	.51
Systolic blood pressure, mm Hg	120.02 ± 8.05	124.09 ± 5.65	.51
Diastolic blood pressure, mm Hg	80.02 ± 6.39	79.22 ± 3.08	.10
Heart rate, beats/min	67.98 ± 5.44	67.39 ± 9.28	.06
Serum glucose, mg/dL	87.55 ± 8.07	84.92 ± 5.18	.29
Serum creatinine, mg/dL	0.71 ± 0.14	0.76 ± 0.08	.04
Left ventricle ejection fraction, (%)	63.4 ± 2.36	64.22 ± 4.17	.22
Left ventricle end diastolic volume, mL	120 ± 6	119.62 ± 5.05	.44
Left ventricle end systolic volume, mL	44.05 ± 5.8	44.76 ± 5.16	.01
S' global, (cm/s)	11.5 ± 0.97	11.19 ± 1.36	.43
E/A ratio	1.35 ± 0.1	1.25 ± 0.15	.64
Left ventricle global longitudinal strain, %	21.3 ± 1.88	21.17 ± 3.6	.55
Estimated pulmonary artery pressure, mm Hg	27.87 ± 3.53	26.57 ± 3.17	.13
Tricuspid annular plane systolic excursion, cm	19.45 ± 1.32	20.08 ± 2.39	.26
cQT, ms	411.94 ± 27.18	406.94 ± 11.72	.23
Tp-e, ms	66.17 ± 6.17	66.83 ± 10.09	.63
Tp-e/cQT, ms	0.18 ± 0.02	0.18 ± 0.03	.54
Duration of symptom onset, mo	5.8 ± 3.1		
Number of tender joints	5.5 ± 3.0		
Number of swollen joints	4.5 ± 2.7		
Visual analog scale, patient global health, 0-100 mm	58.0 ± 25.2		
DAS28-CRP score	5.3 ± 1.8		
Erythrocyte sedimentation rate, mm/h	29.0 ± 18		
C-reactive protein, mg/L	6.0 ± 2.5		
Anti-citrullinated protein antibodies, %	36 (46.8%)		
Rheumatoid factor positivity, %	37 (48%)		

Note: Data are shown as mean ± SD.
Abbreviations: DAS28-CRP, Disease Activity Score of 28 joints – C-reactive protein.

failed to meet either 1 or more of these indicators and formed the non-severe RA group. LVGLS (17.98 ± 1.24 vs 21.29 ± 1.03, $P < .001$), cQT (428.71 ± 9.05 vs 394.61 ± 17.83, $P > .001$), Tp-e/cQT (0.19 ± 0.02 vs 0.16 ± 0.01, $P < .001$), number of tender joints (4.64 ± 1.74 vs 5.88 ± 2.13, $P = .03$) and age (46.07 ± 3.95 vs 50.18 ± 6.01, $P = .02$) were the significantly different factors between groups (Table 3).

PMLE logistic regression analysis revealed LVGLS as the only significantly independent predictor of severe RA disease (OR 0.70, CI 95% 0.52-0.92, $P = .001$, Table 4). ROC curves of the LVGLS for prediction of RA disease severity was 19.9 as GLS discriminative value with 88.8% PPV (72.5% sensitivity, 75% specificity and 0.818 AUC) (Figure 1). The partial effect plots show fitted curve on the probability scale as log-odds (linear predictor) for LVGLS in Figure 2. Severe RA risk increases when log-odds value was over 0, and corresponds to LVGLS value less than 18.

4 | DISCUSSION

This is the first study evaluating the PPV of the cardiac function and arrhythmia markers in new and treatment-naive RA patients without any CVD. The main novel findings in our study were: (a) the patients with high disease severity (patients with DAS28-CRP ≥ 5.1 and both ACPA and RF positivity) had increased LVGLS, cQT and Tp-e/cQT compared with the patients who had both lower disease activity and mono or dual seronegativity at the first diagnosis; (b) LVGLS was superior to cQT and Tp-e/cQT for the prediction of RA disease severity.

TABLE 2 Patients group (N = 77) divided into: first, ACPA positive patients and negative patients; second, RF positive patients and negative patients; third, DAS28-CRP ≥ 5.1 patients and <5.1 patients

	ACPA positive patients, n = 36	*P* value	RF positive patients, n = 37	*P* value	DAS28-CRP ≥ 5.1 patients, n = 50	*P* value
Left ventricle ejection fraction, %	63.4 ± 2.3	.98	63.4 ± 2.3	.98	63.3 ± 2.3	.94
Left ventricle end diastolic volume, mL	120.2 ± 5.3	.73	120.2 ± 6.6	.71	120.1 ± 6.5	.70
Left ventricle end systolic volume, mL	45.9 ± 1.9	.77	45.9 ± 3.7	.73	45.9 ± 3.3	.72
S' global, cm/s	10.5 ± 0.9	.61	10.5 ± 0.8	.72	10.3 ± 0.7	.32
E/A ratio	1 ± 0.07	.65	1 ± 0.14	.65	1 ± 0.13	.63
Left ventricle global longitudinal strain, %	21.3 ± 1.0	.34	20.1 ± 2	.41	20.0 ± 2.1	.48
Estimated pulmonary artery pressure, mm Hg	27.0 ± 1.9	.65	28 ± 4.0	.07	27.5 ± 3.5	.46
Tricuspid annular plane systolic excursion, cm	19.5 ± 1.5	.38	19.2 ± 1.3	.40	19.6 ± 1.5	.74
cQT, ms	390.5 ± 11.5	.33	403.0 ± 27.6	.35	411.6 ± 29.4	.78
Tp-e/cQT, ms	0.18 ± 0.01	.42	0.17 ± 0.02	.55	0.17 ± 0.02	.47
Duration of symptom onset, mo	5.8 ± 2.3	.94	5.9 ± 3.5	.58	6.0 ± 3.0	.28
Number of tender joints	5.8 ± 1.8	.17	5.1 ± 2.1	.18	5.3 ± 2.0	.56
Number of swollen joints	4.2 ± 1.2	.63	4.9 ± 2.3	.38	4.7 ± 2.0	.48
Visual analog scale, patient global health, 0-100 mm	56.5 ± 17.4	.97	58.2 ± 18.5	.27	57.1 ± 17.8	.49
Heart rate, beats/min	68.4 ± 5.6	.30	67.1 ± 5.3	.47	68.8 ± 5.9	.17
Body mass index, kg/m^2	28.8 ± 2.3	.11	27.9 ± 3.1	.61	28.5 ± 2.6	.60
Age, y	49.3 ± 5.3	.01	45.9 ± 4.9	.52	46.6 ± 4.1	.01

Note: Three distinct comparisons were performed.

Data are shown as mean ± SD.

Abbreviations: ACPA, anti-citrullinated protein antibodies; DAS28-CRP, Disease Activity Score of 28 joints – C-reactive protein.

RA disease activity was found related to cardiovascular events irrespective of conventional risk factors. Higher disease activity was associated with increased odds of acute coronary syndrome, 53% increased risk of CV events, 33% higher risk of CVD.[21-23] Also, significant correlation was previously observed between RA disease activity and cardiac left ventricular functions in new onset treatment-naive RA.[10] Autoantibodies of RA (ACPA and RF) are evidently pathogenic and are not just innocent bystanders. Dual seropositive RA patients usually had destructive and severe disease. Furthermore, both ACPA and RF can be found 3 or 4 years prior to the first clinical signs of RA.[24-26] Proposed mechanisms of increased risk of CVD in RA include increased systemic inflammation and inflammatory mediators, post-translational modifications of peptides/proteins and immune responses against these altered peptides/proteins, oxidative stress-related myocyte dysfunction, interstitial fibrosis and impaired perfusion due to endothelial dysfunction.[7] Moreover, these oxidative stress and inflammatory processes might lead to cardiomyocyte necrosis, with subsequent structural and electrical remodeling. Lastly, ACPA and RF mediated cellular events may inhibit cardiac conductive and pacemaker activity and lead to cardiac arrhythmia.[27]

The present study was meticulously designed to establish the very early effects of high RA disease severity on cardiac functions of echocardiographic and ECG parameters. Conventional cardiac ventricle functions and arrhythmia parameters in patients and the control cohort were normal. However, there was linkage to disease severity and the development of decreased LV systolic function (determined by LVGLS), altered electrical intervals (determined by cQT and Tp-e/cQT) documented in severe RA patients who had both ACPA, RF positivity and high disease activity in comparison to patients with lower disease severity. In a previous study higher disease activity was found to be associated with lower LV systolic myocardial function in active vs remission RA measured by LVGLS, and also the active group included more RF positive patients.[28] LV strain was decreased in treatment-naive RA patients in parallel with ACPA titers who classified as intermediate, low titers and ACPA negatives.[10] This result negatively correlates to our study but the difference in grouping of patients, higher mean LVGLS and age of the patient cohort of the above-mentioned study might be the causes of different results. According to the literature, age has been reported as one of the major determinants of subclinical atherosclerosis in different types of arthritis.[29] Even though our severe RA group patients had

TABLE 3 Echocardiographic, electrocardiographic and rheumatological parameters dichotomized in relation to severe rheumatoid arthritis (ACPA, RF positive and DAS28-CRP ≥ 5.1) patients vs non-severe rheumatoid arthritis patients

Parameters	Severe rheumatoid arthritis n = 23	Non-severe rheumatoid arthritis n = 54	*P*
Left ventricle ejection fraction, %	63.57 ± 2.34	63.33 ± 2.39	.75
Left ventricle end diastolic volume, mL	120 ± 7.77	120 ± 5.23	.23
Left ventricle end systolic volume, mL	46 ± 4.47	46 ± 1.77	.30
S' global, cm/s	10.5 ± 0.76	10.5 ± 1.06	.35
E/A ratio	1 ± 0.17	1 ± 0.06	.50
Left ventricle global longitudinal strain, %	17.98 ± 1.24	21.29 ± 1.03	<.001
Estimated pulmonary artery pressure, mmHg	28.14 ± 5.02	27.76 ± 2.76	.68
Tricuspid annular plane systolic excursion, cm	19.36 ± 1.28	19.48 ± 1.35	.72
cQT, ms	428.71 ± 9.05	394.61 ± 17.83	<.001
Tp-e/cQT, ms	0.19 ± 0.02	0.16 ± 0.01	<.001
Duration of symptom onset, mo	6.29 ± 3.97	5.55 ± 2.21	.75
Number of tender joints	4.64 ± 1.74	5.88 ± 2.13	.03
Number of swollen joints	5.29 ± 2.55	4.18 ± 1.1	.08
Visual analog scale, patient global health, 0-100 mm	58.71 ± 17.3	57.7 ± 18.06	.63
Heart rate, beats/min	68.43 ± 5.56	67.79 ± 5.46	.76
Body mass index, kg/m^2	28.29 ± 3.22	28.82 ± 2.56	.42
Age, y	46.07 ± 3.95	50.18 ± 6.01	.02

Note: Data are shown as mean ± SD.

Abbreviations: ACPA, anti-citrullinated protein antibodies; DAS28-CRP, Disease Activity Score of 28 joints – C-reactive protein; RF, rheumatoid factor.

TABLE 4 Penalized maximum likelihood estimation logistic regression

Variables	Odds ratio[a]	95% CI[a]	*P* value
Left ventricle global longitudinal strain	0.70	0.52-0.92	.001
Tp-e/cQT	0.65	0.11-5.33	.49
Age	0.92	0.81-1.03	.89
Visual analog scale, patient global health	0.98	0.92-1.46	.95
cQT	0.60	0.10-5.19	.59
Number of swollen joints	1.01	0.74-1.39	.50
Number of tender joints	0.99	0.75-1.29	.16

[a]Penalized logistic regression adjusted.

significantly lower LVGLS values as compared to the non-severe group, all values were within normal limits (higher than 15.9%).[30] Also, they were independent from both LV diastolic and RV functions. The prolongation of both cQT and Tp-e intervals and Tp-e/cQT ratio were formerly established as ECG predictors for increased risk of arrhythmia and sudden death in the general population. Contemporary association was observed between cQT and CRP levels, and would suggest that the prolonged cQT interval was driven by a high inflammatory burden.[31,32] The Tp-e interval and Tp-e/cQT ratio were also increased in RA patients.[33]

Long-term effects of RA on the CV system has been well documented in recent studies, reviews and meta-analyses.[7,9,28,33,34] Moreover, in 2 very recent reports, inflammation was found a consistent and independent predictor of coronary atherosclerosis progression, myocardial blood flow reduction and LV remodeling.[35,36] However, patients in these studies had disease duration 3 to 20 years. In addition, none of the cardiac markers had prognostic significance in early stage RA disease. We implemented multivariate analysis to reveal whether the evaluated cardiac parameters could predict disease severity. Unlike LVGLS, cQT and Tp-e/cQT was found not associated with RA disease severity. We demonstrated that LVGLS, as a marker of systolic dysfunction, was an independent predictor of RA disease severity (patients with RF and ACPA positivity and DAS28-CRP value ≥ 5.1) with 88.8% PPV. In newly diagnosed RA patients, ECG strain assessment could be performed for prediction of disease severity.

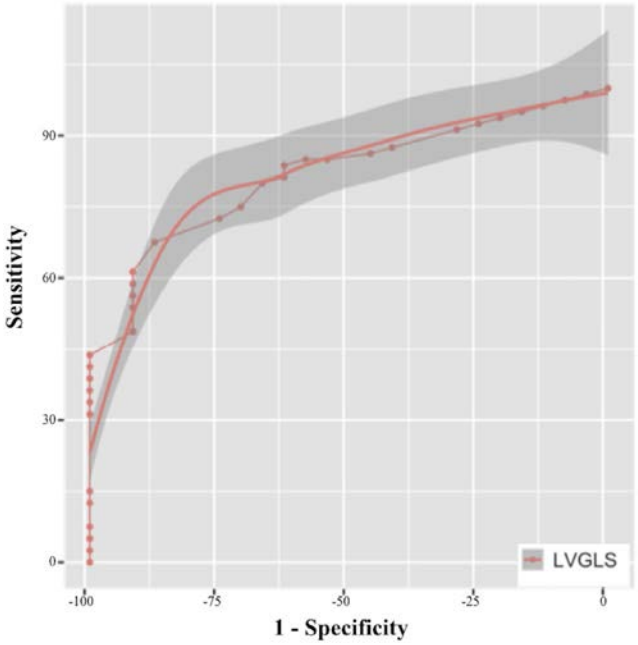

FIGURE 1 Receiver operating characteristic (ROC) curves of the left ventricle global longitudinal strain (LVGLS) for prediction of severe rheumatoid arthritis disease

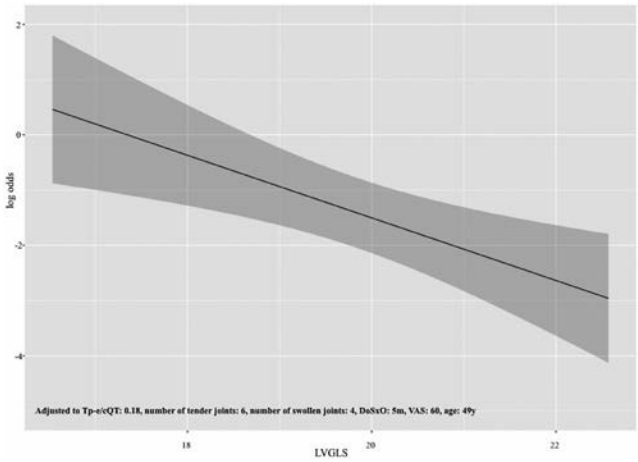

FIGURE 2 Partial effect plot of the left ventricle global longitudinal strain (LVGLS). DoSxO, duration of symptom onset, VAS, visual analog scale, patient global health

Our study has several limitations. First, a limited number of patients was enrolled to the study, this was the result of rigorous inclusion criteria to ascertain that the patients had no diabetes, hypertension or CVD; however, all patients were treatment-naive and newly diagnosed. Our findings therefore require confirmation in larger studies and with an adequate follow-up. Second, circumferential and radial strains were not studied in our patients as these parameters are less reproducible than longitudinal strain. Third, we excluded subclinical CVD by symptoms of the patient, treadmill stress test and ECG. This might have caused us to miss some CVD diagnoses.

5 | CONCLUSION

ACPA and RF positivity with higher disease activity was associated with lower LV systolic myocardial function and increased arrhythmia parameters. Only LVGLS was a significantly independent predictor of RA disease severity. However, the importance of our findings should be proven in prospective outcome studies.

CONFLICT OF INTEREST

All of the authors have no conflict of interest.

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Subclinical atherosclerosis in systemic sclerosis: Different risk profiles among patients according to clinical manifestations

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Abstract

Introduction: Like other autoimmune diseases, systemic sclerosis (SSc) has been described to be associated with accelerated atherosclerosis (ATS). Before clinical manifestations of cardiovascular disease (CVD) occur, subclinical ATS can be investigated in different ways.

Aim: To evaluate the presence of subclinical ATS in a group of patients with SSc, and to identify different risk profiles among patients.

Methods: Subclinical ATS was reviewed in 43 SSc patients and 27 healthy controls, using 2 methods: carotid ultrasound and flow mediated dilation (FMD) of the brachial artery.

Results: Plaques were statistically more frequent in SSc patients than in controls (65% vs 30%, $P = .006$); intima-media thickness of common carotid artery (CCA-IMT) resulted in statistically higher (median value 0.8 mm vs 0.55 mm; $P < .0001$) while FMD was significantly lower (median value 9% vs 14%; $P = .0086$) in patients compared to healthy controls. Among the SSc patients, thickening of CCA-IMT was significantly associated with the presence of diastolic dysfunction of left ventricle (absence of diastolic dysfunction: odds ratio [OR] 0.2, 95% CI 0.04-0.92, $P = .038$) and with a higher Framingham score (OR 1.3, 95% CI 1.03-1.6], $P = .024$). The diffuse cutaneous form was slightly protective against pathological FMD (OR 0.12, 95% CI 0.022-0.71, $P = .019$).

Conclusions: This study confirms the involvement of macrocirculation in SSc patients, detecting the presence of subclinical ATS markers more frequently in patients compared to healthy controls. Framingham score, diastolic dysfunction of left ventricle and limited cutaneous form of the disease appeared to be associated with a higher risk of developing ATS.

KEYWORDS

atherosclerosis, carotid ultrasound, flow mediated dilatation, microcirculation, systemic sclerosis

1 | INTRODUCTION

Systemic sclerosis (SSc) is a connective tissue disease characterized by early vascular involvement. Although the typical vasculopathy of SSc occurs mainly at small vessel levels, with endothelial dysfunction being a relevant component, diffuse cardiovascular disease (CVD) with an increased risk of atherosclerosis (ATS) is also described, as in a large part of other autoimmune diseases (eg, rheumatoid arthritis, systemic lupus erythematosus, systemic vasculitis) leading to higher morbidity and mortality.[1-3] For this reason the term "accelerated ATS" has been created because of the interaction of traditional and non-traditional risk factors (ie, inflammation, increased lipid oxidation, anti-oxidized low-density lipoprotein autoantibody production, endothelial dysfunction, glucocorticoid assumption) in the development of ATS in autoimmune diseases..[1,2,4]

The frequency of death due to CVD in SSc was estimated around 29% of patients by the European Scleroderma Trials and Research group database,[5] and in SSc patients with ATS mortality was higher when compared both to SSc patients without ATS and to patients affected by systemic lupus erythematosus or rheumatoid arthritis with CVD.[3] A recent study showed an overlap between pulmonary arterial hypertension (PAH) and coronary artery disease in SSc patients, suggesting the need of a more invasive approach with right heart catheterization and coronary angiography to symptomatic patients in order to better define the type of cardiac involvement.[6] A systematic review with meta-analysis, dealing in macrovascular involvement in SSc, showed that although different methods have been used to evaluate the presence of subclinical ATS, such as carotid ultrasound, flow mediated dilatation (FMD), nitroglycerin mediated dilatation, pulse wave velocity, augmentation index and ankle-brachial pressure index, the greater number of authors confirms a more frequent macrovascular involvement in SSc patients with respect to controls, with an increased number of atherosclerotic plaques.[7] Therefore a complete CV risk assessment of patients is warranted.[8] Plaques have been detected in SSc patients even without increased IMT. Frerix showed that even in the absence of IMT thickening, plaques could anyway be present mostly in the bulb, and that patients with plaques had a high relative risk to develop CVD.[9] It has been also described that SSc patients show an endothelial dysfunction, pointing out the decreased FMD of the brachial artery in comparison to healthy controls.[10] FMD has been found to inversely correlate with microvascular damage, thus being significantly decreased in patients with late scleroderma pattern at nailfold capillaroscopy.[11] Ozen and colleagues demonstrated that subclinical ATS was frequent in patients with SSc as well as in patients with rheumatoid arthritis and that it was not detected by the traditional CV risk indices. These authors found that subclinical atherosclerosis was associated with older age, elevated erythrocyte sedimentation rate and with PAH.[12] In a more recent comparative study, carotid IMT and pulse wave velocity were measured in 2 SSc and rheumatoid arthritis matched cohorts. The results showed that subclinical ATS was comparable between SSc patients and rheumatoid arthritis patients.[13] Age, anti-centromere antibody (ACA) positivity and higher cumulative dose of glucocorticoids have been described to be associated with a higher risk to develop early ischemic events and premature ATS.[14,15]

Although macrovascular involvement seems to be clear in SSc, the heterogeneity of the methodology used to perform studies determines discordant results. For this reason further investigations are needed.[16]

The aim of this study is to evaluate the presence of subclinical ATS in a group of SSc patients, using non-invasive methods, and to identify any possible association between ATS and clinical features.

2 | MATERIALS AND METHODS

Forty-three consecutive SSc patients and 27 healthy controls, with no statistically significant difference in age, gender and traditional CV risk factors, were enrolled. The diagnosis of SSc was formulated according to the European League Against Rheumatism/ American College of Rheumatology 2013 criteria.[17] Exclusion criteria were represented by a positive history for CV or cerebrovascular events, and by a previously diagnosed CVD. The study received ethics approval by the local institutional review board (IRB): IRB code n°416/11 (Ethic Committee of Policlinico Umberto I of Rome). All subjects gave their informed consent and underwent a complete clinical and laboratory assessment, including cardiac (through electrocardiography and echocardiography), pulmonary (through respiratory function test, chest radiography or pulmonary computed tomography scan) and disease activity assessment (using the European Scleroderma Study Group-EScSG activity index).[18] Traditional CV risk factors, such as family history, obesity, smoking, diabetes, dyslipidemia, arterial hypertension, were also investigated. General CV risk was estimated using both Framingham score and SCORE (Systematic Coronary Risk Evaluation) index, because the latter is representative of the European population.[19,20]

Microcirculatory abnormalities were detected by nailfold videocapillaroscopy and classified into the proper pattern: early, active, late or not specific pattern.[21] Pharmacological treatments at the moment of the enrolment were recorded.

To evaluate the presence of subclinical ATS, patients and controls underwent carotid B-mode ultrasonography and non-invasive measurement of endothelial function, measuring brachial artery diameter variation after application of shear stress (FMD).[22] To measure carotid IMT (c-IMT) and to detect the presence of carotid plaques (c-plaques) carotid ultrasound was used. IMT has been measured at the level of the back wall of the common carotid artery (CCA), 1 cm proximal to the bulb. The mean value of left and right measures was obtained, and thickening of IMT was defined when IMT was ≥0.9 mm, according to 2011 Mannheim consensus.[23] According to the same consensus, c-plaque was defined as a focal structure projecting into the lumen of at least 0.5 mm of thickness or 50% of the surrounding IMT value, or demonstrating a thickness >1.5 mm as measured from the media-adventitia interface to the intima lumen interface. The presence of plaques was explored in the CCA, bulb and internal carotid artery. To measure

TABLE 1 Clinical-demographic and laboratory features of 43 systemic sclerosis patients, including ongoing treatments

Parameters	Values
Gender (M/F)	4/39
Age (y) (median, IQR)	66, 16
Cutaneous form (limited/diffuse)	30/13
Disease duration (y) (median, IQR)	10, 8
Raynaud's phenomenon (n/%)	43/100
Puffy hands (n/%)	21/49
mRSS (median, IQR)	7, 6
Digital ulcers (n/%)	10/23
Pitting scars (n/%)	16/37
Calcinosis (n/%)	6/14
PAH (n/%)	1/2
ILD (n/%)	20/47
EF < 55% (n/%)	6/14
Diastolic dysfunction (n/%)	15/35
Conduction abnormalities (n/%)	4/9
Gastrointestinal involvement (n/%)	28/65
EScSG activity index ≥ 3 (n/%)	6/14
Early NVC pattern (n/%)	5/12
Active NVC pattern (n/%)	15/35
Late NVC pattern (n/%)	20/47
Non-specific NVC pattern (n/%)	3/7
Calcium channel blockers (n/%)	40/93
Acetyl salicylic acid (n/%)	32/74
DMARDs (n/%)	17/40
Intravenous prostanoids (n/%)	22/51
Glucocorticoids (n/%)	15/35
Lipid-lowering drugs (n/%)	10/23
ERAs (n/%)	8/19
Anti-PDE5 (n/%)	4/9
ESR (mm/1 h) (median, IQR)	24, 18
CRP (mg/dL) (median, IQR)	0.3, 0.4
ANA positive (n/%)	43/100
Anti-topo I Ab positive (n/%)	13/30
ACA positive (n/%)	21/49
Anti-RNA polymerase 3 Ab positive (n/%)	1/2
Anti-CL Ab positive (n/%)	5/12
Anti-β2GPI Ab positive (n/%)	7/16
Lupus anticoagulant (n/%)	4/9
Total cholesterol (mg/dL) (median, IQR)	217, 54.5
HDL cholesterol (mg/dL) (median, IQR)	61, 23.2
LDL cholesterol (mg/dL) (median, IQR)	126, 56
Triglycerides (mg/L) (median, IQR)	96, 42

Abbreviations: ACA, anti-centromere antibodies; ANA, anti-nuclear antibodies; Anti-CL Ab, anti-cardiolipin antibodies; anti-PDE5, phosphodiesterase 5 antagonists; Anti-Topo I Ab, anti-topoisomerase I antibodies; Anti-β2GPI Ab, anti-β2 glycoprotein I antibodies; CRP, C-reactive protein; DMARDs, disease-modifying anti-rheumatic drugs; EF, ejection fraction; ERAs, endothelin-receptor antagonist; EScSG, European Scleroderma Study Group activity index; ESR, erythrocyte sedimentation rate; Gastrointestinal involvement, esophageal reflux and small intestinal bacterial overgrowth; HDL, high-density lipoproteins; ILD, interstitial lung disease; IQR, interquartile range; LDL, low-density lipoproteins; mRSS, modified Rodnan skin score; NVC, nailfold videocapillaroscopy; PAH, pulmonary arterial hypertension; Prostanoids, prescribed for severe Raynaud's phenomenon, digital ulcers or PAH.

TABLE 2 Cardiovascular risk factors and ultrasound features of systemic sclerosis patients and healthy controls

Features	Patients (N = 43)	Healthy controls (N = 27)	*P*
Age (y) (median, IQR)	66, 16	56, 21	.23
Gender (M/F)	4/39	7/20	.09
Hypercholesterolemia (n/%)	11/26	7/26	1
Arterial hypertension (n/%)	23/53	11/41	.33
Type II DM (n/%)	2/5	2/7	.63
Obesity (n/%)	4/9	4/15	.7
Current smoking habits (n/%)	9/22	5/19	1
Plaques n/%	28/65	8/ 30	.006
CCA-IMT (mm) (median, IQR)	0.8, 0.2	0.55, 0.11	<.0001
FMD (%) (median, IQR)	9, 11	14, 6	.0086

Abbreviations: CCA-IMT, intima-media thickness of common carotid artery; DM, diabetes mellitus; FMD, flow mediated dilation; IQR, interquartile range.

FMD, a compression of the forearm was applied by sphygmomanometer for 5 minutes, followed by rapid decompression. FMD was calculated as the percentage difference between the maximum post-ischemic diameter of the brachial artery and the mean basal diameter. Normal values were defined when FMD > 10%.[24] For both techniques a 7-10 Mhz ultrasound probe was used (ESAOTE GPX).

Statistical analysis was performed using the program IBM-SPSS Statistics version 25. Significance threshold was set at $P < .05$.

3 | RESULTS

Our group of 43 SSc patients was represented by 39 females and 4 males, with a median age of 66 years and a median disease duration of 10 years from the first non-Raynaud symptom. Thirty patients had a limited form of the disease and 13 a diffuse one, while 6 (14%) patients had an active disease as defined by the EScSG activity index. All the patients were positive for anti-nuclear antibodies, 21 (49%) were ACA positive, 13 (30%) were anti-topoisomerase I

positive and 1 (2%) was anti-RNA polymerase 3 positive. The main clinical-demographic and laboratory features, including ongoing treatments, of the SSc patients are listed in Table 1.

The most frequent CV risk factor among patients was arterial hypertension, present in 23 cases (53%), while 16 (37%) of them reported actual or former smoking habits, 12 (28%) had family history for CV events, and 11 (26%) presented with dyslipidemia. The majority of the patients were within normal range of weight (median body mass index 24, interquartile range 6); only 2 patients had type II diabetes (1 patient with the limited form of the disease, the other with the diffuse form of the disease). Considering the subgroup of patients with limited form of the disease (30 patients), arterial hypertension and smoking habits were slightly more frequent compared to the overall patients group (respectively 60% and 46.6%). However, prevalence of traditional CV risk factors was not statistically different between SSc patients and healthy controls, as well as between limited SSc patients and diffuse SSc patients. Carotid B-mode ultrasonography showed plaques in 28 SSc patients (65%), multiple in 9 of them. Plaques were statistically more frequent in SSc patients compared to control subjects (65% vs 30%, $P = .006$). IMT in CCA was statistically higher in patients than in controls (median value 0.8 mm vs 0.55 mm; $P < .0001$) while FMD was significantly lower compared to healthy controls (median value 9% vs 14%; $P = .0086$). Data are shown in Table 2. Fisher's test found that the risk to develop carotid plaques was almost 4 times higher among patients compared to control subjects (odds ratio [OR] 4.43, 95% CI 1.57-12.5). The risk to develop a thickening of carotid IMT was almost 22 times higher in patients compared to controls (OR 22.6, 95% CI 2.81-181.92), while the risk to develop a reduction of FMD was almost 5 times in patients compared to controls subjects (OR 4.61, 95% CI 1.47-14.42).

Vascular ultrasound parameters of the patients have been compared with their clinical and laboratory data. The presence of plaques was found to be statistically associated with arterial hypertension, left ventricle diastolic dysfunction (LVDF) and SCORE as well as with Framingham indices. LVDF was defined as an impaired LV relaxation due to myocardial fibrosis and has been assessed according to recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging.[25] No other statistical differences with the examined features were observed.

SSc patients with IMT thickening in CCA were significantly older than patients with normal IMT, had a higher SCORE index and showed more frequently a diastolic dysfunction of the left ventricle. SSc patients with reduced FMD had more frequently a limited cutaneous form of the disease as well as puffy hands. They showed a lower modified Rodnan skin score and less frequently had interstitial lung disease. Moreover they used less frequently lipid-lowering drugs and intravenous prostanoids. These results are showed in Table 3.

No association among the ultrasound features and the autoantibody profiles or the nailfold videocapillaroscopic features of the patients was found.

Logistic regression showed that thickening of CCA-IMT was associated with diastolic LV dysfunction and with Framingham score. In particular, absence of diastolic dysfunction of LV resulted to be slightly protective against the thickening of CCA-IMT (OR 0.2, 95% CI 0.04-0.92, $P = .038$), while Framingham score resulted to be a risk factor (OR 1.3, 95% CI 1.03-1.6, $P = .024$). As regards the reduction of FMD, the diffuse cutaneous form of the disease resulted to be slightly protective against its occurrence (OR 0.12, 95% CI 0.02-0.7, $P = .019$). Goodness of fit has been evaluated with Hosmer and Lemeshow's test, as shown in Table 4.

4 | CONCLUSIONS

Association among subclinical ATS and chronic inflammatory rheumatic diseases is now largely supported in the scientific literature,[26] and is related to interaction of traditional CV risk factors and disease specific factors such as inflammation, oxidative stress and autoantibody production.[1,2,26] Although the hallmark of SSc is the involvement of microcirculation, epidemiological studies show an increasing frequency of death due to cardiovascular events.[27-29]

Our study confirms that subclinical ATS is more frequent in SSc patients compared to healthy controls, despite similar rates of traditional CV risk factors. Carotid plaques were mainly localized in the bulb, as reported by Frerix and colleagues.[9] Patients with carotid plaques had more frequently arterial hypertension and LV diastolic dysfunction as well as higher values of CV risk score (Framingham and SCORE). Similarly, carotid IMT thickening was more frequent in patients with older age, LV diastolic dysfunction and higher SCORE index. No significant association with any disease feature has been detected. Brachial artery FMD was significantly reduced in SSc patients compared to the control group, especially among patients with the limited cutaneous form of the disease.

With regard to nailfold videocapillaroscopic features, as mentioned above, we could not find any association with ultrasound characteristics although quite recently other authors demonstrated that arterial stiffness indices (namely augmentation index) were significantly higher in patients with late and active nailfold capillaroscopy pattern compared to patients with the early one.[30]

Our findings support data from the literature, where treatment with prostacyclin-like drugs and statins were protective toward endothelial dysfunction, both in SSc and in non-autoimmune disease.[31-33] In fact we found a statistically significant less frequent endothelial dysfunction in patients taking intravenous prostacyclin and in those treated with statins, although the statistical significance was not confirmed in the multivariate analysis. We did not find association between subclinical ATS and corticosteroid cumulative intake, as reported in another study.[15]

Although SSc is a rare disease, the small number of patients and healthy controls enrolled represents a limitation of the study, which could be considered as a pilot one. Moreover age differences between patients and controls group, although substantial, was not

TABLE 3 Clinical-demographical and laboratory features of patients according to ultrasound parameters

Features	SSc patients with plaques (n = 28)	SSc patients without plaques (n = 15)	*P*	SSc patients with CCA-IMT ≥ 0.9 (n = 16)
Age (y) (median, IQR)	67, 12	60, 17	.06	68, 6
Gender (M/F)	4/24	0/15	.28	1/15
Disease duration (y) (median, IQR)	9, 6	12, 9	.19	11, 8
Diffuse/limited cutaneous form	7/21	6/9	.32	3/13
BMI (median, IQR)	23, 5	25, 4	.84	25, 6
Arterial hypertension (n/%)	19/68	4/27	**.013**	11/69
Diabetes (n/%)	2/7	0/0	.53	2/13
Actual or former smoking habit (n/%)	12/43	4/27	.34	8/50
SCORE index (median, IQR)	2, 2	1, 2	**.02**	2, 2
Framingham score (median, IQR)	6, 5	2, 4	**.008**	5, 3
FMD % (median, IQR)	9, 10	11, 12	.6	9, 10
CCA-IMT mm (median, IQR)	0.8, 0.1	0.7, 0.2	.25	-
ILD (n/%)	11/39	9/60	.21	8/50
PAH (n/%)	¼	0/0	1	0/0
EF < 55% (n/%)	5/18	1/7	0.4	1/6
Diastolic dysfunction of LF (n/%)	13/46	2/20	**.04**	9/56
Conduction abnormalities (n/%)	4/14	0/0	.28	0/0
Puffy fingers (n/%)	16/57	5/33	.2	6/38
mRSS (median, IQR)	7, 4	10, 11	.12	7, 4
Total cholesterol mg/dL (median, IQR)	220, 68	213, 43	.88	215, 65
HDL cholesterol mg/dL (median, IQR)	62, 21	59, 34	.95	63, 21
LDL cholesterol mg/dL (median, IQR)	126, 70	126, 33	.5	131, 75
TG mg/dL (median, IQR)	98, 38	85, 42	.09	96, 43
DMARDs (n/%)	10/36	7/47	.52	4/25
GC (n/%)	11/39	4/27	.51	4/25
Cumulative dose GC g (median, IQR)	6, 11	8, 22	.69	8, 25
Lipid-lowering drugs (n/%)	7/25	4/27	1	7/44
Intravenous prostanoids (n/%)	12/43	10/67	.2	9/56
Calcium channel blockers (n/%)	26/93	14/93	1	15/94
ERAs (n/%)	7/25	1/7	.22	3/19
Anti-PDE5 (n/%)	2/7	2/13	.6	1/6
ASA (n/%)	21/75	11/73	1	11/69

Statistically significant values ($P < .05$) are in bold.

Abbreviations: anti-PDE5, anti-phosphodiesterase 5 antagonists; ASA, acetylsalicylic acid; BMI, body mass index; CCA-IMT, intima-media thickness of common carotid artery; DMARDs, disease-modifying anti-rheumatic drugs; EF, ejection fraction; ERAs, endothelin-receptor antagonist; EScSG, European Scleroderma Study Group; FMD, flow mediated dilation; GC, glucocorticoids; GI, gastrointestinal; HDL, high-density lipoproteins; ILD, interstitial lung disease; IQR, interquartile range; LDL, low-density lipoproteins; LF, left ventricle; mRSS, modified Rodnan skin score; PAH, pulmonary arterial hypertension; SCORE, Systematic Coronary Risk Evaluation; SSc, systemic sclerosis; TG, triglycerides.

statistically significant, as well as the frequencies of traditional CV risk factors. Nevertheless it can represent a possible limit of our study and a better age matching should be sought.

The strength of this study is the investigation for possible association between subclinical ATS and disease related features. Patients with LV diastolic dysfunction and/or with the limited cutaneous form

SSc patients with CCA-IMT < 0.9 (n = 27)	*P*	SSc patients with FMD ≤ 10 (n = 23)	SSc patients with FMD > 10 (n = 20)	*P*
60, 18	**.001**	66, 13	67, 14	.8
3/24	1	2/21	2/18	1
10, 8	.19	10, 7	11, 11	.77
10/17	.3	2/21	11/9	**.002**
24, 5	.57	23, 6	24, 4	.72
12/44	.2	14/61	9/45	.36
0/0	.13	1/4	1/5	1
8/30	.2	9/39	7/35	1
1, 2	**.022**	2, 2	1, 1	0.24
4, 7	.17	5, 6	4, 4	.62
10, 11	.9	-	-	-
-	-	0.9, 0.2	0.8, 0.2	.09
12/44	.76	7/30	13/65	**.033**
1/4	1	1/4	0/0	1
5/19	.38	2/9	4/20	.39
6/22	**.04**	10/44	5/25	.33
4/15	.27	1/4	3/15	.32
15/56	.34	15/65	6/30	**.032**
8, 7	.56	7, 4	10, 11	**.017**
224, 47	.84	226, 46	201, 55	.23
59, 30	.65	62, 30	59, 21	.43
126, 41	.58	128, 51	119, 52	.32
98, 38	.94	98, 33	90, 46)	.98
13/48	.19	7/30	10/50	.22
11/41	.34	8/35	7/35	1
6, 11	1	5, 14	5, 10	.72
4/15	.06	2/9	9/40	**.027**
13/48	.75	7/30	15/75	**.0058**
25/92	1	22/96	18/90	.59
5/19	1	2/9	6/30	.11
3/11	1	2/9	2/10	1
21/78	.71	20/87	12/60	.07

of the disease seem to represent a subset at higher risk to develop ATS, while intravenous prostanoids and lipid-lowering drugs could be useful to modulate endothelial function both in small and large vessels. These preliminary data, obtained from a limited cohort of patients, must be confirmed with a larger study, in order to perform a stronger analysis.

TABLE 4 Results from logistic regression model. Goodness of fit evaluated with Hosmer and Lemeshow's test

Carotid plaques	
Variables	**OR**
No hypertension	0.24
No diastolic dysfunction	0.2
Framingham score	1.26
SCORE index	1.26
Age	0.97
Female gender	0
Thickening of carotid IMT	
Variables	**OR**
Female gender	95.4
Age	1.07
No diastolic dysfunction	0.2
Framingham score	1.3
SCORE index	1.26
FMD < 10	
Variables	**OR**
Age	0.94
Female gender	9.6
No puffy hands	0.24
mRSS	0.95
No ILD	0.78
No Iloprost treatment	3.7
Diffuse cutaneous form	0.12

Abbreviations: FMD, flow mediated dilation; ILD, interstitial lung disease; IMT, intima-media thickness; mRSS, modified Rodnan skin score; OR, odds ratio; SCORE, Systematic Coronary Risk Evaluation.

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AUTHORS' CONTRIBUTIONS

IS and MV equally contributed to the work and share the first authorship. IS, MV and VR conceived of the study. AGS and GV participated in the design of the study. AC performed ultrasonographic evaluations. IS and MV collected the clinical data of the patients and performed clinical capillaroscopic examination. KS and NI collected the data of follow up. KS and CA performed the literature review. IS established the database and performed the statistical analysis. IS and MV drafted the manuscript. VR helped to draft the manuscript, which was critically revised by GV. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

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Left ventricular hypertrophy predicts poorer cardiovascular outcome in normotensive normoglycemic patients with rheumatoid arthritis

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Abstract

Introduction: Patients with rheumatoid arthritis (RA) develop early changes in left ventricular (LV) geometry and experience cardiovascular events in excess than in the general population. This study was designed to assess prevalence, predictors and prognostic role of LV hypertrophy (LVH) in a selected group of RA patients with normal blood pressure and glycemia who should be at low risk for LVH.

Methods: We prospectively analyzed 241 normotensive normoglycemic RA patients (mean age 53 ± 12 years, 61% women) involved in a primary prevention program for cardiovascular diseases who were followed-up for 40 (24-56) months. LVH was detected by echocardiography and defined as LV mass ≥49.2 g/m$^{2.7}$ for men and ≥46.7 g/m$^{2.7}$ for women. Primary outcome was a composite of cardiovascular death/hospitalization.

Results: LVH was detected in 39 patients (16%). Older age (>53 years), greater body mass index (BMI > 25 kg/m^2), longer duration of RA disease, anti-cyclic citrullinated peptide antibody (ACPA) positivity and concentric LV geometry were the variables associated with LVH. During the follow-up, a cardiovascular event occurred in 12 of 39 (31%) patients with LVH and in 22 of 202 (11%; $P < .001$) patients without LVH. LVH independently predicted cardiovascular events (hazards ratio 3.28 [95% CI 1.03-9.20], $P = .03$) at Cox regression analysis together with C-reactive protein and ACPA positivity.

Conclusions: Nearly one-sixth of normotensive normoglycemic RA patients analyzed in a primary prevention program for cardiovascular diseases has LVH which is associated with obesity and older age, and strongly predicts cardiovascular event in these subjects.

KEYWORDS

ACPA, cardiovascular risk, left ventricular hypertrophy, prognosis, rheumatoid arthritis

1 | INTRODUCTION

Left ventricular hypertrophy (LVH) is the main adaptive process that the human heart puts in place to respond to physiologic (physical exercise, state of pregnancy) or pathologic stimuli triggering left ventricular (LV) mass growth. These stimuli are largely represented by arterial hypertension and type 2 diabetes mellitus,[1,2] 2 of the most common causes of LVH together with overweight and obesity in the clinical setting.[3-5]

As a rule, LVH develops and progresses for long time in an asymptomatic way and predicts poorer cardiovascular (CV) outcomes in the general population[6] as well as in several settings of patients at increased risk for CV events.[1-3,7-9]

In patients with rheumatoid arthritis (RA), even when evaluated fairly early in a context of primary prevention, some maladaptive cardiac changes including concentric remodeling, LVH and/or dysfunction have been documented,[10,11] particularly whereas arterial hypertension and/or diabetes mellitus coexist. However, in clinical practice, LVH is often detected in patients with RA who have none of these pathologies. Non-hemodynamic reasons (mainly including chronic inflammation) may be possible predisposing conditions.[12-15] Conflicting information exists on the prevalence and clinical significance of LVH in RA patients.[10,11,16,17]

Accordingly, we designed this study to assess prevalence and factors associated with LVH in a large cohort of normotensive normoglycemic patients with RA involved in a primary prevention program for cardiovascular diseases. Furthermore, we tested the hypothesis that LVH unrelated to hypertension and/or hyperglycemia is associated with increased CV adverse events in these patients.

2 | METHODS

2.1 | Study population

The design of the study was observational prospective. The study population was formed by non-institutionalized subjects >18 years of age with RA diagnosed according to the 2010 American College of Rheumatology / European League Against Rheumatism classification criteria,[18] who had normal blood pressure and serum glucose values at baseline evaluation and were not receiving any anti-hypertensive or anti-diabetic medication. The study population initially consisted of 410 RA patients consecutively referred to our center and considered for this investigation. Hypertension and diabetes mellitus were diagnosed in 40% and 9% of them. Among these 410 patients, 241 (59%) were normotensive normoglycemic, so that they epitomized the definite population of the study. Participants were consecutively recruited from March 2014 to March 2016 at the Division of Rheumatology, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona (Italy) in which patients underwent clinical, laboratory and echocardiographic evaluations as part of a primary prevention program for cardiovascular diseases. Full details of all inclusion and exclusion criteria used for selecting our study population have been published previously.[19] All patients gave written informed consent signing a specific institutional consent form; the study was approved by Ethical Committees of the Verona University and conforms to the ethical guidelines of the Declaration of Helsinki as revised in Brazil 2013.

2.2 | Definitions

Normotensive status was defined as systolic blood pressure <140 mm Hg and/or a diastolic blood pressure <90 mm Hg in absence of anti-hypertensive pharmacological treatment.[20] For evaluating the blood pressure values in all patients we applied the standard "office" blood pressure measurement technique, according to the recommendations of the current international clinical guidelines for the management of arterial hypertension. For this purpose, validated oscillometric or auscultatory semiautomatic sphygmomanometers were used, with all patients kept at 5-minute rest in a sitting position.[20] Normoglycemic status was defined as fasting serum glucose levels <100 mg/dL in absence of anti-diabetic pharmacological treatment or hypoglycemic diet. Obesity was recognized when body mass index (BMI) ≥30 kg/m^2. Dyslipidemia was defined as levels of total serum cholesterol >190 mg/dL and or triglycerides >150 mg/dL or pharmacologically treated high lipid serum levels. To assess renal function we considered the glomerular filtration rate (GFR) estimated with the Chronic Kidney Disease Epidemiology Collaboration equation and defined renal dysfunction as estimated GFR <60 mL/min 1.73 m^2.[21] The RA activity disease was evaluated by the clinical disease activity index (CDAI) score.[22] Patients with a CDAI score >10 were defined as subjects with activated pattern of the disease having moderate-high disease activity, those with CDAI score >22 having high disease activity. Biologic disease-modifying antirheumatic drugs (bDMARDs) refractory disease was identified according to the definition of the British Society of Rheumatology (BSR) as published in the BSR-Biologics Register in Rheumatoid Arthritis.[23] In detail, patients who had been exposed to at least 3 different classes of bDMARD (irrespective of reason for failure to prior bDMARD) were classified as bDMARDs refractory disease.

2.3 | CV Outcome and follow-up

Hospitalizations and vital status were recorded every 3 months during the scheduled visited for clinical check or during hospital access for therapy or by 3-month telephone calls. Follow-up ended on 30 November 2019. The pre-specified outcome of the study was defined as CV death/hospitalization due to both cardiac events (unstable angina, myocardial infarction, paroxysmal atrial fibrillation associated with reduced work capacity/dyspnea, heart failure, severe chest pain due to acute pericarditis, elective percutaneous coronary intervention and coronary artery bypass grafting), and non-cardiac vascular events (stroke, transient ischemic attack, thromboembolism, peripheral vascular intervention and stent thrombosis). For each

patient, the follow-up was stopped at the time of the first hospitalization or death. All clinical events were examined by an independent outcome classification committee. Each clinical event was diagnosed and classified by 2 expert clinicians who analyzed in detail the clinical reports, validated the event and formally generated the information which was migrated into the database.

2.4 | Echocardiography

All Doppler-echocardiographic studies were performed using Alpha Esaote Biomedica machine (Florence, Italy) following a standardized protocol by experienced cardiologists. LV chamber dimensions and wall thicknesses were measured by the American Society of Echocardiography guidelines and LV mass was calculated using a validated formula.[24] LV mass was normalized for height to the 2.7 power and LV hypertrophy was defined as LV mass ≥49.2 g/m$^{2.7}$ for men and ≥46.7 for women.[25] Relative wall thickness was calculated as the ratio 2*end-diastolic posterior wall thickness/LV diameter and indicated concentric LV geometry if ≥0.43 (the 97.5 percentile in a normal population).[26] LV end-diastolic and end-systolic volumes were measured by the biplane method of disks from 2D apical 4 and 2 chamber view and used to calculate LV ejection fraction. Assessment of LV diastolic function was based on widely accepted diastolic function parameters and LV diastolic dysfunction was recognized using validated cut-offs of prognostic relevance, as previously reported.[27]

2.5 | Statistical analysis

Data are reported as mean values ±1 SD (medians and interquartile ranges for asymmetric variables) or percentages. Unpaired Student's test and χ^2 statistics were used for descriptive statistics. Between-group comparisons of categorical and continuous variables were performed by χ^2 test and analysis of variance (ANOVA) with comparison between each group by Scheffè test for unequal samples, as appropriate. The study population was stratified by LVH at baseline. Variables that were significantly related to LVH in univariate tests ($P < .05$) were included in an initial explorative multivariate logistic regression analysis. Receiver operating characteristic (ROC) analyses were performed to find the cut-offs for age and BMI which emerged as the variables independently associated with LVH in this first analysis. Thus, the sub-group with the highest risk for LVH was recognized and characterized by a second multivariate logistic regression analysis which identified the factors independently related to LVH. Log cumulative hazard functions were computed by univariate and multivariate Cox proportional hazards analyses to identify the prognosticators of CV events. Variables that were significantly related to CV events in univariate tests ($P < .05$) were included in the multivariate models and probabilities of event-free survival and Kaplan-Meier survival curves were obtained (differences between the curves were tested for significance by the log-rank test). All analyses were performed using statistical package SPSS 19.0 (SPSS Inc., Chicago, IL, USA) and statistical significance was identified by 2-tailed $P < .05$.

3 | RESULTS

3.1 | Study population and risk factors for LVH

The clinical and echocardiographic features of the 241 normotensive normoglycemic RA patients enrolled into the study are reported in Table 1. Among these 241 patients (mean age of 53 ± 12 years, 61% women), LVH was detected in 39 subjects (16%) who were characterized and compared with the 202 patients without LVH (Table 1). Patients who had LVH were older with higher BMI, waist circumference, serum levels of C-reactive protein, higher prevalence of anti-cyclic citrullinated peptide antibodies (ACPA) positivity and LV diastolic dysfunction than patients who had not (univariate analysis).

3.2 | Characterization of patients at higher risk for LVH

The variables included in the first explorative multivariate logistic regression analysis were age, BMI, dyslipidemia, C-reactive protein and ACPA positivity (LV diastolic dysfunction was excluded because this totally depended on LVH). The analysis showed that older age and higher BMI were the independent variables associated with LVH, ACPA positivity showed a borderline statistical significance, while C-reactive protein and dyslipidemia lost statistical significance (Table 2, first model). Taking into consideration age and BMI, ROC analysis indicated that the best predictive cut-offs for LVH were 53 years (area under the curve [AUC] 0.73 [95% CI 0.66-0.81], sensitivity 77%, specificity 65%) and 25.0 kg/m^2 (AUC 0.64 [95% CI 0.54-0.74], sensitivity 67%, specificity 58%). Thus, to better define the sub-group of patients with the highest risk for having LVH, the whole study population was divided into 3 sub-groups according to the presence/absence of these 2 risk factors. Prevalence of LVH was 4% (2 of 55) in patients who had no risk factor, 13% (15 of 118) in those with 1 risk factor and 32% (22 of 68, all $P < .01$) in patients with both risk factors.

Thus, we focused on the 22 patients with both risk factors and the highest risk for having LVH who were compared with the remaining 219 patients at lower risk. Beyond older age (62 ± 10 vs 52 ± 10 years, $P < .001$) and higher BMI (27.8 ± 3.8 vs 23.9 ± 4.6 kg/m^2, $P < .001$), the former had longer duration of RA (15.4 ± 11.9 vs 10.7 ± 8.2 years, $P = .01$), higher prevalence of ACPA positivity (64% vs 38%, $P = .002$) and higher LV relative wall thickness (0.48 ± 0.07 vs 0.43 ± 0.05, $P = .01$) suggesting a higher degree of concentric LV geometry. Thus, a second multivariate logistic regression analysis was performed including age, BMI, disease duration, ACPA positivity and LV relative wall thickness. Duration of disease, ACPA positivity and higher LV relative wall thickness were confirmed to be

TABLE 1 Baseline characteristics of the study population divided into 2 sub-groups according to the presence of left ventricular hypertrophy

Variables	LV hypertrophy Yes 39 patients	LV hypertrophy No 202 patients	*P*	Total study population 241 patients
Age (y)	61 ± 10	51 ± 12	<.001	53 ± 12
Female gender (%)	64	61	.75	61
Body mass index (kg/height^2)	26.4 ± 5.0	23.9 ± 3.7	<.001	24.1 ± 4.3
Waist circumference (cm)	92.6 ± 12.2	87.6 ± 12.1	.02	88.2 ± 12.4
Obese (%)	21	4	<.001	7
Systolic blood pressure (mm Hg)	127 ± 15	124 ± 14	.09	125 ± 14
Diastolic blood pressure (mm Hg)	81 ± 8	80 ± 7	.54	80 ± 8
Smoker (%)	38	38	.89	38
Dyslipidemia (%)	58	43	.04	46
Glycemia (mg/dL)	92 ± 8	91 ± 9	.90	91 ± 9
eGFR (mL/min/m^{2a} 1.73)	95 ± 20	99 ± 20	.16	97 ± 21
Hemoglobin (g/dL)	14.0 ± 1.3	13.8 ± 1.3	.07	13.9 ± 1.3
Cholesterol LDL (mg/dL)	127 [88-162]	123 [87-155]	.50	124 [89-159]
Triglycerides (mg/dL)	119 [80-159]	108 [75-148]	.15	113 [79-151]
C-reactive protein (mg/dL)	4.3 [1.4-8.9]	2.6 [1.0-6.2]	.04	3.6 [1.6-8.5]
ESR (mm/h)	17 [7-28]	16 [8-29]	.73	16 [7-27]
Rheumatoid factor positivity (%)	50	46	.85	47
ACPA positivity (%)	59	42	.05	48
Duration of disease (y)	12.0 ± 9.3	10.5 ± 8.2	.22	11.2 ± 9.0
CDAI	8 [2-16]	8 [1-15]	.87	8 [2-14]
Moderate/high disease activity (%)	23	19	.47	20
High disease activity (%)	5	4	.89	4
DAS28	2.5 ± 1.0	2.1 ± 0.9	.19	2.2 ± 0.8
LV ejection fraction (%)	64 ± 9	66 ± 6	.10	65 ± 6
LV mass (g/h$^{2.7}$)	57.5 ± 7.5	36.8 ± 6.3	<.001	40.1 ± 8.2
LV diastolic dysfunction (%)	28	13	.01	15
Medications				
Statins (%)	8	10	.77	10
Anti-platelets agents (n, %)	6	4	.71	4
NSAIDs (%)	42	33	.32	35
Methotrexate (%)	48	34	.12	33
Hydroxychloroquine (%)	9	7	.73	7
Leflunomide (%)	17	14	.62	15
Corticosteroids (%)	31	50	<.001	37
Biologic DMARDs at enrolment (%)	64	69	.57	68
Biologic DMARDs class				
Anti-TNFα (%)[a]	68	65	.86	69
Anti-interleukin 6 (%)[a]	14	12		13
CTLA 4Ig (%)[a]	13	14		13
Anti-CD 20 (%)[a]	5	6		5
Biologic DMARDs (number of drugs overtime for single patient)	1.75 ± 0.58	1.81 ± 1.57	.84	1.80 ± 1.57
Refractory biologic DMARDs disease (%)	17	16	.86	16

Note: High disease activity, CDAI > 22; Moderate/high disease activity = CDAI > 10.

Abbreviations: ACPA, anti-cyclic citrullinated peptide antibodies; CD, cluster of differentiation; CDAI, clinical disease activity index; CTLA, cytotoxic T-lymphocyte antigen; DAS, Disease Activity Score; DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; LV, left ventricular; NSAIDs, non-steroidal anti-inflammatory drugs; TNF, tumor necrosis factor.

[a]% among patients who were receiving biologic DMARDs.

independent variables associated with LVH (Table 2, second model) (Figure 1).

3.3 | LVH and CV outcome

Analyses were performed by comparison of 39 patients who had LVH versus 202 patients who had not. During the follow-up (median 40 months, interquartile range 24-56 months) an adverse CV event occurred in 34 patients (14.1%): congestive heart failure = 2 (both patients were hospitalized for and died due to heart failure), paroxysmal and symptomatic atrial fibrillation associated with reduced work capacity/dyspnea requiring hospitalization for early cardioversion or heart rate control = 4, myocardial infarct = 3 (all treated with primary percutaneous trans-catheter coronary angioplasty), severe chest pain due to acute pericarditis = 5, unstable angina = 1, elective percutaneous coronary intervention = 2 and coronary artery bypass grafting = 1, stroke = 5, peripheral thromboembolism = 5, transient ischemic attack = 3, acute peripheral ischemia requiring amputation = 3. The rate of CV events was significantly higher in patients with LVH (12 of 39 = 31%) than in those without LVH (22 of 202 = 11%; $P < .001$).

At univariate Cox regression analyses, C-reactive protein, ACPA positivity and LVH were the variables associated with CV events. Multivariate model (adjusted for age, gender and BMI) confirmed LVH as an independent predictor of CV events (hazards ratio [HR] 3.28 [95% CI 1.03-9.20], $P = .03$) together with C-reactive protein and ACPA positivity (Table 3). Figure 2 shows CV event-free survival curves in groups of patients with and without LVH.

TABLE 2 Variables associated with left ventricular hypertrophy (LVH): multiple logistic regression analyses

	HR	CI	*P*
First initial explorative model 39 patients with LVH vs. 202 patients without LVH variables			
Age (y)	1.08	1.03-1.23	.001
Body mass index (kg/m^2)	1.13	1.02-1.25	.02
ACPA positivity (yes/no) (%)	1.10	1.00-1.19	.048
C-reactive protein (mg/dL)	1.02	0.96-1.08	.58
Dyslipidemia (%)	1.73	0.71-4.20	.22
Second model (adjusted for age and body mass index) 22 patients with the highest risk for LVH vs. 219 patients at lower risk variables			
ACPA positivity (yes/no) (%)	2.93	1.11-7.74	.001
Disease duration (y)	1.04	1.00-1.08	.04
Left ventricular relative wall thickness	5.95	1.89-18.72	.002

Abbreviations: ACPA, anti-cyclic citrullinated peptide antibodies; HR, hazards ratio.

4 | DISCUSSION

In this study we searched for LVH in a selected group of normotensive and normoglycemic RA patients involved in a primary prevention program for CV diseases who should have had, on a theoretical basis, a low probability for LVH. LVH was found in nearly one-sixth of patients and strongly predicted CV events at mid-term follow-up. Older age, greater BMI, longer disease duration, ACPA positivity and more concentric LV geometry were the variables associated with the presence of LVH at baseline evaluation.

The main finding of our study consists of the prognostic role that LVH has in RA patients even if stripped from traditional

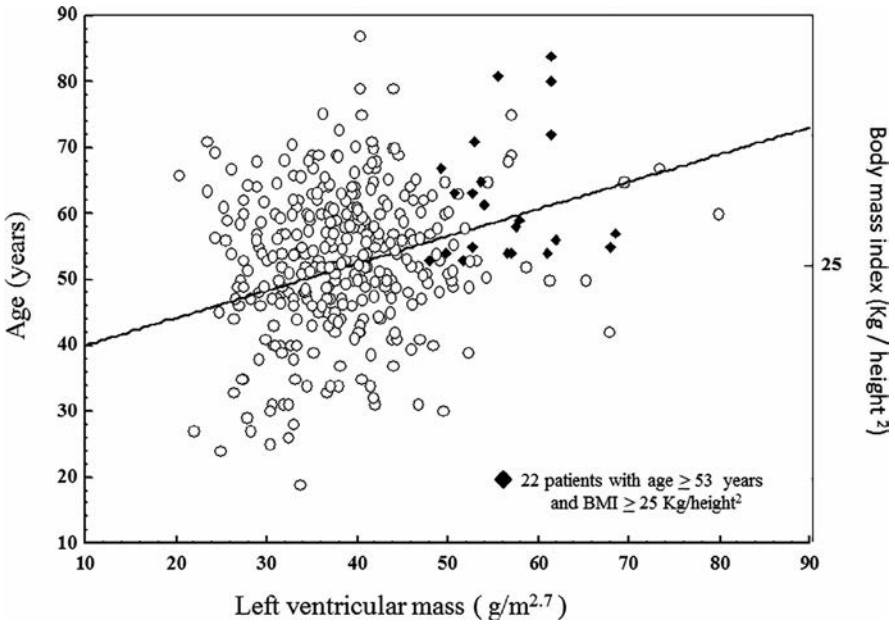

FIGURE 1 Graphic representation of the 22 patients who belonged to the subgroup with the highest risk for having left ventricular hypertrophy (LVH) at baseline evaluation. These patients were older than 53 y and had body mass index higher than 25 kg/height^2

TABLE 3 Variables associated with cardiovascular death/hospitalization: univariate and multivariable Cox regression analyses

CV death/hospitalization	CV event No 207 patients	CV event Yes 34 patients	Univariate			Multivariate[a]		
			HR	CI	*P*	HR	CI	*P*
Left ventricular hypertrophy yes/no (%)	14/86	50/50	3.98	1.39-11.40	.01	3.28	1.03-9.20	.03
C-reactive protein (mg/dL)	3.1 [1.3-8.1]	8.9 [2.8-16.4]	1.09	1.03-1.15	.003	1.08	1.02-1.14	.004
ACPA positivity yes/no (%)	36/64	60/40	3.57	1.10-11.57	.03	5.09	1.20-15.22	.03

Abbreviations: ACPA, anti-cyclic citrullinated peptide antibodies; CV, cardiovascular; HR, hazards ratio.

[a]Multivariate analysis was adjusted for age, gender and body mass index.

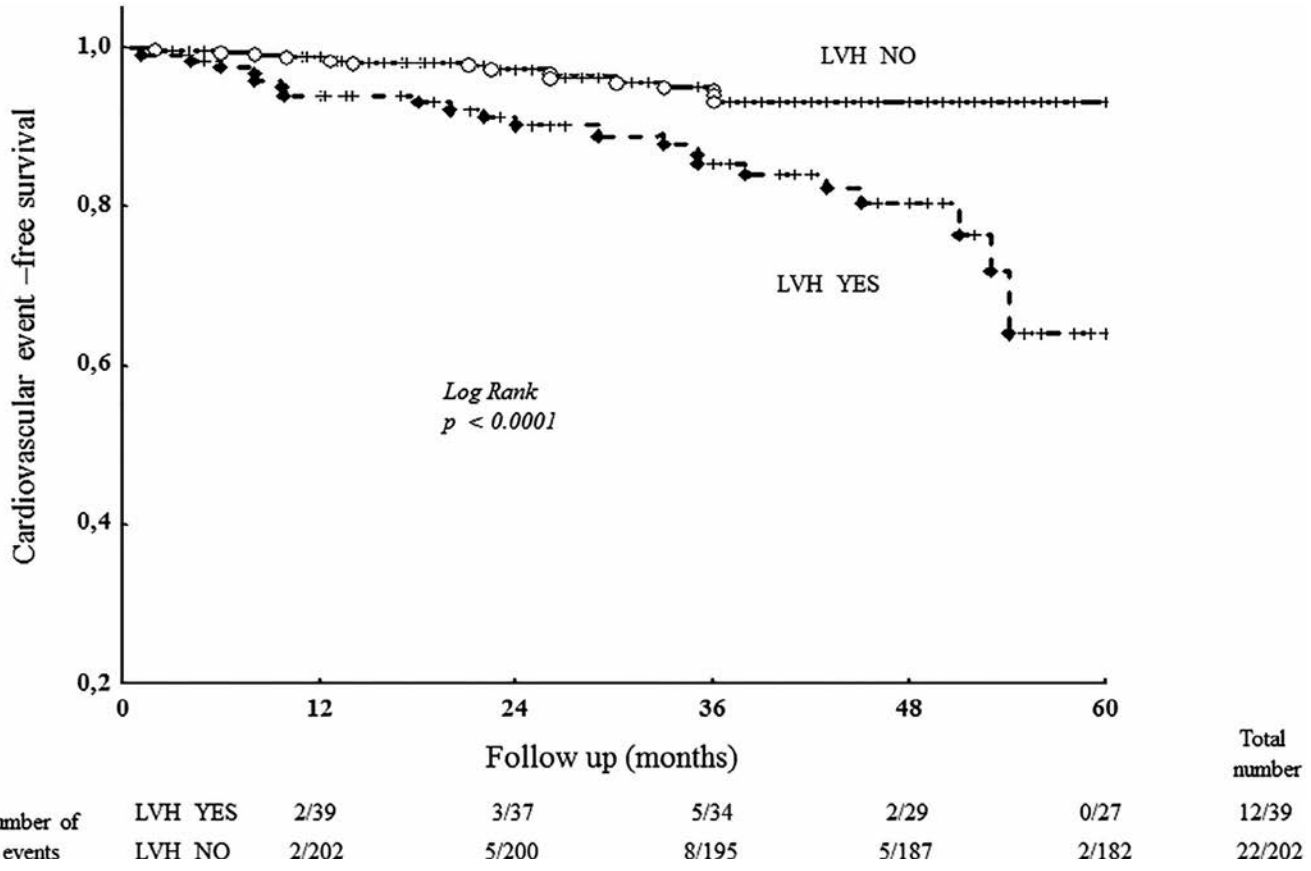

FIGURE 2 Cardiovascular event-free survival curves in non-hypertensive non-diabetic patients with rheumatoid arthritis divided according to the diagnosis of left ventricular hypertrophy (LVH) at echocardiographic baseline evaluation

hemodynamic and non-hemodynamic CV risk factors. The negative impact of LVH on CV adverse events in normotensive normoglycemic RA patients has not been reported before, thereby the present data has to be considered original and may suggest the use of LVH as a surrogate CV end-point for clinical trials involving these patients. We demonstrated that CV event rate was more than threefold higher in patients who had LVH than in those who had not. As already evident in the general population analyzed in the Framingham project,[6] echocardiographically determined LVH per se provides additional prognostic information to the traditional clinical risk factors even in the sub-group of normotensive normoglycemic RA patients at lower risk for adverse CV events. This is in line with the data reported by Litwin et al. who analyzed longitudinal changes in cardiac structure and function in obese patients.[28] Similarly, Davis et al.[29] showed the lesser contribution of conventional CV risk factors on CV clinical outcomes in patients with than those without RA. As an example, the clinical presentation and the outcome of heart failure (a major contributor to mortality for patients with RA) significantly differs between RA and non-RA subjects from the same population. Among RA subjects, the presentation of heart failure is more subtle, it is much less associated with hypertension and/or ischemic heart disease, myocardial systolic function is more likely preserved, while mortality from heart failure is significantly higher. On the other hand, the detection of LVH in our patients coincides with myocardial

diastolic dysfunction, a state widely present in RA patients.[29-31] Both our research group[30] and researchers from the Mayo Clinic[31] previously showed that sub-clinical changes in diastolic function frequently occur overtime and more rapidly in RA patients than in the general population. Collectively, all these findings suggest that diastolic dysfunction may play an important role in the pathophysiology of heart failure and worse prognosis for RA patients, and that chronic immune activation and inflammatory mechanisms may be the substrate for the development and progression of myocardial hypertrophy, fibrosis, and impairment of diastolic distensibility, relaxation, or ventricular filling.

In this experience we focused on LVH unrelated to LV pressure/volume overload due to abnormally high blood pressure as well as that induced by hyperglycemia, which represent 2 of the most common causes of LV mass growth in humans. We found that this condition was not uncommon in our normotensive normoglycemic RA patients. Clinical data showed that hypertension and/or hyperglycemia did not represent the needed condition promoting LVH in RA patients, and that inflammation during active RA had long-term consequences on molecular cardiac remodeling and mass growth.[32-36] Norton et al.[33] reported that in RA humans, a pro-inflammatory state (ie higher circulating serum tumor necrosis factor[TNF]-α levels) was more closely related to concentric LVH than a systemic hypertensive state. Recently, our research group showed that the prevalence of inappropriately high LV mass was fourfold higher in RA patients than matched controls, and that this phenomenon depended on RA per se rather than the traditional CV risk factors.[34] Furthermore, interventional studies showed that anti-inflammatory drugs such as the bDMARDs etanercept (TNF-α inhibitor) and tocilizumab (interleukin-6 inhibitor) significantly reduced LV mass in patients with RA without changes in blood pressure or pulse wave velocity.[35,36] All these findings were confirmed by a recent systematic review and meta-analysis.[37]

Older age, greater BMI and ACPA positivity were the clinical conditions associated with LVH at baseline evaluation in our patients. C-reactive protein was significantly higher in patients who had LVH at baseline evaluation than those who had not, but this association lost statistical significance at multivariate analysis when clinical variables were considered. The large use of bDMARDs in our population (two-thirds of patients were receiving bDMARDs at enrolment and 80% of them were in remission or had low disease activity) would be the main reason why C-reactive protein together with all other biochemical and/or clinical indexes of disease activity did not emerge as covariates of LVH in our study. Greater BMI was an expected finding mainly in light of the large body of information now available on the detrimental role of underweight and obese states on the disease activity of patients suffering from RA.[10,28,38-40] Clinical studies and meta-analyses, indeed, demonstrated that greater BMI was closely associated with persistent higher degree of disease activity by means of several mechanisms including the loss of balanced production of adipose-derived products (simply termed adipokines, like leptin, adiponectin, visfatin and resistin which are all involved in inflammation and immunity), the reduction of the response rate to bDMARDs and the decreased odds of achieving remission and sustaining remission in RA.[23,41-44] Greater BMI has been also associated with several pathophysiological processes potentially leading to LVH in RA patients including hemodynamic alterations, activation of the renin-angiotensin-aldosterone system, hyperleptinemia due to leptin resistance, low circulating adiponectin levels, insulin resistance with hyperinsulinemia, and possibly cardiac lipotoxicity.[45]

The clinical phenotype of RA patients with these characteristics corresponds to an individual with longer disease duration, higher degree of concentric LV geometry and ACPA positivity.[31,46] Looking at ACPA positivity, we found that this condition was closely associated with both LVH and worsening CV outcomes in our normotensive normoglycemic RA patients, extending its important prognostic role, definitely demonstrated in previous clinical studies on unselected RA populations,[46-49] and also in our RA sub-group at lower risk.

4.1 | Study limitations and strengths

Several limitations have to be underlined. First, our data refer to the prognostic role of LVH at 40-month follow-up (no longer time) and the number of CV events was relatively small, although really enough to have statistically stable results. Second, the biochemical pathways leading to LVH (which are actually not clear in RA patients even today) were not specifically searched for and clarified in our study. Third, we could not enter in the database some changes in pharmacological treatment for RA disease because of the current practice to administer the non-steroidal anti-inflammatory drugs as needed, so that this could have prejudiced our findings. Fourth, we used standard echocardiography for the detection of LVH. We did not get the chance to use cardiac magnetic resonance which has been shown to be a reliable technique to detect changes in cardiac structure and function in patients with chronic inflammatory disease earlier than other methods, including echocardiography.[50,51] These changes include increased passive myocardial stiffness (proportional to collagen deposition degree), cardiac remodeling and hypertrophy with possible development of heart failure, particularly in patients with normal ejection fraction. Strengths of our study consist of its prospective nature and design, the large number of participants recruited consecutively, the reliable methods for the assessment of LVH, the complete nature of the (baseline and follow-up) dataset.

4.2 | Clinical implications and conclusions

The present findings add information to better define the pathophysiologic links between chronic inflammation, maladaptive changes in cardiac structure and the development of adverse CV events in patients with RA. LVH is not uncommon and strongly predicts cardiovascular events in normotensive normoglycemic RA patients which can be recognized at an early stage of disease by standard

echocardiography, a reliable, well proven, flexible, low cost, environmentally safe, and easy to manage diagnostic technique.

CONFLICT OF INTEREST

The authors declare they have no competing interests.

AUTHORS' CONTRIBUTION

Conception and design: Giovanni Cioffi, Ombretta Viapiana, Maurizio Rossini, Alessandro Giollo. Generation of clinical data: Giovanni Cioffi, Ombretta Viapiana, Giovanni Orsolini, Federica Ognibeni, Andrea Dalbeni, Davide Gatti, Giovanni Adami, Angelo Fassio, Maurizio Rossini, Alessandro Giollo. Analysis and interpretation of data, or both: Giovanni Cioffi, Ombretta Viapiana, Giovanni Orsolini, Federica Ognibeni, Andrea Dalbeni, Davide Gatti, Giovanni Adami, Angelo Fassio, Maurizio Rossini, Alessandro Giollo. Drafting of the manuscript or revising it critically for important intellectual content: Giovanni Cioffi, Ombretta Viapiana, Giovanni Orsolini, Federica Ognibeni, Andrea Dalbeni, Davide Gatti, Giovanni Adami, Angelo Fassio, Maurizio Rossini, Alessandro Giollo. Final approval of the manuscript submitted: Giovanni Cioffi, Ombretta Viapiana, Giovanni Orsolini, Federica Ognibeni, Andrea Dalbeni, Davide Gatti, Giovanni Adami, Angelo Fassio, Maurizio Rossini, Alessandro Giollo. Agreement for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved: Giovanni Cioffi, Ombretta Viapiana, Giovanni Orsolini, Federica Ognibeni, Andrea Dalbeni, Davide Gatti, Giovanni Adami, Angelo Fassio, Maurizio Rossini, Alessandro Giollo.

ETHICAL APPROVAL

All patients gave written informed consent signing a specific institutional consent form; the study was approved by Ethical Committees of the Verona University and conforms to the ethical guidelines of the Declaration of Helsinki as revised in 2000.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article, please contact the corresponding author for data requests.

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ORIGINAL ARTICLE

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Tonsillitis as a possible predisposition to synovitis, acne, pustulosis, hyperostosis and osteitis (SAPHO) syndrome

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Abstract

Aim: To present the prevalence of tonsillitis in synovitis, acne, pustulosis, hyperostosis and osteitis (SAPHO) patients, to compare the clinical characteristics and disease activities between SAPHO patients with and without tonsillitis and to preliminarily explore the efficacy of tonsillectomy in SAPHO syndrome.

Method: A total of 58 SAPHO patients were included. Clinical data were collected, including demographic characteristics and acute phase reactants (erythrocyte sedimentation rate, high-sensitivity C-reactive protein). The visual analog scale (VAS), Palmoplantar Pustule Psoriasis Area Severity Index (PPPASI) and Nail Psoriasis Severity Index (NAPSI) were used to measure the severity of bone pain, skin lesions and nail lesions, respectively. Patients were referred to the otolaryngology department for tonsil examinations, including tonsil hypertrophy (grade ≥ 2), chronic congestion, inflammatory secretion and tonsil stones. The patients who underwent tonsillectomy were followed up after the surgery.

Results: A total of 67.2% of patients had tonsillitis. Patients with tonsillitis had markedly higher PPPASI (1.2 [0, 7.4] vs. 7.6 [1.75, 15.5], $P = .018$) and NAPSI (0 [0, 21] vs. 8 [3, 28], $P = .032$) scores. After tonsillectomy, the patients experienced significantly improved bone pain (VAS, 5 [4, 7] vs. 3 [1, 4], $P = .034$) and skin lesions (PPPASI, 16.2 [7.05, 18.35] vs 1.8 [0.7, 3.7], $P = .028$).

Conclusion: Approximately 2/3 of SAPHO patients had tonsillitis. Patients with tonsillitis had more severe skin and nail lesions. Tonsillectomy might be associated with improved bone and skin symptoms in SAPHO patients. Future prospective controlled studies are warranted.

KEYWORDS

acne, hyperostosis and osteitis (SAPHO) syndrome, pustulosis, surgery, synovitis, tonsillectomy, tonsil-related disease, treatment

Xiang, Wang and Cao contributed equally to this work and should be considered co-first authors.

1 | INTRODUCTION

Synovitis, acne, pustulosis, hyperostosis and osteitis (SAPHO) syndrome, a rare autoinflammatory disease combining osteoarticular and cutaneous manifestations, was first described in 1987.[1] The pathogenesis of SAPHO syndrome remains unknown, and the theory of persistent chronic infection with low-virulence pathogens is intriguing. Pustulotic arthro-osteitis (PAO) and palmoplantar pustulosis (PPP), sharing overlapping features with SAPHO syndrome, were demonstrated to be related to focal infections and were exacerbated following tonsillitis and other infections. In addition, after tonsillectomy, marked clinical improvements in skin lesions were observed in several previous PPP studies,[2] and patients experienced improved PAO-induced arthralgia after the operation.[3,4] These diseases are denoted as tonsil-induced autoimmune/inflammatory syndrome (TIAS), since they might be triggered by chronic indigenous infection in the tonsil. Therefore, whether chronic infection in the tonsil plays a role in the pathogenesis of SAPHO syndrome warrants further investigation.

There is no standardized treatment for SAPHO syndrome. Since the efficacy of tonsillectomy has been verified in PPP, in-depth research on whether tonsillectomy could be adopted in SAPHO patients is necessary. Therefore, the objectives of this study were: (i) to present the prevalence of tonsillitis in SAPHO patients; (ii) to compare clinical characteristics and disease activities between SAPHO patients with and without tonsillitis; and (iii) to preliminarily explore the effectiveness of tonsillectomy in SAPHO syndrome.

2 | MATERIALS AND METHODS

2.1 | Patients

We consecutively enrolled SAPHO patients who came to our SAPHO syndrome-special clinic at Peking Union Medical College Hospital (PUMCH) from August 2019 to October 2019. Inclusion criteria were as follows: (1) fulfilling the classification criteria for SAPHO syndrome proposed by Kahn;[5] (2) aged no less than 18 years old. A total of 58 patients were included. Patients provided informed consent and were referred to the otolaryngology department for tonsil examinations. Up to February 1, 2020, among the 58 patients, 7 patients underwent tonsillectomy in the otolaryngology department, and we retrospectively collected the follow-up data of these patients. This study was approved by the PUMCH Ethics Committee as ZS-944.

2.2 | Clinical assessments

Baseline data included gender, age, disease duration, osteoarticular symptoms, skin lesions and nail involvement. We also determined baseline laboratory findings, including rheumatoid factor (RF), antinuclear antibody (ANA) and human leukocyte antigen B27 (HLA-B27), and collected information on treatment strategies, including nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, disease-modifying antirheumatic drugs (DMARDs), biological agents and bisphosphonate.

We obtained erythrocyte sedimentation rates (ESRs) and serum high-sensitivity C-reactive protein (hsCRP) levels, both of which were examined most recently within 1 month before the tonsil examination. The visual analog scale (VAS) for pain (0-10) was also administered. The Palmoplantar Pustular Psoriasis Area Severity Index (PPPASI)[6] was used to measure the severity of skin lesions in patients presenting with PPP, and the Nail Psoriasis Severity Index (NAPSI)[7] was adopted to evaluate nail lesions.

In the otolaryngology department, first, the otolaryngologist asked the patients about their tonsillitis medical histories, which were whether they had a severe tonsillitis history (confirmed by physicians) accompanied by exacerbating symptoms related to SAPHO syndrome (worsening of bone pain or skin lesions). Second, the otolaryngologist conducted tonsil examinations, that is, tonsil hypertrophy (grade ≥ 2), chronic congestion and inflammatory secretion or tonsil stones (typical tonsillitis examination shown in Figure 1). Patients were diagnosed with tonsillitis if they: (1) presented with chronic tonsil congestion or inflammatory secretion or tonsil stones on the present examination; or (2) reported that they had tonsillitis diagnosed by physicians in the past along with exacerbating symptoms related to SAPHO syndrome.

2.3 | Statistical analysis

Categorical variables are expressed in numbers (%). Continuous variables are presented as the mean (SD) or median (first quartile,

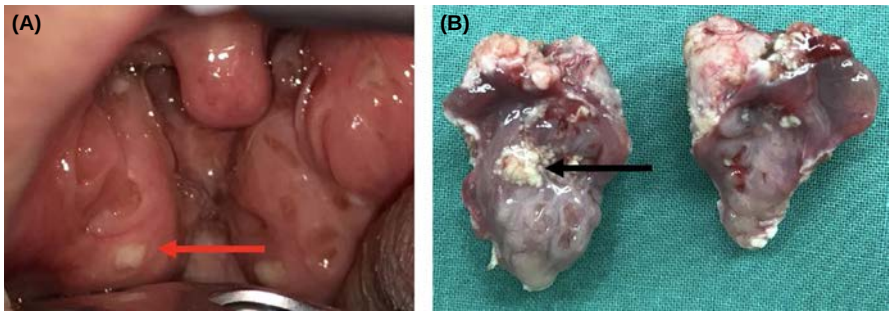

FIGURE 1 Tonsillitis assessment. A, Patient 1: tonsil hypertrophy with tonsil stones (red arrow: tonsil stones); B, Patient 2: excised tonsil sample with inflammatory secretion in tonsil crypt (black arrow: inflammatory secretion)

third quartile). The normality was tested using the Shapiro-Wilk test. To compare the differences between patients with or without tonsillitis, we used Student's *t* test for continuous variables and the Chi-square test (or Fisher's exact test, if required) for categorical variables, while the Mann-Whitney *U* test was adopted for rank variables and nonnormal variables. The conditions of patients before and after tonsillectomy were compared by the Wilcoxon signed-rank test.

3 | RESULTS

3.1 | Tonsil examinations

On examination, the otolaryngologist found 26 (44.8%) patients with congested tonsils, while inflammatory secretion or tonsil stones were found in 29 (50%) patients. Regarding the tonsillitis medical history, 6 of the 58 patients reported tonsillitis symptoms related to exacerbated SAPHO symptoms in the past, among which 3 reported tonsillitis diagnosed by physicians. In summary, 39 (67.2%) patients were diagnosed with tonsillitis. Among these patients, 13 (33.3%) complained of symptoms for the same period, including sore throat and abnormal sensation.

3.2 | Patient characteristics

The demographic and clinical characteristics are presented in Table 1. The group with the patients with tonsillitis had a lower proportion of females (74.4% vs. 94.7%, $P = .063$) than the group with patients without tonsillitis and were more likely to have nail lesions (43.6% vs. 21.1%, $P = .094$), although the difference was not statistically significant.

3.3 | Disease activity

Figure 2 shows that the patients with tonsillitis presented markedly higher PPPASI (1.2 [0, 7.4] vs. 7.6 [1.75, 15.5], $P = .018$) and NAPSI (0 [0, 21] vs. 8 [3, 28], $P = .032$) scores. However, no significant differences were observed between the 2 groups for acute phase reactants (ESR: 16.3 [10.6] vs. 19 [10.5, 29], $P = .249$; hsCRP: 2.06 [0.97, 3.755] vs. 2.57 [1.09, 7.695], $P = .407$) and pain VAS (4.3 [2.7] vs. 4.3 [2.6], $P = .907$).

3.4 | Clinical evaluations before and after tonsillectomy

Seven patients underwent tonsillectomy. The clinical characteristics, indications for tonsillectomy, and follow-up data of these patients are presented in Table 2. One patient (Patient 6) had recurrent and severe tonsillitis attacks; 1 patient (Patient 2) underwent the operation because of the recurrent tonsillitis along with exacerbation of SAPHO symptoms; the other 5 patients had chronic tonsillitis with severe or recurrent SAPHO symptoms.

The changes in VAS, PPPASI and NAPSI scores in these 7 patients are shown in Table 2 and Figure 3. Approximately 3 months after the surgery, the patients presented significant alleviation of bone pain (VAS, 5 [4, 7] vs. 3 [1, 4], $P = .034$) and skin lesions (PPPASI, 16.2 [7.05, 18.35] vs. 1.8 [0.7, 3.7), $P = .028$). However, no significant change was found for nail lesions (NAPSI, 10 [7, 31.5] vs. 2 [0, 24], $P = .249$). Figure 4 shows a typical change in skin and nail lesions before and after tonsillectomy in 1 patient.

TABLE 1 Demographic and clinical characteristics of SAPHO patients with and without tonsillitis

	With tonsillitis (n = 39)	Without tonsillitis (n = 19)	*P* value
Gender, female	29 (74.4)	18 (94.7)	0.063
Age, y	34.5 (43.0, 29.0)	37.0 (33.4, 48.6)	0.567
Disease duration, y	2 (1, 5)	3 (1.5, 8.5)	0.142
Osteoarticular involvement			
Anterior chest wall	38 (97.4)	19 (100)	1.000
Spine	19 (48.7)	11 (57.9)	0.512
Sacroiliac joint	20 (52.3)	13 (68.4)	0.216
Peripheral skeleton	20 (51.3)	13 (68.4)	0.216
Nail involvement	17 (43.6)	4 (21.1)	0.094
Skin involvement			
PPP	37 (94.9)	17 (89.5)	0.591
SA	6 (15.4)	1 (5.3)	0.407
Laboratory findings			
ANA positive	2 (5.1)	0	1.000
RF positive	0	0	-
HLA-B27 positive	3 (7.7)	1 (5.3)	1.000
Past treatments			
NSAIDs	30 (76.9)	15 (78.9)	0.862
Glucocorticoids	9 (23.1)	4 (21.1)	0.862
DMARDs	12 (30.8)	5 (26.3)	0.727
Biological agents[#]	7 (17.9)	3 (15.8)	0.838
Bisphosphonates	2 (5.1)	1 (5.3)	0.199

Note: Data are presented as the median (first quartile, third quartile) or number (%).

Abbreviations: ANA, antinuclear antibody; DMARDs, disease-modifying antirheumatic drugs; HLA-B27, human leukocyte antigen subtypes B27; NSAIDs, nonsteroidal anti-inflammatory drugs; PPP, palmoplantar pustulosis; PV, Psoriasis vulgaris; RF, rheumatoid factor; SA, severe acne; Biological agents: tumor necrosis factor-α inhibitors, interleukin (IL)-6 inhibitors and IL-17 inhibitors.

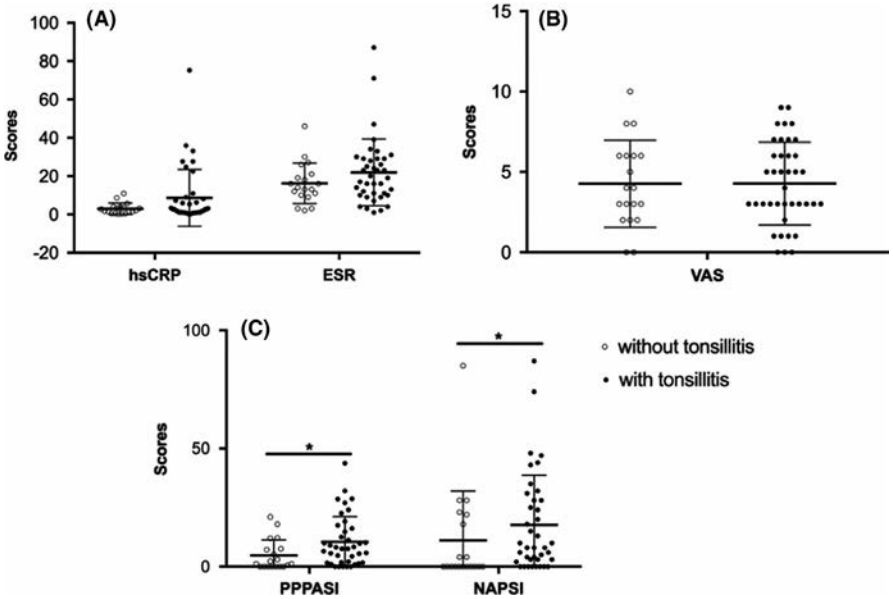

FIGURE 2 Clinical assessments of SAPHO patients with and without tonsillitis. A, hsCRP and ESR; B, pain VAS; and C, PPPASI and NAPSI. Asterisk indicates $P < .05$. ESR, erythrocyte sedimentation rates; hsCRP, serum high-sensitivity C-reactive protein; NAPSI, Nail Psoriasis Severity Index; PPPASI, Palmoplantar Pustular Psoriasis Area Severity Index; VAS, Visual Analog Scale

4 | DISCUSSION

Our study presents several findings. First, we found that 67.2% of SAPHO patients had presented with tonsillitis. Second, the patients with tonsillitis seemed to have more severe skin and nail lesions. Third, tonsillectomy might be associated with improved bone and skin symptoms in SAPHO patients.

The possible role of chronic infection in the pathogenesis of SAPHO syndrome has gained considerable attention among researchers. Several different types of bacteria were revealed to be relevant in previous studies, for example, *Staphylococcus aureus*[8] and *Propionibacterium acnes*,[9] both of which are common recurrent tonsillitis microbes.[10] Rozin et al.[8] suggested a relationship between *S. aureus* and SAPHO syndrome and successfully treated patients with cotrimoxazole. Additionally, Assmann et al. demonstrated positive microbiological cultures for *P. acnes* in 67% of bone biopsy specimens from SAPHO patients,[11] and they found that antibiotics seemed to be effective in some patients, although relapses occurred after discontinuation. It was also hypothesized that these microbes triggered autoimmune processes via molecular mimicry or Toll-like receptors,[12,13] which caused tissue damage. Thus, chronic infection is a controversial but important point in the pathogenesis of SAPHO syndrome.

The tonsil is a common site for occult chronic infection, and tonsillitis has been demonstrated to be associated with a variety of rheumatic diseases, namely TIAS.[2] In the present study, we first investigated tonsillitis in SAPHO patients. We identified that approximately 2/3 patients had tonsillitis through physical examination or history of tonsillitis during exacerbation of SAPHO syndrome. Our previous study[14] demonstrated that in positron emission tomography / computed tomography, approximately 1/3 of patients with SAPHO syndrome had abnormal[15] fluorine 18-labeled deoxyglucose uptake in the tonsils. Our findings supported the hypothesis that occult tonsillitis is frequent in patients with SAPHO syndrome. Detailed history taking and physical examination regarding the tonsils should be performed when evaluating SAPHO patients, although there may not be clinical manifestations for a tonsillitis outbreak. Additionally, we found that the patients with tonsillitis had higher PPPASI and NAPSI scores than patients without tonsillitis, which indicated that chronic infection in the tonsils might be responsible for recurrent skin and nail lesions and exacerbation of the symptoms. Interestingly, only a small proportion (15%) of patients with tonsillitis recalled exacerbated SAPHO symptoms during a bout of tonsillitis. We speculated that asymptomatic tonsillitis might play a role in this phenomenon. The chronic inflammation in the tonsil could be silent without any symptoms.[16] As a result, tonsillitis attacks may not be noticed by the patients. We proposed that tonsillitis might be a possible predisposition to SAPHO syndrome.

There are many studies discussing the efficacy of tonsillectomy on rheumatic diseases. In immunoglobin A nephropathy (IgAN), tonsillectomy combined with steroid pulse therapy was revealed to induce significant remission of the disease.[15,17,18] Additionally, several other diseases, such as psoriatic diseases,[19-21] reactive arthritis[22] and IgA vasculitis,[23] were reported to be tonsil-related. Additionally, PPP is known to cause typical tonsillar focal diseases, as shown in multiple studies.[3,24,25] Regarding the molecular mechanism, the migration of tonsillar T cells expressing homing receptors caused a hyperimmune response against indigenous bacteria, which was critical in the pathogenesis of tonsillar focal diseases.[25] The hyperimmune response to keratin and heat shock proteins in tonsillar crypt epithelium might also play a role.[2]

Emerging evidence has suggested potential benefits of tonsillectomy in some patients with SAPHO syndrome. Horiguchi et al.[26] reported that a 75-year-old woman with refractory SAPHO symptoms showed improvement in arthralgia and life quality after tonsillectomy. Previously, we also reported a case[27] in which a 29-year-old female SAPHO patient with refractory palmoplantar pustulosis underwent tonsillectomy and showed remission

TABLE 2 Demographic and clinical characteristics of the 7 operated patients who underwent tonsillectomy

					Tonsil			Follow-up				Treatment strategies	
	Sex	Age	Duration (y)	Indication for tonsillectomy	Hypertrophy	Congestion	Secretion	Time	VAS	PPPASI	NAPSI	Before surgery	After surgery
1	F	43	1.9	Severe and refractory palmoplantar pustulosis			+	0w	5	16.2	43	Etanercept, 50 mg qw	Etanercept, 25 mg qw
								3w	3	3.3	24		
								8w	6	1.8	2		
2	F	25	0.2	Recurrent tonsillitis along with SAPHO-related rash outbreaks	+		+	0w	8	19.2	10	Etanercept, 50 mg qw	Etanercept, 25 mg qw
								8w	2	12.4	26		
3	F	29	0.5	Severe unbearable bone pain	+		+	0w	9	43.8	87	Etanercept, 50 mg qw	None
								8w	8	20			
								18w	4	5.6	42		
4	F	37	0.5	Recurrent tonsillitis and suffered from severe bone pain and rashes	+	+	+	0w	6	5.9	10	Methotrexate, 10 mg qw	None
								10w	3	0.9	0		
5	F	27	4	Recurrent palmoplantar pustulosis outbreaks	+		+	0w	3	17.5	4	Leflunomide, 20 mg qd	None
								11w	0	0.5	0		
6	F	25	1.2	Recurrent tonsillitis (approximately 1 attack per month) and suffered from severe sore throat	+	+		0w	5	8.2	20	Etoricoxib, 60 mg qd	None
								10w	4	1.8	22		
7	F	33	11	Long disease duration and suffered from several rash outbreaks	+	+		0w	2	0	0	Etoricoxib, 60 mg qd	None
								12w	1	0	0		

Abbreviations: NAPSI, Nail Psoriasis Severity Index; PPPASI, Palmoplantar Pustular Psoriasis Area Severity Index; VAS, visual analog scale.

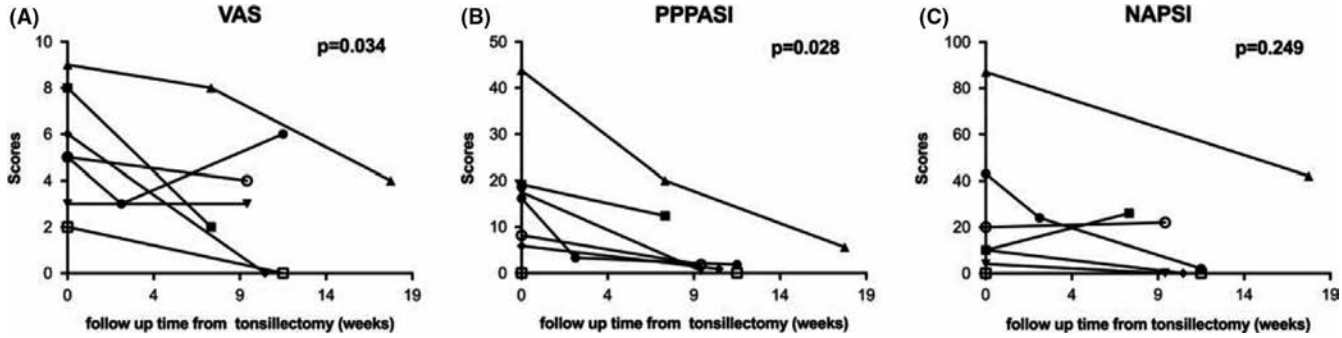

FIGURE 3 Changes in the VAS, PPPASI and NAPSI scores in the 7 patients undergoing tonsillectomy. *P* values indicate comparisons between baseline and the latest follow-up. NAPSI, Nail Psoriasis Severity Index; PPASI, Palmoplantar Pustular Psoriasis Area Severity Index; VAS, visual analog scale

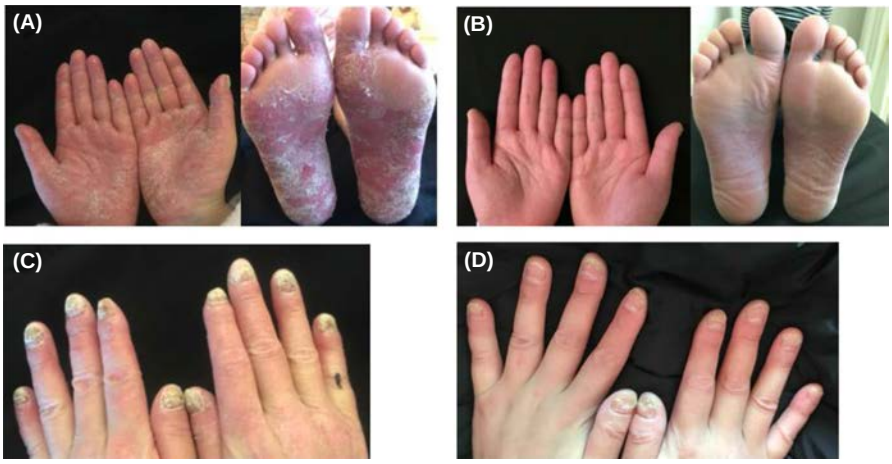

FIGURE 4 Changes in skin and nail lesions before and after tonsillectomy in a 29-y-old woman with SAPHO syndrome. She suffered severe PPP on the bilateral palms and soles and bone pain involving the anterior chest wall, sacroiliac joint and shoulder joint for 7 mo. Her tonsil examination showed tonsil hypertrophy (grade 2) and inflammatory secretion. Bilateral tonsillectomy was performed. Four months later, her skin lesion almost disappeared (PPPASI from 43.8 to 5.6), and the bone pain was relieved (pain VAS from 9 to 4). In addition, the nail lesions improved as NAPSI decreased from 87 to 42. A, PPP before tonsillectomy; (B) PPP after tonsillectomy; (C) nail lesions before tonsillectomy; (D) nail lesions after tonsillectomy. NAPSI, Nail Psoriasis Severity Index; PPP, Palmoplantar Pustular Psoriasis; PPPASI, Palmoplantar Pustular Psoriasis Area Severity Index; SAPHO, Synovitis, Acne, Pustulosis, Hyperostosis and Osteitis; VAS, visual analog scale

of skin lesions and bone marrow edema after the surgery. A recent case series recruited 17 patients with pustulotic arthro-osteitis, an entity considered to be the same as SAPHO syndrome, who underwent tonsillectomy. They found remarkable VAS and PPPASI improvement 1 month after the surgeries.[28] In the present research, we retrospectively studied 7 SAPHO patients who underwent tonsillectomy, either because of frequent attacks of severe tonsillitis, or because of chronic or recurrent tonsillitis accompanied by refractory or recurrent SAPHO-related symptoms. Despite a reduction or discontinuation of drug treatments after the operation, most patients experienced decreased bone pain and improved skin lesions. We think these findings warrant further studies on this issue, including confirming the results with prospective controlled studies and identifying patients who are most likely to benefit from tonsillectomy.

Our study has several limitations. First, we only took a preliminary step to explore tonsillitis prevalence and characteristics in SAPHO syndrome with only a limited sample size. Second, we included patients with a history of tonsillitis related to SAPHO syndrome, which might introduce recall bias. Third, we explored the outcomes of patients who underwent tonsillectomy in a retrospective way, and the lack of a control group limits interpretation of the results. We believe that our results suggest the need for further prospective controlled studies on this issue with larger sample sizes.

5 | CONCLUSIONS

In summary, we conclude that: (1) approximately 2/3 of SAPHO patients had presented with tonsillitis; (2) the patients with tonsillitis had more severe skin and nail lesions; and (3) tonsillectomy might be associated with improved bone and skin symptoms in SAPHO patients.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

YRX, YTW, YW and CL participated in the conception and design of the study and contributed to data acquisition. YRX, YTW, YHC, YW, CL, ZHL, DKX, and LW participated in data analysis and interpretation. YRX drafted the manuscript, and YHC, XFZ, WZ, YW and CL critically revised the manuscript. YHC, YW and CL supervised the study. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

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A rheumatologic approach to granulomatous mastitis: A case series and review of the literature

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Abstract

Aim: Idiopathic granulomatous mastitis (IGM) is an enigmatic inflammatory breast disorder. IGM responds to immunomodulatory treatment and may be associated with systemic manifestations such as arthritis and erythema nodosum. These patients are increasingly referred to rheumatologists for management, but IGM is rarely discussed in the rheumatology literature. The objective of this report is to familiarize rheumatologists with the treatment and systemic manifestations of IGM. We report here a case series of IGM at our institution, and a literature review of IGM treated with methotrexate (MTX).

Method: Patients with IGM at our institution were identified and described using a retrospective chart review. A literature review of PubMed and Google Scholar identified studies of IGM patients treated with MTX.

Results: We identified 28 IGM patients at our institution. Inflammatory arthritis/arthralgia were present in four patients (14%), and five patients (18%) had erythema nodosum. Patients treated with MTX had the highest rates of relapse-free remission; relapse-free remission occurred in four of the five (80%) MTX-treated patients, compared with 5 of 12 (42%) patients treated with steroids alone, and two or three (66%) patients treated with steroids and surgery. In the literature review, 116 patients treated with MTX were identified, and the rate of relapse-free remission ranged from 58% to 100%. Arthritis/arthralgia and erythema nodosum were more common at our institution than reported in the literature.

Conclusion: Methotrexate is a promising treatment for IGM. Arthritis/arthralgias and erythema nodosum may be under-recognized when IGM patients are managed outside rheumatology. Prospective studies are needed to characterize clinical features and optimum treatment of IGM.

KEYWORDS
arthritis, erythema nodosum, granuloma, mastitis, methotrexate

Sarah Ringsted and Marcia Friedman are equal contributors to this work.

1 | INTRODUCTION

Idiopathic granulomatous mastitis (IGM) is an inflammatory disease of breast tissue that occurs primarily in young to middle-aged women with a history of recent pregnancy and/or breastfeeding.[1,2] IGM is a poorly understood disease, and there is no consensus regarding underlying cause, risk factors, or optimal treatment. Historically, breast surgeons have primarily managed this disease; however, it is increasingly recognized as an inflammatory disease and is therefore being referred to rheumatologists. The primary purpose of this article is to help rheumatologists recognize, understand, and manage this disease. We present here our single academic medical center experience, including more frequent co-occurrence of arthritis and erythema nodosum than has been previously described, and the successful use of anti-rheumatic drugs in the treatment of IGM. We also report a literature review of the use of methotrexate (MTX) to treat IGM.

Idiopathic granulomatous mastitis typically presents with a tender, firm breast mass accompanied by erythema, pain, and drainage. IGM may be unilateral or bilateral, a self-limited or persistent and recurring disease, and may leave disfiguring scars. The diagnosis of IGM is made by breast biopsy (ideally by core biopsy) showing a non-caseating granuloma with epithelioid histiocytes and multinucleated giant cells within breast lobules and may contain micro-abscesses (Figure 1).[1,2] Tissue needs to be sent for bacterial, mycobacterial, and fungal cultures to exclude infection. Imaging findings are non-specific and may mimic breast carcinoma and infection.[3]

It is not known what causes IGM; however, risk factors may include trauma, hormones (oral contraceptives, pregnancy, and birth), breastfeeding (which may include both trauma and hormones), elevated prolactin levels, infections (particularly *Corynebacterium*), and autoimmunity.[1,4] There additionally seems to be geographic and ethnic variance in the prevalence of IGM. The majority of large case series come from Asian and Mediterranean countries.[1] In the three largest case series from the USA, the majority of IGM patients were of Hispanic descent and IGM was less commonly seen in Caucasian and African American patients.[5-7] This geographic variation suggests that there could be an underlying unidentified infectious trigger or genetic predisposition, although neither has been identified.

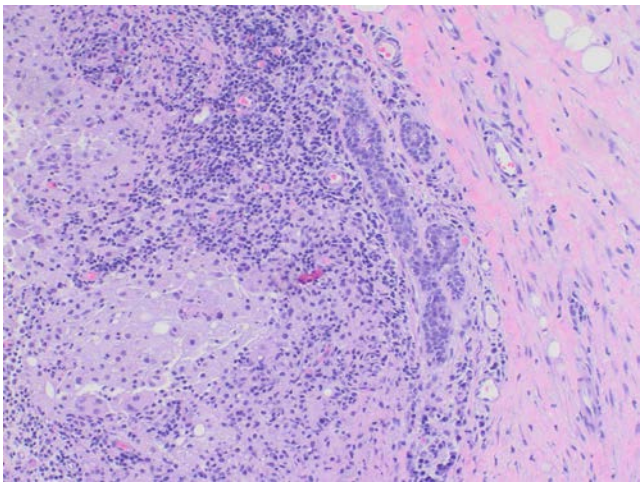

FIGURE 1 Microscopic examination shows well-formed granulomas, with giant cells and associated chronic active inflammation, centered within lobules or adjacent to duct space

Idiopathic granulomatous mastitis is a diagnosis of exclusion. Tuberculosis (TB) can cause a granulomatous mastitis and needs to be ruled out in addition to other fungal and bacterial infectious causes. Corynebacteria infection has been associated with a cystic neutrophilic granulomatous mastitis, which many authors suggest is a subtype of IGM that can be differentiated based on characteristic histopathology and positive *Corynebacterium* culture.[4] Rheumatic granulomatous diseases including sarcoidosis and granulomatosis with polyangiitis have been reported to cause granulomatous inflammation of the breast, although both are exceedingly rare.[8,9] Other non-infectious, inflammatory mimics to consider include squamous metaplasia of lactiferous ducts (associated with smoking), fat necrosis (associated with previous trauma or surgery), and foreign body reaction (associated with silicone and sutures).[4]

The optimal treatment of IGM in the literature is highly variable and often contradictory, ranging from surgical resection, observation alone, antibiotic therapy, steroids, and more targeted immunosuppressive therapy such as MTX and biologic therapy.[6,10-15] However, the vast majority of these data come from the breast surgery literature, and rheumatologists' perspective and experience is lacking.

2 | MATERIALS AND METHODS

2.1 | Retrospective case series

The Oregon Health & Science University (OHSU) Cohort Discovery tool was used to identify individuals seen at OHSU carrying a diagnosis of "granulomatous mastitis". Patients with known IGM seen in the rheumatology clinic were also included. Included patients had been evaluated at OHSU between 2007 and 2018. A retrospective chart review was used to ensure that each participant's diagnosis was correct. Criteria for this diagnosis include a breast biopsy showing non-caseating granulomas, and evidence that infection was not the underlying etiology of disease. Thirty patients were identified and two were excluded (one who was ultimately felt to have granulomatous mastitis secondary to fat necrosis and one because of lack of adequate follow up).

Clinical data were collected by chart review and included baseline characteristics (ethnicity, age at diagnosis, follow-up period, specialty of treating providers, smoking, gestational history, breastfeeding history, and use of hormonal birth control), IGM lesion details at time of diagnosis (lesion size, bilaterally, infectious studies), symptoms at diagnosis, presence of inflammatory arthralgia/arthritis, and erythema nodosum. Treatment data were collected in the following categories: high-dose steroids alone (prednisone >20 mg/day), surgery plus high-dose steroids, MTX plus high-dose steroids, and other treatments. Remission was defined as a 3-month period without recurrence of symptoms or imaging suggestive of symptom

recurrence. Relapse was defined as recurrence of disease after 3 months of remission. Persistent disease was defined as patients who never achieved remission. Patients whose treatment course was still ongoing or who were lost to follow up were excluded from analysis of treatment courses/outcomes.

2.2 | Review of the literature

A Pubmed and Google Scholar search was done using the search terms "idiopathic granulomatous mastitis AND methotrexate" and "granulomatous mastitis AND methotrexate" to identify patients with IGM who received MTX (alone or in combination with other treatments). All full-length case series, cohort studies, or clinical trials with three or more patients were included. Studies were excluded if they did not use MTX as a treatment, if treatment outcomes were not available, if they were not written in English, or if they were abstracts only. Articles focusing only on radiographic or pathologic findings with no report of clinical or therapeutic outcomes were excluded. Review articles were excluded.

3 | RESULTS

3.1 | Retrospective case series

3.1.1 | Baseline characteristics

Thirty patients seen between 2007 and 2019 were identified. Two were excluded (one diagnosed with alternative condition and one without adequate follow up). All patients were female, with a mean age at diagnosis of 32 years, the majority (61%) were Hispanic (Table 1); 89% were treated by providers in the surgical oncology/breast health center and 29% were treated by rheumatologists. Over 90% had a history of pregnancy and breastfeeding. Few patients used tobacco (14%) or were on hormone-based contraception at the time of diagnosis (29%).

TABLE 1 Baseline characteristics of 28 patients with idiopathic granulomatous mastitis

Baseline characteristic[a]	N (%)
Mean age at diagnosis	32 years (range 16-42)
Mean follow-up period	27 months (range 5-63)
Female	28 (100)
Race/Ethnicity	
White, Hispanic	17 (61)
White, non-Hispanic	6 (21)
Asian	2 (7.1)
Other	3 (11)
Specialties of treating MDs[b]	
Surgical oncology/Breast health	25 (89)
Rheumatology	8 (29)
Other[c]	5 (18)
Smoking (ever)	4 (14)
Gestational history	
Ever	26 (93)
In past 5 years before symptom onset	19 (83)
Breastfeeding history	
Ever	23 (92)
In past 5 years before symptom onset	15 (83)
Hormonal-based birth control	
Ever	14 (56)
At time of symptom onset	6 (29)

[a]Data not available in the following numbers of patients, as listed by baseline characteristic: mean follow-up period, 2; Gestational history/in past 5 years before symptom onset, 5; breastfeeding history/ever, 3; breastfeeding history/in past 5 years before symptom onset, 10; hormonal-based birth control/ever, 3; hormonal-based birth control/at time of symptom onset, 4.

[b]Patients sometimes treated by more than one specialist.

[c]Other = primary care (3), general surgery (1), obstetrics-gynecology (1).

3.1.2 | Clinical features of patients with IGM

Most women had pain on presentation (96%) and many others had overlying erythema (57%; Table 2). Interestingly, over the course the disease 14% had inflammatory arthritis/arthralgia and 18% had erythema nodosum. The presence of both of these features was often inferred by review of the reported symptoms and clinical examination—there was not often documented synovitis or a picture of a rash. The mean maximum size of the lesion at diagnosis was 4.7 cm and most were unilateral, although 25% had bilateral involvement throughout the course of the disease. Seven patients (26%) had positive bacterial culture and/or Gram stain of breast tissue, and of these, two had positive *Corynebacterium* cultures and two had Gram staining for Gram-positive bacilli, which is suggestive of *Corynebacterium*. Of these four patients, two had a diagnosis of cystic neutrophilic granulomatous mastitis on pathology. None of the patients who had positive bacterial cultures responded to antibiotics alone. Four patients had positive tuberculin skin test and/or interferon-γ release assay (two with a history of treated TB, two with no prior TB treatment) but none of these patients had TB identified on acid-fast bacilli staining of the breast biopsy tissue. Of the two patients with positive TB testing without a history of TB treatment, one had a positive tuberculin skin test but negative chest X-ray and negative acid-fast bacilli staining of the IGM biopsy specimen, and one had positive tuberculin skin test and interferon-γ release assay as well as chest X-ray with transient hilar lymphadenopathy and axillary lymphadenopathy. In the latter case, biopsy of both the axillary lymph node and breast tissue were negative for acid-fast bacilli by staining and culture. This patient received treatment for active TB as well as IGM treatment and it is notable that breast symptoms did not completely resolve after TB treatment.

3.1.3 | Treatment outcomes in IGM patients

Of the 28 patients in this study, treatment outcome data are available for 25 (Table 3). The three patients that were excluded had insufficient follow up to determine outcomes. The majority of patients were treated with high-dose steroids alone (n = 12), which was defined as a starting dose of >20 mg/day of prednisone. Five patients were treated with MTX, at a dose of 15-20 mg/week orally. All patients treated with MTX had also received high-dose steroids but in all cases, they had not had a complete response to steroids and four of these patients had also undergone surgery for the IGM lesion. One of the patients treated with MTX also had concurrent treatment for active TB. She had a positive TB interferon-γ release assay and tuberculin skin test with axillary lymphadenopathy; however, biopsy of the axillary lymph node and breast tissue was negative for acid-fast bacilli.

In total, 56% of patients had remission without relapse. Of those, patients treated with MTX had the highest rate of remission without relapse (80%). The duration of follow up for MTX-treated patients ranged from 13 to 34 months. The one MTX-treated patient with persistent disease actually responded well to MTX and was able to taper prednisone from 40 mg daily to 2.5 mg daily over the course of 4 months. At the end of these 4 months, she had an increase in symptoms, was seen by a breast surgeon and a new 3.3 cm mass was seen in the breast that had been affected by IGM. She was tapered off of all immunosuppressants, underwent a lumpectomy, and was found to have a phyllodes tumor although there was also residual IGM at one of the surgical margins. She has been followed for 15 months without immunosuppressants, and has become pregnant in the interim. She has some mild residual pain over the left breast and ultrasound shows a residual 1.2 cm nodule as well as heterogeneous areas of decreased echogenicity at the site of the previous IGM that may be ongoing residual IGM.

TABLE 2 Clinical features of idiopathic granulomatous mastitis at time of diagnosis in 28 patients

Clinical feature[a]	N (%)
Maximum diameter at diagnosis (mean)	4.7 cm
Bilateral disease	
At time of diagnosis	1 (3.6)
Ever	7 (25)
Positive bacterial culture or Gram stain	
Any positive	7 (25)
Corynebacterium[b]	4 (14)
Positive for tuberculosis[c]	4 (14)
Symptoms at diagnosis	
Pain	27 (96)
Discharge	5 (18)
Erythema	16 (57)
Inflammatory arthritis/arthralgia	4 (14)
Erythema nodosum	5 (18)

[a]Data not available in the following numbers of patients, as listed by clinical feature: maximum size at diagnosis, 6; positive bacterial culture or Gram stain/any positive, 4 had no Gram stain or bacterial culture although these 4 patients had acid fast bacilli and fungal staining; positive tuberculosis, 2 had no acid fast bacilli stain or culture.

[b]Defined as positive *Corynebacterium* culture or Gram stain showing gram positive bacilli in breast tissue.

[c]Defined as positive acid-fast bacilli culture, interferon-γ release assay, tuberculin skin test, and/or chest X-ray with suggestive findings.

3.2 | Review of the literature

Twenty-two studies were identified, 14 were excluded (nine case reports with fewer than three patients, three review articles, one without patient outcomes reported and one not written in

TABLE 3 Idiopathic granulomatous mastitis treatment outcomes in 25 patients

	All treatment groups[a] N = 25 N (%)	High-dose steroids + surgery N = 3 N (%)	High-dose steroids alone N = 12 N (%)	MTX N = 5 N (%)	Other[b] N = 5 N (%)
Relapse-free remission	14 (56)	2 (66)	5 (42)	4 (80)	2 (40)
Relapse	7 (28)	0	5 (42)	0	2 (40)
1 relapse	5 (20)	0	4 (33)	n/a	1 (20)
>1	2 (8)	0	1 (8)	n/a	1 (20)
Mean length of time until first remission (mo)	7.8	15	7.3	8.3	5.3
Mean length of time until first relapse (mo)	11.4	n/a	9.6	n/a	16
Persistent disease	5 (20)	1 (33)	2 (17)	1 (20)	1 (20)
Treatment-free remission for ≥6 mo	20 (80)	2 (66)	10 (83)	4 (80)	4 (80)

[a]All patients received varying courses of antibiotics, without significant clinical improvement except in one case (listed in "other").

[b]Other treatments were: observation, surgery alone, antibiotics/surgery alone, antibiotics alone.

TABLE 4 Literature review of case series of patients with idiopathic granulomatous mastitis treated with methotrexate (MTX)

Article	No. of patients	Country of origin	Mean follow-up (mo)	Extra-mammary manifestations n (%)[a]	MTX regimen, mg/week	Outcomes[b]	
						Remission without relapse n (%)	Persistent disease n (%)
Sheybani et al[3]	12	Iran	11.9 ± 4.4	9 (41%) Inflammatory arthritis/arthralgia 2 (9%) Erythema nodosum	7.5-10 escalate to maximum of 15	12 (100%)	0 (0%)
Postolova et al[7]	19	USA	36 (12-84)	2 (11%) Pre-existing rheumatologic conditions including tenosynovitis and erythema nodosum	10-15, escalate to maximum of 25	11 (58%)	1 (5.3%)
Haddad et al[16]	13	Iran	16.4 ± 9.2	1 (8%) Bilateral ankle arthritis and erythema nodosum	Unknown dose	10 (77%)	0 (0%)
Akbulut et al[17]	4	Turkey	2-9	None	7.5	4 (100%)	0 (0%)
Akbulut et al[18]	4	Turkey	8.6 (6-14)	None	7.5-15	4 (100%)	0 (0%)
Kim et al[19]	5	Australia	n/a	None	10-15	3 (60%)	0 (0%)
Aghajanzadeh et al[13]	56	Iran	3-18	None	7.5-10	40 (71%)	16 (29%)
Raj et al[12]	3	UK	6-12+	None	7.5-15	2 (66%)	0 (0%)

[a]Extra-mammary manifestations were based on all patients included in a series, regardless of whether they received methotrexate or not.

[b]Other patients had relapse and then remission, were still on treatment at the conclusion of the study, were lost to follow-up or stopped methotrexate due to adverse effects.

English).[3,7,12,13,16-19] All studies were retrospective case series except for one, which was a prospective case series.[3] In total, there were 116 patients treated with MTX. The majority of these studies originated from Middle Eastern countries, one study was from the USA. Fourteen (12%) patients had co-existent inflammatory arthritis or erythema nodosum. Several studies used MTX as monotherapy, although most patients were also on steroids.[7,17,18] The majority of patients had tried/failed other treatments including steroids, anti-TB treatment, and/or surgery. One study used MTX as initial therapy[16] and in another, 7 out of the 12 patients received MTX as initial therapy.[3] Patients were treated with doses of MTX ranging from 7.5 to 25 mg weekly. The average rate of relapse-free remission was 79% (range 58%-100%). Duration of follow up ranged from 2 to 36 months (Table 4).

4 | DISCUSSION

Consistent with previous reports, the patients in our series were young to middle aged females, mostly of Hispanic ancestry, and the vast majority had recent history of childbirth and/or lactation. Interestingly, we found in our cohort a stronger association with inflammatory arthritis and erythema nodosum than has been previously described. A recent systematic review of 3060 cases found the incidence of erythema nodosum to be 8%.[20] Our cohort's higher incidence of erythema nodosum and arthritis may be partly explained by the fact that rheumatologists are more likely to identify these features; however, it adds evidence that this disease is a systemic inflammatory condition and may benefit from rheumatologists' care.

Rheumatologists care patients with for other granulomatous diseases such as sarcoidosis, and are familiar with an arsenal of immunomodulatory therapy, which may not be within the realm of expertise for breast surgeons. Although corticosteroids have been used by many as a first-line therapy for IGM, they are associated with significant risks when used long-term and it is imperative to have additional treatment options for IGM. The utility of MTX in the treatment of IGM was also highlighted by the review of the literature and current case series. In our case series, the rate of remission-free relapse with MTX-treated patients was 80%, which is within the range of 57%-100% seen in the review of the literature.

There are several limitations to this report. Our case series was a retrospective analysis, so some data had to be inferred from chart review. An exact time to remission or relapse could not be determined due to the retrospective nature of the data and the varied intervals at which patients were seen in follow up. Additionally, precisely defining remission is challenging because of limitations in confidently identifying active IGM on history, examination, and breast imaging, particularly in patients with previous breast surgery or multiple breast conditions.

Infection needs to be excluded before the diagnosis of IGM. TB is of particular concern in patients from regions where it is endemic. In our retrospective data, there was not a standardized way that infection was excluded; in many cases, breast tissue was stained for bacteria, fungi, and acid-fast bacilli, but cultures were not always performed. We feel that none of the cases in this series were likely to have infection as the primary driver of breast disease, given the lack of response to antibiotics alone and in many cases, an excellent response to immunosuppressant agents; however, there were seven patients who had positive Gram stain and/or bacterial culture of breast tissue. This could be due to super-infection of inflamed and draining breast tissue rather the primary etiology of disease. We had four patients with confirmed or suspected *Corynebacterium* on breast tissue. Of these four patients, two had pathology consistent with cystic neutrophilic granulomatous mastitis. Further studies are still needed to define the role of *Corynebacterium* in IGM and to investigate if cystic neutrophilic granulomatous mastitis is a subset that is best treated with antibiotic therapy.

Although our data suggest that MTX-treated patients have a higher rate of remission, randomized prospective studies are urgently needed to understand optimal IGM treatment. The literature review included studies that were also retrospective, not randomized, and were highly variable in terms of diagnostic evaluation, treatment, and follow up. A prospective, randomized trial comparing MTX to prednisone for the treatment of this condition would be useful. Tumor necrosis factor inhibitors also may have a role in treating resistant cases.[21] Lastly, azathioprine should be explored as a treatment, particularly because it can be used in the context of pregnancy and breastfeeding.

Idiopathic granulomatous mastitis is an enigmatic orphaned disease. Patients diagnosed with this condition often suffer through cancer scares, numerous biopsies, and multiple rounds of unsuccessful treatments. Once they are finally diagnosed, these patients are faced with a frustrating paucity of high-quality data to guide their management, and a lack of certainty as to who should be treating them. We have found at our center that signs of systemic inflammation (erythema nodosum and arthritis) may be more common than previously thought, and that MTX is a very useful treatment. Together, these findings suggest that rheumatologists can and should be partners in the management of these patients. Management of IGM is an ideal opportunity for interdisciplinary care, leading to timely diagnosis, rapid treatment, and ultimately an increase in expertise and understanding of this challenging disease.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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ORIGINAL ARTICLE

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Musculoskeletal sarcoidosis: A single center experience over 15 years

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Abstract

Background: Musculoskeletal (MSK) sarcoidosis presents with a variety of clinical phenotypes. Four subtypes of MSK sarcoidosis have been identified to date: Lofgren syndrome, chronic sarcoid arthritis, osseous sarcoidosis, sarcoid myopathy. Each subtype has been reported with varying incidence mainly due to lack of universal classification criteria.

Methods: We performed a retrospective chart review of patients with MSK sarcoidosis at a single academic center between January 2000 and December 2014. Descriptive statistics were used to describe the proportion of patients with sarcoidosis who had the 4 MSK syndromes of interest, demographic characteristics and therapeutic agents used.

Results: A cohort of 58 patients with MSK manifestations were identified among 1016 patients with sarcoidosis. Frequency of subtypes include: Lofgren syndrome 46.6%, osseous sarcoidosis 25.9%, chronic sarcoid arthritis 24.1% and sarcoid myopathy 6.9%. The cohort was predominantly female (43/58 patients, 74%) and Caucasian (48/58 patients, 82.8%). Mean age was 47.2 years. One patient had overlap of osseous sarcoidosis and chronic sarcoid arthritis, another patient initially had Lofgren syndrome and later developed chronic sarcoid arthritis. Sarcoid myopathy patients presented with myalgia more often than muscle weakness.

Conclusion: We identified a large cohort of MSK sarcoidosis and determined the prevalence of all 4 subtypes. In patients who do develop MSK manifestations of sarcoidosis, they are commonly a part of the initial presentation of sarcoidosis. There is an unmet need to establish standardized classification criteria for the 4 MSK sarcoidosis syndromes.

KEYWORDS

Lofgren syndrome, osseous sarcoid, rheumatologic manifestations, sarcoid arthritis, sarcoid myopathy, sarcoidosis, tumor necrosis factor inhibitors

Abbreviations: DMARDs, disease-modifying anti-rheumatic drugs; ICD, International Classification of Diseases; MSK, musculoskeletal; NSAIDs, Non-steroidal anti-inflammatory drugs; Th-1, Type 1T helper cell; US, United States; VA, Veterans Affairs.

1 | INTRODUCTION

Sarcoidosis is a disease characterized by non-caseating granulomatous inflammation, with predominantly pulmonary involvement but can affect multiple different organs. Granuloma formation involves a Th-1 response with infiltration of $CD4^+$ T lymphocytes and macrophages.[1,2] It occurs most frequently in African-Americans and least commonly in the Japanese population, with an incidence of 71 and 0.73 per 100 000, respectively.[3-5] Musculoskeletal (MSK) sarcoidosis refers to sarcoidosis involving bones, muscles, axial and peripheral joints. Four major types of MSK sarcoidosis have been identified: acute sarcoid arthritis or Lofgren syndrome (triad of acute inflammatory arthritis, bilateral hilar lymphadenopathy, erythema nodosum), chronic sarcoid arthritis, osseous sarcoidosis and sarcoid myopathy.[6-9] There is a paucity of large-scale observational cohort studies focusing on MSK sarcoidosis. MSK manifestations of sarcoidosis are rare, with 1 multicenter study from the United States noting 0.95% of patients developed these manifestations within 6 months of sarcoidosis diagnosis (7 out of 736 cases).[4] Each subtype of MSK sarcoidosis has been reported with varying incidence depending on the study design and the lack of universal criteria for diagnosing each subtype has been a limitation in the literature. Previous studies have often focused on 1 subtype of MSK sarcoidosis in isolation without comprehensive description of all the 4 subtypes. Additionally, there are no internationally accepted definitions of each subtype and this has led to heterogenous inclusion criteria. Given the above limitations, the aim of this study is to describe the epidemiology, clinical characteristics of a cohort of patients with MSK sarcoidosis by each subtype, as well as describe their treatment and outcomes.

2 | METHODS

This is a retrospective analysis of all patients with MSK sarcoidosis managed between January 2000 and December 2014 at the University of Iowa Hospitals & Clinics and Veterans Affairs (VA) Medical Center, Iowa City. The study was approved by the Institutional Review Board at the University of Iowa Hospitals & Clinics and the Iowa City VA Medical Center. Data were obtained from electronic medical records using International Classification of Diseases (ICD)9 codes for sarcoidosis. Manual data extraction was performed by 5 co-authors (SP, MA, SJ, EF, NS). Sarcoidosis diagnosis was accepted if patients had a biopsy from any tissue site demonstrating non-caseating granulomatous inflammation, or if the treating pulmonologist established a diagnosis of pulmonary sarcoidosis without tissue confirmation (such as patients with Lofgren syndrome or asymptomatic patients with bilateral hilar adenopathy not otherwise explained). Patients were included in the study if they met at least 1 of the following criteria:

1. a diagnosis of Lofgren syndrome by a pulmonologist or rheumatologist; patients without erythema nodosum were included as incomplete Lofgren syndrome
2. biopsy-proven sarcoidosis and chronic inflammatory arthritis > 6 months, not attributed to other causes (rheumatoid arthritis, spondyloarthritides, systemic lupus erythematosus and gout)
3. patients with sarcoidosis and evidence of bone involvement either on imaging or bone biopsy
4. biopsy-proven granulomatous inflammation involving muscle tissue.

We defined effectiveness as clinical improvement in symptoms or signs noted by the treating physician, given that there are no standardized treatment outcome measures. Descriptive statistics were used to describe the results. Age was described utilizing means. Proportions were used to describe the following: patients with a diagnosis of sarcoidosis who had the 4 MSK syndromes of interest, demographic characteristics and therapeutic agents that were used to treat each MSK syndrome. Clinical data from MSK sarcoid patients were entered into a standardized questionnaire created in a secure web application, Research Electronic Data Capture (REDCap, versions 6-8). Data were collected independently by at least 2 authors for each patient with MSK sarcoidosis, and the conflicts and queries were resolved by discussion among the authors.

3 | RESULTS

A total of 1614 patients with a diagnosis of sarcoidosis were initially captured; 598 patients were excluded as they did not meet criteria for sarcoidosis. Out of the remaining 1016 patients, 958 were excluded as they did not meet criteria for MSK involvement of systemic sarcoidosis, leaving a study population of 58 patients (Figure 1). Among the 58 patients who met inclusion criteria, mean age was 47.2 years (SD ± 12.2), majority were females (74%) and Caucasian (82.8%). Lofgren syndrome was the most frequent clinical phenotype with 27 cases (46.6%), osseous sarcoidosis with 15 cases (25.9%), chronic sarcoid arthritis with 14 cases (24.1%) and sarcoid myopathy with 4 cases (6.9%). One patient was identified with an overlap of osseous sarcoidosis and chronic sarcoid arthritis, another patient was identified with Lofgren syndrome initially and 9 months later developed chronic sarcoid arthritis. There were 4 patients identified with incomplete Lofgren syndrome (Figure 2).

Lofgren syndrome was the initial manifestation of sarcoidosis in 25/27 (92.6%) patients. Joints were involved as oligoarthritis and polyarthritis in 14/27 and 13/27 patients, respectively (Table1). The most commonly involved joints were ankle (96.3%), knee (59.3%), followed by wrist, hand joints and elbow in that order. There were 2/27 patients with shoulder joint pain and 2/27 patients with hip joint pain (Figure 3). The involvement of shoulder or hip joints in these patients was not confirmed by imaging, but the joint involvement was attributed to sarcoidosis based on clinical judgment.

Chronic sarcoid arthritis was most often in the form of polyarthritis (12/14). There were 2/14 patients with monoarthritis and

TABLE 1 Demographic and clinical characteristics

Demographic and clinical characteristics	Lofgren syndrome N = 27	Chronic sarcoid arthritis N = 14	Osseous sarcoidosis N = 15	Sarcoid myopathy N = 4
Age at diagnosis (y), mean	44	47.4	51.5	52.4
Female	n = 43 (74%)			
Race, n (%)				
Caucasian	48 (82.8)			
African American	6 (10.3)			
Asian	1 (1.7)			
South-Asian	1 (1.7)			
Unknown	2 (3.4)			
Joint involvement, n (%)				
Sternoclavicular	0 (0)	1 (7.1)		
Shoulder	2 (7.4)	4 (28.6)		
Elbow	6 (22.2)	4 (28.6)		
Wrist	12 (44.4)	9 (64.3)		
Hand joints	7 (25.9)	10 (71.4)		
Hip	2 (7.4)	4 (28.6)		
Knee	16 (59.3)	6 (42.9)		
Ankle	26 (96.3)	8 (57.1)		
Foot joints	4 (14.8)	4 (28.6)		
Bone involvement in osseous sarcoidosis, n (%)				
Cranium			3 (20)	
Maxilla			1 (6.7)	
Sternum			2 (13.3)	
Ribs			4 (26.7)	
Scapula			3 (20)	
Humerus			7 (46.7)	
Cervical spine			1 (6.7)	
Thoracic spine			7 (46.7)	
Lumbar spine			9 (60)	
Sacrum			6 (40)	
Pelvis			8 (53.3)	
Femur			6 (40)	
Tibia			3 (20)	
Bones of foot			1 (6.7)	
Sarcoid myopathy, n (%)				
Neck				1 (25)
Upper arm				4 (100)
Forearm				1 (25)
Hip				1 (25)
Thigh				4 (100)
Leg				2 (50)

none with oligoarthritis. The most commonly involved joints were hand joints (71.4%), wrist (64.3%), followed by ankle, knee, shoulder, elbow, hip, toe joints and sternoclavicular joint, in that order. There were 4 patients with inflammatory symptoms in hips (Figure 4). Chronic sarcoid arthritis was the initial manifestation of sarcoidosis in 5/14 (35.7%) patients.

TABLE 2 Pharmacologic agents used in treatment of musculoskeletal sarcoidosis

Pharmacologic agents used, n (%)	Lofgren syndrome N = 27	Chronic sarcoid arthritis N = 14	Osseous sarcoidosis N = 15	Sarcoid myopathy N = 4
(i) Not treated	4 (14.8)		4 (26.7)	1 (25)
(ii) NSAIDs and corticosteroids				
NSAIDs	8 (29.6)	1 (7.1)		
Prednisone	16 (59.3)	8 (57.1)	9 (60)	3 (75)
Corticosteroid joint injection		1 (7.1)		
Repository corticotropin gel (subcutaneous injection)			1 (6.7)	
(iii) csDMARDs				
Hydroxychloroquine		4 (28.6)	3 (20)	1 (25)
Chloroquine		1 (7.1)		
Sulfasalazine		2 (14.3)		
Methotrexate	1 (3.7)	5 (35.7)	8 (53.3)	1 (25)
Leflunomide		1 (7.1)	1 (6.7)	
Azathioprine		1 (7.1)	2 (13.3)	
(iv) bDMARDs				
Infliximab		1 (7.1)	1 (6.7)	1 (25)
Adalimumab		1 (7.1)	1 (6.7)	
Etanercept		1 (7.1)	1 (6.7)	

Abbreviations: bDMARD, biologic DMARD; csDMARD, conventional synthetic disease-modifying anti-rheumatic drug; NSAIDs, Non-steroidal anti-inflammatory drugs.

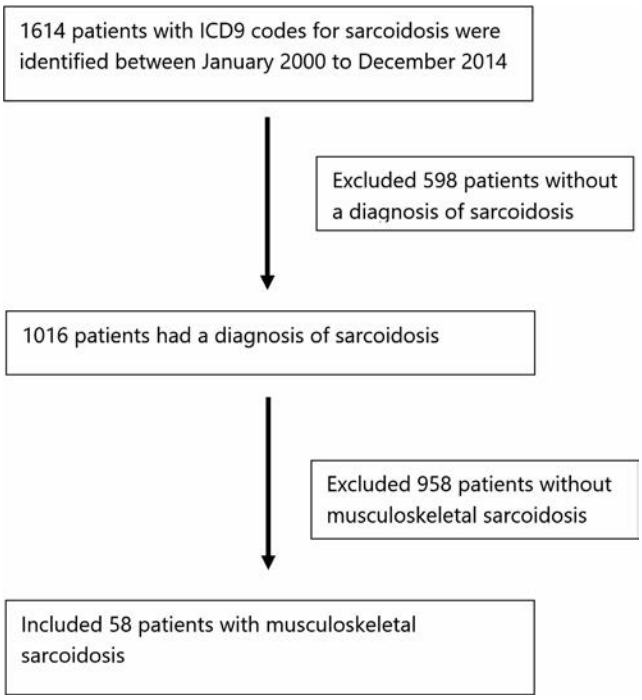


FIGURE 1 Cohort selection: patients with International Classification of Diseases (ICD)9 codes for sarcoidosis were identified, manual data extraction was performed and 58 patients with musculoskeletal sarcoidosis were included

Osseous sarcoidosis was symptomatic in 10/15 patients, most commonly as back pain, followed by thigh, maxillary, sternum and toe pain. The remaining patients (5/15) were identified incidentally by imaging. Diagnosis was biopsy-proven in 9/15 patients. Osseous sarcoidosis most commonly affected the lumbar spine (60%), followed by pelvis (53.3%), thoracic spine (46.7%), humerus (46.7%), sacrum (40%), femur (40%), ribs (26.7%), cranium (20%), scapula (20%), tibia (20%). Other areas were involved in a minority of patients (Figure 5). Osseous sarcoidosis was the initial manifestation of sarcoidosis in 7/15 (46.7%) patients.

Sarcoid myopathy was symptomatic in all patients, 4/4 presented with myalgia, 2/4 experienced weakness. The distribution of symptoms was in proximal muscle groups of upper and lower extremities in 4/4, distal lower extremities in 2/4, distal upper extremities in 1/4, and cervical muscles in 1/4 patients (Figure 6). Palpable muscular nodules were not reported in any of the patients. Sarcoid myopathy was the initial manifestation of sarcoidosis in 3/4 (75%) patients. All sarcoid myopathy patients had only moderate elevations in creatine kinase. Other enzymes such as aldolase, alanine transaminase, aspartate transaminase or lactate dehydrogenase were not tested prior to initiation of treatment in any of the patients. Electromyogram (EMG) was done in 2/4, out of which 1 was abnormal, showing rapidly recruiting motor unit potentials, suggestive of myopathic process. Imaging was

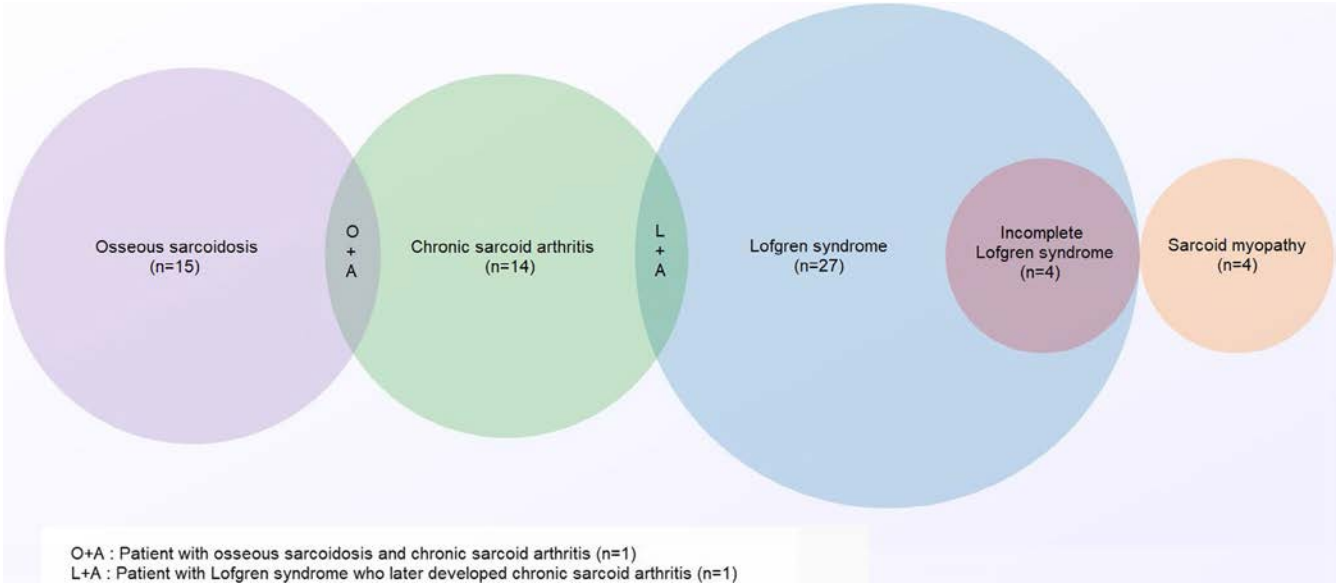


FIGURE 2 Venn diagram showing overlap between the different types of musculoskeletal sarcoidosis in our patient population

FIGURE 3 Joint involvement in Lofgren syndrome

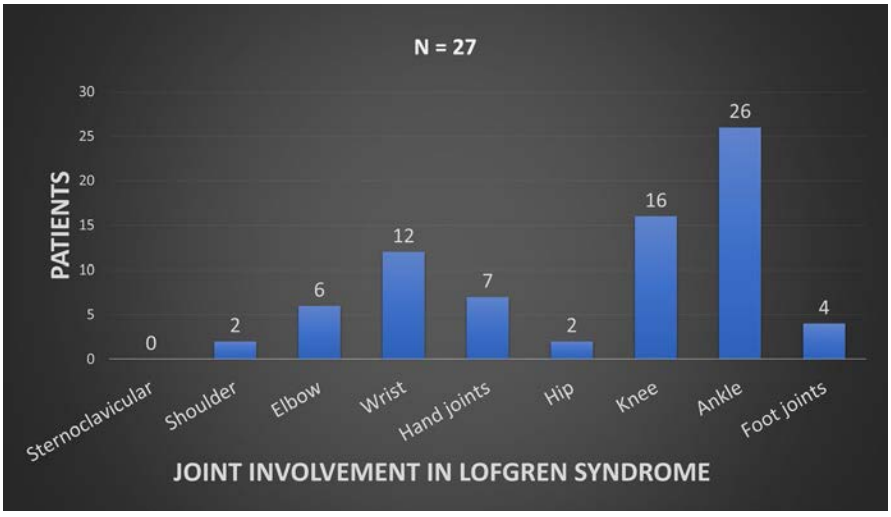
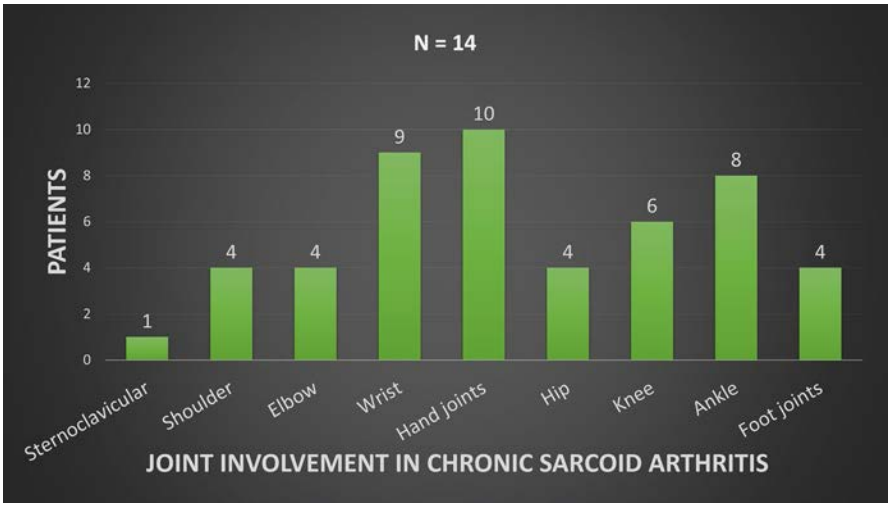


FIGURE 4 Joint involvement in chronic sarcoid arthritis



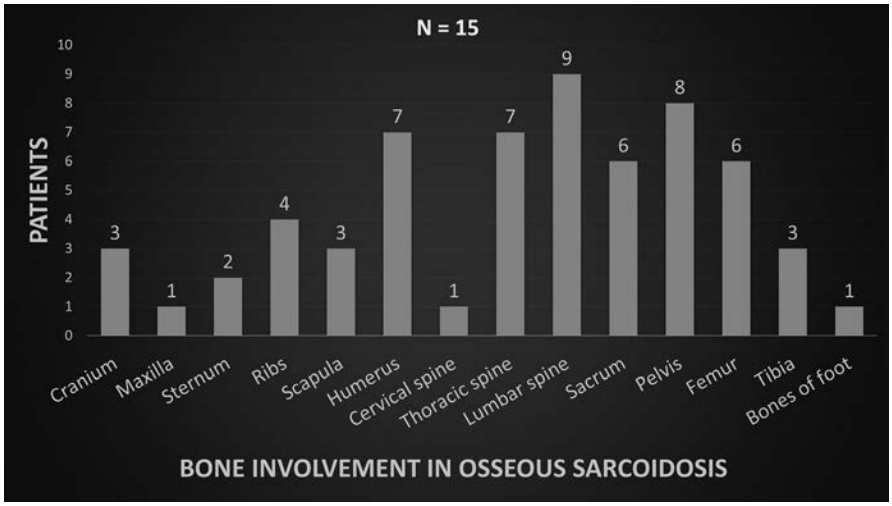


FIGURE 5 Sites of bone involvement in osseous sarcoidosis

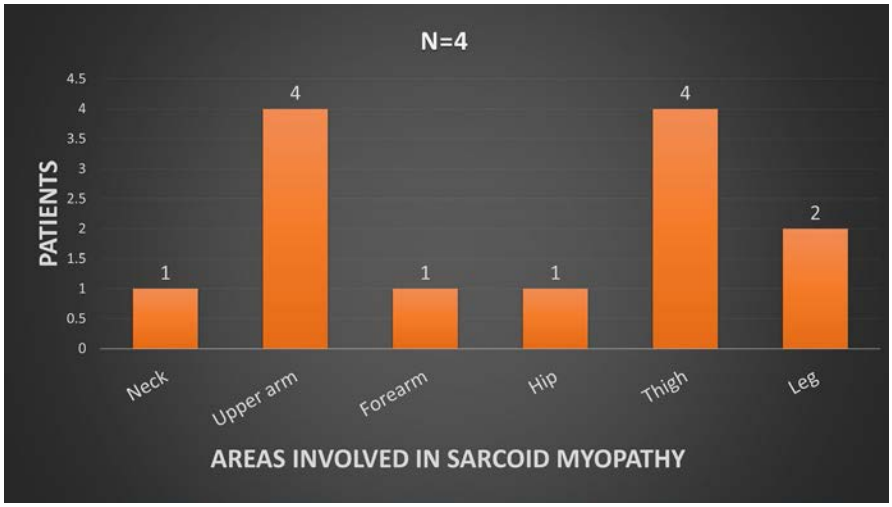


FIGURE 6 Sites affected in sarcoid myopathy

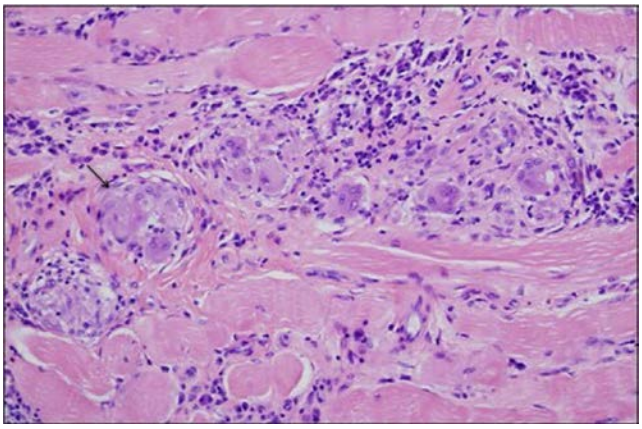


FIGURE 7 Muscle biopsy with hematoxylin and eosin stain showing non-caseating granulomas (magnification 200×)

not pursued in any of the cases. All patients had the diagnosis proven on muscle biopsy. A section from the muscle biopsy of a patient with sarcoid myopathy showing non-caseating granulomas is shown in Figure 7. On histopathology report, non-caseating granulomatous inflammation was noted in perimysium in 3/4, perivascular area in 2/4, and endomysium in 1/4 patients.

There was variation in the pharmacologic agents used in the treatment of MSK manifestations of sarcoidosis (Table 2). Lofgren syndrome was most commonly treated with prednisone, followed by non-steroidal anti-inflammatory drugs (NSAIDs). Treatment was considered effective in 6/8 patients treated with NSAIDs and 16/16 patients treated with prednisone. Four patients had resolution of symptoms at the time of evaluation, hence not requiring treatment. Conventional synthetic disease-modifying antirheumatic drug (csDMARD) was used in 1 patient. This patient was treated with methotrexate in addition to prednisone. In patients who were treated, arthritis resolved within 6 months. Median duration of treatment with prednisone was 11 weeks (interquartile range [IQR] 8-18). We did not capture any recurrence of Lofgren syndrome.

Initial treatment in chronic sarcoid arthritis consisted of prednisone alone in 3 patients, prednisone and csDMARD in 5 patients, and csDMARD alone in 4 patients. The most commonly used csDMARD was methotrexate, followed by hydroxychloroquine. Biologic DMARD (bDMARD) was used in 1 patient after the initial

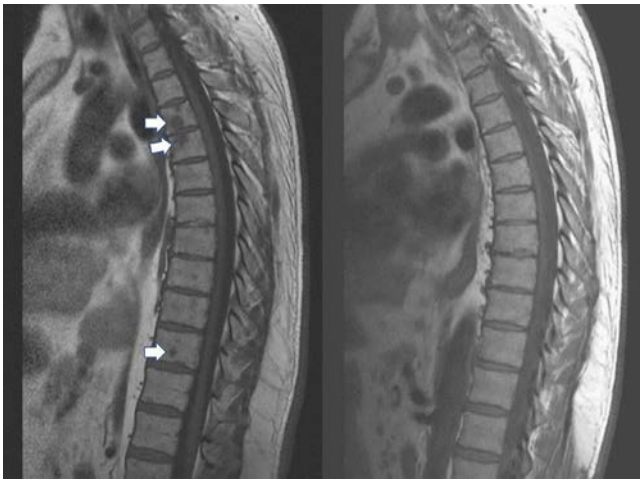


FIGURE 8 Sagittal T1 weighted, non-fat suppressed magnetic resonance imaging showing multiple hypo-intense lesions in thoracic spine before treatment (left, see arrows) and 2 y after treatment with anti-tumor necrosis factor inhibitor (right). Note: resolution of osseous sarcoid lesions post-treatment

treatment with csDMARD lost effectiveness. This patient was sequentially treated with etanercept, infliximab and then adalimumab. Prednisone was considered to be effective in 7/7, while methotrexate was in 4/5 patients. All 3 bDMARDs were considered effective but required switching due to secondary failure.

Most common initial treatment in osseous sarcoidosis was prednisone followed by addition of csDMARD for maintenance. Methotrexate was the most commonly used csDMARD. Biologic DMARD (infliximab followed by adalimumab and then etanercept) were used in 1 patient who failed initial csDMARD. Five patients with asymptomatic osseous sarcoidosis were not treated. Prednisone and methotrexate were considered effective in 5/6 and 3/5 patients, respectively. Similar to the patient with chronic sarcoid arthritis, bDMARDs were effective in osseous sarcoidosis but required to be switched due to the treating clinician's impression off loss of effectiveness (Figure 8).

In sarcoid myopathy, patient #1 was lost to follow up after diagnosis and was therefore not treated. Patient #2 was treated with prednisone alone for 1 year; this was noted to be effective. Patient #3 was treated with prednisone and hydroxychloroquine for more than 4 years without significant improvement in myalgia and weakness. This patient was reluctant to try any other DMARD due to concerns for adverse effects. Patient #4 was treated with prednisone initially; methotrexate and infliximab were later added for steroid sparing. Effectiveness of methotrexate was not reported. Prednisone and infliximab were both reported as effective in improving myalgia.

In contrast to patients with Lofgren syndrome, patients with the other 3 forms of MSK sarcoidosis required to be followed for longer term on maintenance therapy. The median follow up for chronic sarcoid arthritis and osseous sarcoidosis were 5.3 years (IQR 3.2-7.9) and 2.4 years (IQR 0.25-5.8) respectively. The 3 patients with sarcoid myopathy were followed between 3.7-9.8 years.

4 | DISCUSSION

The frequency of MSK involvement in patients with sarcoidosis has been reported in 1%-40% of patients.[8,10-12] Furthermore, MSK involvement has been reported to be higher in women, with racial variation noted in several studies.[6] Large studies have typically described each MSK sarcoidosis syndrome in isolation, at times combining acute and chronic sarcoid arthritis.[3] We herein describe all 4 forms of MSK sarcoidosis in a single academic center. The proportion of patients with MSK sarcoidosis was 5.7%, the majority were females and Caucasians. When comparing the proportion of African American populations in the state of Iowa (4.1%) with the proportion of African American patients with MSK sarcoidosis in this cohort (10.3%), the latter was higher, which may be reflective of higher prevalence of sarcoidosis in this racial group. Little can be said about the racial predominance of MSK sarcoidosis given the protean racial distribution of the disease and scarcity of data on reported MSK sarcoidosis among different racial groups.

Lofgren syndrome was first described in 1946, defined formally in 1953 and is the most commonly reported MSK syndrome in sarcoidosis.[13] Acute sarcoid arthritis has been defined in the literature as the combination of erythema nodosum, symptom duration of <2 months, age < 40 and ankle arthritis.[9] Others have described acute sarcoid arthritis as separate from Lofgren syndrome or described acute sarcoid arthritis as a Lofgren syndrome variant.[7,8,10,14-17] In this cohort we considered acute sarcoid arthritis and classic Lofgren syndrome as synonymous. Given that erythema nodosum is not present in all patients at the time of onset of inflammatory arthritis/periarthritis, we used the term "incomplete Lofgren syndrome" to refer to such patients. Due to lack of specific description of periarthritis in the clinical notes, we are unable to report the frequency of periarthritis in this cohort. Prior studies have shown a female predominance for Lofgren syndrome, a higher prevalence of Lofgren syndrome in Caucasians while showing a low prevalence in Japan and India.[3,7,14,15,17-20] We found Lofgren syndrome predominantly affecting the ankle joint (96.3%), which is similar to previous studies (reported between 75%-90%). Following the ankle joint, we found knee, wrist, hand joints and elbow to be involved in that order, which is consistent with other studies.[3,7,9,10,12,14,21] Chatham et al. reported shoulder involvement to be rare in Lofgren syndrome;[6] this aligns with our results in which 2/27 patients had shoulder involvement. To our knowledge this is the first time Lofgren syndrome has been reported with hip involvement. In all studies cited, treatment with NSAIDs and steroids were effective, and resolution of Lofgren syndrome occurred within 1.5 years of treatment, although most patients obtained remission within 6 months. We found similar therapeutic responses in our cohort.

In a Caucasian US population, chronic sarcoid arthritis has been described in 0.8% of patients diagnosed with pulmonary sarcoidosis[3] and most literature cites this entity as "rare". In the study by Salari et al. involving 26 patients with sarcoid arthritis, there were no patients with chronic sarcoid arthritis identified.[8] To our knowledge,

this is the largest cohort of patients with chronic sarcoid arthritis described in the USA. Chronic sarcoid arthritis has been described as commonly affecting shoulder, wrist, hand joints, foot joints, knee and ankle, and having a symptom duration of >6 months.[9,10,14,18,21] We identified it commonly affecting hand joints followed by the wrist, ankle, knee, shoulder, hip and elbow joints. Again, to our knowledge, this is the first report of hip joint involvement in chronic sarcoid arthritis. The prevalence of chronic sarcoid arthritis in our cohort is 1.3% (14/1016) which is higher than the 0.2% quoted by Nessrine et al.[21] Literature on treatment for chronic sarcoid arthritis is scant. In this cohort, patients were treated initially with prednisone and csDMARD followed by bDMARD were added for maintenance. Methotrexate and hydroxychloroquine were the most commonly used DMARDs.

Osseous sarcoidosis has been found to affect between 1% and 15% of patients with sarcoidosis,[9,11,17,21,22] and up to 50% of patients have been reported to be asymptomatic.[25] We estimated the prevalence of 1.4% and it is the second most common MSK involvement after Lofgren syndrome. We found 33.3% of patients with osseous sarcoidosis to be asymptomatic. The largest study done by Hassine et al. found a slight predominance in Caucasian individuals and a high incidence of pulmonary and extra-pulmonary involvement with osseous sarcoidosis, for example, lymph nodes and skin involvement. Additionally, they found the 2 most commonly affected sites were the spine and pelvis,[11] which aligns with our results, where 60% of cases had lumbar spine and 53.3% had pelvic involvement. In contrast to Hassine et al. who described humerus involvement in 19% of patients, we found the humerus was the third most commonly affected at 46.7%. We also found a higher prevalence of sacral and femoral involvement (40% each). In our cohort we did not capture any patients with involvement of the small bones of hand, but this has been reported in other studies as a common site of involvement.[5,9-11,19,23-26] Similar to our study, treatment has involved use of steroids, methotrexate and hydroxychloroquine,[11] with prednisone and methotrexate appearing most effective in our cohort.

Sarcoid myopathy has been reported to affect 25%-75% of patients in an asymptomatic manner.[5,9,10,19,23,24,26] Symptomatic sarcoid myopathy has been reported to affect <0.5% or up to 3% of patients with sarcoidosis.[21,24,27] In our study, sarcoid myopathy affected 0.3% of patients. Similar to previously reported phenotypes, proximal upper and lower extremities were more commonly affected; however, in our study there were 2 patients with distal lower extremities involvement and 1 patient with cervical muscle involvement. Three subtypes of sarcoid myopathy have been described in the literature, chronic myopathy being the most common, nodular myopathy and acute myositis as the least common. Most recently Cohen Aubart et al. described a large cohort of sarcoid myopathy patients, identifying 4 subtypes of sarcoid myopathy: nodular, smoldering, myopathic, and combined myopathic and neurogenic pattern.[23] Our patients best fit the myopathic pattern described by Cohen Aubart et al., based on presence of motor weakness and lack of palpable muscular nodules or neurologic involvement. There is an unmet need for standardized classification of sarcoid myopathy subtypes.

Treatment for MSK involvement of sarcoidosis has not been well studied and the typical course described in the literature starts with NSAIDs and low-dose steroids as first line, followed by methotrexate and high-dose steroids as second line, followed by bDMARDs.[8,11] A similar approach to treatment of MSK sarcoidosis was noted in this cohort, with the exception of Lofgren syndrome which usually does not require addition of DMARDs.

To our knowledge this is the largest cohort of MSK sarcoidosis described to date in the USA. It is also the largest cohort following strict inclusion criteria and describing all 4 subtypes of MSK sarcoidosis. This allowed us the opportunity to describe the relative proportion of each subtype of MSK involvement in sarcoidosis. We described a higher prevalence of chronic sarcoid arthritis in our cohort as compared to prior studies. Since this is a poorly described and quantified subtype of MSK involvement, larger studies are required to determine true prevalence. There are several limitations to our study inherent to its retrospective design. The exact prevalence of osseous sarcoidosis in this cohort is likely underestimated as skeletal imaging was not routinely obtained. This highlights the challenge in estimating prevalence of osseous sarcoidosis given that a significant proportion of cases are asymptomatic. It remains unclear if routine screening for asymptomatic osseous sarcoidosis adds any clinical or prognostic value. The true skeletal distribution of osseous sarcoidosis is not clear, as not all patients underwent entire axial and peripheral skeletal imaging. Other studies have also used a combination of serological markers and radiographic imaging to determine disease progressions/response to treatment, although there are no clear guidelines that either of these are useful, and this would be an area that could be further explored in the future.

5 | CONCLUSION

In conclusion, the 4 MSK sarcoidosis syndromes are of rather low prevalence among sarcoidosis patients. In patients who do develop MSK manifestations of sarcoidosis, they are commonly a part of the initial presentation of sarcoidosis (with 69% of patients with MSK sarcoidosis having MSK symptoms as the initial manifestation). There is an unmet need for interdisciplinary and multi-institutional efforts to establish standardized classification criteria for the 4 MSK sarcoidosis syndromes.

CONFLICT OF INTEREST

Dr Singh is funded by the Rheumatology Research Foundation. Other authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

SP, study design, acquisition of data, drafting the manuscript, approval of submitted version and personally accountable for their contributions. CH, study design, drafting the manuscript, approval

of submitted version and personally accountable for their contributions. MA, study concept, design, acquisition of data, drafting the manuscript, approval of submitted version and personally accountable for their contributions. SJ, study design, acquisition of data, drafting the manuscript, approval of submitted version and personally accountable for their contributions. EHF, study concept, design, drafting the manuscript, approval of submitted version and personally accountable for their contributions. NS, study concept, design, acquisition of data, drafting the manuscript, approval of submitted version and personally accountable for their contributions.

ETHICAL APPROVAL

This study was approved by the Institutional Review Board at the University of Iowa and the Iowa City VA.

CONSENT FOR PUBLICATION

This is a retrospective study, and no patient consent was obtained.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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ORIGINAL ARTICLE

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Comparison of baseline laboratory findings of macrophage activation syndrome complicating systemic juvenile idiopathic arthritis and multisystem inflammatory syndrome in children

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Abstract

Aims: Recently, multisystem inflammatory syndrome in children (MIS-C) has been recognized in association with coronavirus disease 2019 as a cytokine storm syndrome. MIS-C presents with symptoms similar to Kawasaki disease and macrophage activation syndrome (MAS). We aimed to better understand this cytokine storm syndrome by comparing the initial laboratory findings of MIS-C and MAS.

Methods: Patients who were diagnosed with MAS due to systemic juvenile idiopathic arthritis in our clinic between March 2002 and November 2020 and with MIS-C between 20 September and 20 October 2020 were enrolled into the study. The medical files of all patients were reviewed retrospectively.

Results: A total of 13 MAS (9 boys, 4 girls) and 26 MIS-C (16 boys,10 girls) patients were included in the study. Hemoglobin, absolute neutrophil and lymphocyte counts, C-reactive protein (CRP), ferritin, fibrinogen and lactate dehydrogenase (LDH) levels showed significant differences between the two groups ($P < 0.05$). Patients with MAS had lower hemoglobin (10.10 g/dL) and fibrinogen (2.72 g/dL), but higher ferritin (17 863 mg/dL) and LDH (890.61 U/L) at the time of diagnosis. Patients with MIS-C had higher absolute neutrophil count (12 180/mm^3) and CRP (194.23 mg/dL) values, but lower absolute lymphocyte count (1140/mm^3) at the time of diagnosis. Left ventricle ejection fraction was significantly lower in the MIS-C group in echocardiographic evaluation ($P < 0.001$).

Conclusion: Ferritin, hemoglobin, LDH, and fibrinogen levels were significantly changed in MAS compared with MIS-C. However, patients with MIS-C have more severe signs than MAS, such as cardiac involvement.

KEYWORDS

coronavirus disease 2019, cytokine storm syndrome, macrophage activation syndrome, multisystem inflammatory syndrome in children

1 | INTRODUCTİON

Multisystem inflammatory syndrome in children (MIS-C) is a novel syndrome that has been recently recognized in association with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[1] Previous reports of coronavirus disease 2019 (COVID-19) indicated mild or asymptomatic course in the majority of children.[2] In April 2020, pediatric multisystem inflammatory syndrome–temporally associated with SARS-CoV-2 was identified by physicians from the United Kingdom and France in children with a hyperinflammatory condition characterized by fever, cardiovascular shock, and suspected SARS-CoV-2 infection.[3-5] The US Centers for Disease Control and Prevention (CDC) described MIS-C as children with fever, laboratory evidence of inflammation, multisystem organ involvement, severe illness, and SARS-CoV-2 infection or exposure.[1] Patients have clinical signs and laboratory abnormalities that indicate cytokine storm syndrome, such as high fever, confusion, and coagulopathy.[6] MIS-C has variable presentations with features resembling macrophage activation syndrome (MAS), Kawasaki shock syndrome, and toxic shock syndrome.[7]

Pediatric rheumatologists are alert for MAS among their own patient populations (systemic juvenile idiopathic arthritis [sJIA], systemic lupus erythematosus, Kawasaki disease). MAS is a term used as a synonym for secondary hemophagocytic lymphohistiocytosis and is a potentially life-threatening complication of systemic inflammatory diseases usually encountered by rheumatologists, especially during the course of sJIA in childhood.[8] Systemic JIA comprises 10%-20% of all JIA patients and is called adult-onset Still's disease when the onset of the disease is after the age of 16 years.[9,10] MAS is one of the major emergencies of pediatric rheumatology and develops in 5%-10% of sJIA patients. This devastating complication is characterized by sustained fever, hepatosplenomegaly, central nervous system dysfunction, and multiple organ failure. Hyperferritinemia, coagulopathy, pancytopenia, elevated liver enzymes, triglycerides, and C-reactive protein (CRP) with low erythrocyte sedimentation rates and fibrinogen are main laboratory findings of MAS.[11] Soluble CD163 and soluble interleukin-2 (IL-2) receptor increase in active MAS. Hematopoietic cell phagocytosis of macrophages can be shown in bone marrow evaluation, but hemophagocytosis may not be present in early stages. Activation of cytotoxic CD8 T cells and macrophages, proinflammatory cytokine storm, decrease in cytolytic activity of natural killer cells, and increase in soluble IL-2 receptor-α (SCD25) are responsible for pathogenesis.[12] All of the findings in MAS mentioned above are the results of hyperinflammation and these findings may also be encountered in patients with MIS-C. There are no specific clinical or laboratory markers to identify MAS. It is difficult to distinguish MAS from diseases with the same clinical features such as sJIA flares and sepsis-like syndromes.[13] In 2016, Ravelli et al[12] presented MAS classification criteria to facilitate the diagnosis of MAS in patients with sJIA. Early diagnosis and treatment are very important as MAS causes death in 8%-20% of patients. Cytokine-targeting therapy (IL-1 and IL-6 blockade) are crucial in controlling hyperinflammation and are used widely in patients with MAS due to sJIA. MIS-C is also a hyperinflammatory condition and rheumatologists are involved in the follow up of patients with particularly severe course. For this reason, rheumatologists share their practices in the area of anti-cytokine therapy for the management of MIS-C all around the world.[14]

However, the hyperinflammatory syndrome detected in COVID-19 is incompletely identified, it has similarities with other hyperinflammatory disorders such as secondary hemophagocytic lymphohistiocytosis, MAS, MAS-like sepsis syndrome, and cytokine release syndrome. In this study, we aimed to better understand and to be conscious of this newly described cytokine storm syndrome by comparing the initial laboratory findings of MIS-C and MAS.

2 | MATERIALS AND METHODS

Patients who were diagnosed with MAS due to sJIA in our clinic between March 2002 and November 2020 and with MIS-C according to the CDC criteria between 20 September and 20 October 2020 were enrolled into the study. Systemic JIA diagnosis is based on International League of Associations for Rheumatology criteria.[15] The diagnosis of MAS was made according to the classification criteria that were reported by Ravelli et al[12] in 2016: ferritin >684 ng/mL is accompanied by at least two out of the following four criteria; (a) platelet count ≤181 × 10^9/L, (b) aspartate aminotransferase >48 U/L, (c) triglyceride >156 mg/dL, (d) fibrinogen ≤360 mg/dL. CDC recommendations were considered for the diagnosis of MIS-C.[1]

Data were collected from the files of patients. Age, gender, presenting features, and all initial laboratory findings such as hemoglobin, absolute neutrophil and lymphocyte count, platelet count, CRP, erythrocyte sedimentation rates, ferritin, fibrinogen, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase (LDH), albumin, creatinine, sodium, echocardiography and abdomial ultrasonography findings, and consciousness of patients were recorded. The drugs used in the follow up of the disease, the course, and the final status of the patients were included in the data. All findings of the patients in the MAS and MIS-C groups were compared. The study complies with the Declaration of Helsinki and the protocol was approved by the institutional ethics committee.

2.1 | Statistical analysis

Results are given as mean ± SD, median (range) or proportion as appropriate. Kolmogorov-Smirnov/Shapiro-Wilk's test was used to identify whether the data were distributed normally or non-normally. Comparisons of categorical variables were applied by χ^2 test. Comparison between two groups for the non-normally distributed variables was evaluated by Mann-Whitney *U* test. A *P* value less than 0.05 was considered significant. All of the analyses were achieved using SPSS Version 17.0 (SPSS Inc., Chicago, IL, USA).

3 | RESULTS

A total of 13 MAS (9 boys,4 girls) and 26 MIS-C (16 boys,10 girls) patients were enrolled in the study. Mean age at the time of diagnosis was 6.10 ± 4.52 years and 8.80 ± 4.23 years in the MAS and MIS-C groups, respectively ($P = 0.081$). All MIS-C patients had reactive COVID-19 serology. Table 1 shows the demographic and clinical features of the patients.

All patients in the MAS group had fever, rash, and arthritis for at least 15 days at the time of sJIA diagnosis. Eleven (84.6%) patients had organomegaly and four (30.8%) had serositis. All patients in the MIS-C group had fever and gastrointestinal symptoms such as abdominal pain, diarrhea, and vomiting before diagnosis. Fifteen (57.7%) patients had conjunctivitis and rash, 8 (30.8%) had organomegaly, and 12 (46.2%) had serositis. Abdominal ultrasound revealed an increase in thickness and edema in the wall of the intestinal loops, indicating inflammation with free fluid in nine patients. One (3.8%) patient underwent an appendectomy. Organomegaly, serositis, and cardiac involvement, which are clinical findings that may develop in both diseases, were compared. Left ventricle ejection fraction (LVEF; 57.8%) was significantly lower in the MIS-C group in echocardiography evaluations ($P < 0.001$) and the patients with MAS had a higher rate of organomegaly (84.6%, $P = 0.002$). There was no significant difference between the two groups for serositis (Table 1).

Initial laboratory parameters (hemoglobin, absolute neutrophil and lymphocyte counts, platelet count, CRP, erythrocyte sedimentation rates, ferritin, fibrinogen, alanine aminotransferase, aspartate aminotransferase, LDH, albumin, creatinine, sodium) at the time of diagnosis of MAS and MIS-C were compared. The triglyceride levels studied in the MAS group were not routinely checked in the MIS-C

TABLE 1 Clinical and demographic characteristics of patients with MAS and MIS-C

Characteristics	Patients with MAS (n = 13)	Patients with MIS-C (n = 26)	*P* value
Age at diagnosis; mean ± SD (y)	6.10 ± 4.52	8.80 ± 4.23	0.081
Male/female	9/4	16/10	0.64
Hemoglobin (g/dL)	10.10 ± 1.78	11.66 ± 1.47	**0.014**
Absolute neutrophil count (mm^3)	4830 ± 3030	12 180 ± 7580	**<0.001**
Absolute lymphocyte count (mm^3)	2690 ± 1680	1140 ± 840	**<0.001**
Platelet count (mm^3)	229 460 ± 227 900	189 000 ± 102 440	0.67
CRP (mg/dL)	106.75 ± 80.29	194.23 ± 74.82	**0.004**
ESR (mm/h)	45.58 ± 29.36	53.03 ± 31.38	0.59
Ferritin (mg/dL)	17 863 ± 21 846	753 ± 586	**<0.001**
Fibrinogen (g/dL)	2.72 ± 1.24	5.01 ± 1.68	**<0.001**
ALT (U/L)	71.30 ± 60.96	47.24 ± 31.70	0.36
AST (U/L)	111.30 ± 124.15	49.44 ± 37.64	0.45
LDH (U/L)	890.61 ± 627.68	319.08 ± 4.56	**0.002**
Albumin (mg/dL)	31.09 ± 4.56	36.33 ± 12.07	0.207
Creatinine (mg/dL)	0.41 ± 0,19	0.54 ± 0.24	0.078
Sodium (mEq/L)	134.66 ± 3.42	134.80 ± 5.12	0.59
Echocardiography—ejection fraction (%)	69.40 ± 5.15	57.80 ± 6.73	**<0.001**
Organomegaly (%)	11(84.6%)	8 (30.8%)	**0.002**
Hepatosplenomegaly (n)	9	3	
Hepatomegaly (n)	1	4	
Splenomegaly (n)	1	1	
Serositis (%)	4 (30.8)	12 (46.2%)	0.29
Change in consciousness (%)	1 (7.7%)	4 (15.4%)	0.45
Need for intubation (%)	1 (7.7%)	4 (15.4%)	0.45
Exitus	1(7.7%)	1 (3.8%)	0.56

Note: Statistically significant *P* values are highlighted in bold.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rates; LDH, lactate dehydrogenase; MAS, macrophage activation syndrome; MIS-C, multisystem inflammatory syndrome in children.

Statistically significant *P* values are highlighted in bold.

TABLE 2 Treatments in patients with MAS and MIS-C

Treatments	MAS n (%)	MIS-C n (%)	*P* value
Pulse methylprednisolone (30 mg/kg, 3-5 consecutive days, max: 1 g/dose)	13 (100)	23 (88.5)	0.28
Prednisolone (2 mg/kg/day, max:60 mg)	13 (100)	25 (96.2)	0.66
Intravenous immunoglobulin (1-2 g/kg, max:75 g)	4 (30.8)	26 (100)	**<0.001**
Anakinra (4-10 mg/kg/day)	6 (46.2)	18 (69.2)	0.14
Tocilizumab (<30 kg 12 mg/kg, >30kg 8 mg/kg)	1 (7.7)	2 (7.7)	0.72
Cyclosporine (2.5-5 mg/kg/day)	5 (38.5)	–	**0.002**
Plasmapheresis	1 (7.7)	7 (26.9)	0.64

Note: Statistically significant *P* values are highlighted in bold.

Abbreviations: MAS, macrophage activation syndrome; MIS-C, multisystem inflammatory syndrome in children.

group, and the D-dimer, brain natriuretic peptic, and troponin levels studied in the MIS-C group were not routinely checked in the MAS group. Therefore, laboratory tests for those were not included in the comparative analysis because of the missing data.

Hemoglobin, absolute neutrophil and lymphocyte count, CRP, ferritin, fibrinogen, and LDH levels showed significant differences between the two groups ($P < 0.05$). Patients with MAS had lower hemoglobin (10.10 g/dL) and fibrinogen (2.72 g/dL), and higher ferritin (17 863 mg/dL) and LDH (890.61 U/L) values at the time of diagnosis. Patients with MIS-C had higher absolute neutrophil count (12 180/mm^3) and CRP (194.23 mg/dL) values, and lower absolute lymphocyte count (1140/mm^3) at the time of diagnosis. There was no significant difference between the two groups for platelet counts (Table 1).

In our hospital, MIS-C patients requiring intensive care were consulted to the pediatric rheumatology department, and we primarily evaluated these patients. Therefore, all of the MIS-C patients in this study were initially followed up in the intensive care unit because of hypotension, poor general condition, low oxygen saturation, and left ventricular dysfunction. Confusion, respiratory distress, and need for mechanical ventilation developed in four patients in the MIS-C group and in one patient in the MAS group ($P > 0.05$). In the MAS group, only one patient was followed up in the intensive care unit. One patient with MAS and one patient with MIS-C died from multiorgan insufficiency ($P > 0.05$).

All MAS patients were initially given high-dose methylprednisolone and continued with 2 mg/kg/day prednisolone treatment. Four patients (30.8%) received intravenous immunoglobulin (IVIG), 6 (46.2%) patients received anakinra, 1 (7.7%) patient received tocilizumab, 5 (38.5%) patients received cyclosporine treatments. Plasmapheresis was performed in one (7.7%) patient. All MIS-C patients were initially received IVIG. Twenty-three (88.5%) patients required high-dose methylprednisolone, 25 (96.2%) required prednisolone, 18 (69.2%) required anakinra, and 2 (7.7%) required tocilizumab therapy. Plasmapheresis was performed in seven (26.9%) patients. All treatments with doses in two groups are shown in Table 2.

4 | DISCUSSION

We have recognized MIS-C very recently and its pathophysiology is not yet fully understood, but it is thought to have developed as a result of a cytokine storm, as in MAS. However, MAS and MIS-C have both similar and distinct findings, and their identification will provide a better understanding of the pathophysiology and appropriate management of MIS-C. The main objective of this study was to compare the laboratory parameters that existed at the time of diagnosis between the two diseases. We found that there were significant differences in hemoglobin, absolute neutrophil and lymphocyte counts, CRP, ferritin, fibrinogen, and LDH levels between the two groups. Patients with MAS had lower hemoglobin and fibrinogen, and higher ferritin and LDH values, and patients with MIS-C had higher absolute neutrophil count and CRP values, and lower absolute lymphocyte count at the time of diagnosis.

Macrophage activation syndrome is a cytokine storm syndrome that develops as a result of a dysregulated and highly stimulated immune response involving the persistent activation and expansion of T lymphocytes and macrophages, which is mostly encountered by pediatric rheumatologists in patients with sJIA.[16] The signs of MAS present subclinically in 30%-40% of sJIA patients. Early suspicion of MAS is crucial to begin therapy before the injury due to hyper-cytokinemia turns out to be irreversible.[17] However, MAS is a challenging condition to diagnose because of the variability of clinical and laboratory findings and the absence of typical features. Changes in the laboratory parameters of patients followed up with a diagnosis of sJIA are warning signs for the development of MAS. Close monitoring of laboratory biomarkers can provide the diagnosis in the pre-MAS stage. The classification criteria developed for the early recognition of MAS were also based on laboratory tests.[12] Patients with active sJIA often have high platelet count, increased fibrinogen, and sedimentation levels, whereas in MAS, platelet counts, fibrinogen, and sedimentation levels tend to decrease. Meanwhile, CRP levels increase in both condition.[18] In some previous studies, initial laboratory tests of the pre-MAS and MAS stages were compared. Minoia et al[13] reported that platelet

count, liver transaminases, ferritin, LDH, triglyceride, and D-dimer levels showed changes between pre-MAS and MAS visits. Çakan et al[18] found that five laboratory tests differed between pre-MAS and MAS onset: ferritin (11.6 times higher), LDH (6.1 times higher), platelets (5.3 times lower), leukocytes (3.0 times lower), and fibrinogen (2.6 times lower). Based on the studies mentioned above, the high ferritin and LDH and lower fibrinogen values in our MAS group compared with the MIS-C group showed that MIS-C patients may have similar laboratory parameters with patients in the pre-MAS stage. Although the laboratory findings of MIS-C were similar to pre-MAS, patients initially presented with more severe cardiac findings. Initial echocardiography assessments showed a lower LVEF in the MIS-C group than in MAS patients (57.8% vs 69.4%, $P < 0.001$). In MIS-C, the cardiovascular system is frequently affected and is one of the main factors determining disease severity. Several cardiac findings were reported such as left ventricular dysfunction, coronary artery enlargement or aneurysms, arrhythmias, valvular dysfunction, and pericardial effusion. Depressed LVEF has been reported in approximately 40%-60% of patients.[19-21] Matsubara et al[22] reported that patients with MIS-C had lower LVEF than normal control individuals and patients with Kawasaki disease. In addition, our results showed that the MIS-C group had lower LVEF than the MAS group. Cardiac involvement often develops as pericarditis in MAS patients.[15] In this study, the patients had 30.8% and 46.2% serositis, respectively, in the MAS and MIS-C groups ($P = 0.29$).

The COVID-19-associated MIS-C is a cytokine storm syndrome with excessive cytokine release from the uncontrolled immune response. As a result of reduced cytotoxicity, the clearance of infected cells and elimination of active macrophages is impaired and proinflammatory mediators are released massively. Patients frequently present with fever, cytopenias, coagulopathy, high transaminase levels, hyperferritinemia, and multiorgan dysfunction.[23] The hyperinflammation encountered in COVID-19 is not MAS and is relatively different among other infectious cytokine storm syndromes. There are modest ferritin elevations compared with other cytokine storm syndromes.[23,24] This study has supported this information that patients with MAS complicating sJIA had significantly higher mean ferritin levels than MIS-C patients (17 863 mg/dL vs 753 mg/dL, $P < 0.001$). There was hypofibrinogenemia in the MAS group, and fibrinogen levels were increased in the MIS-C group ($P < 0.001$). Hemoglobin values were significantly lower in the MAS group ($P = 0.014$). Additionally, organomegaly was significantly higher in the MAS group ($P = 0.002$). We attribute these differences to the presence of an inflammatory disease such as sJIA before the onset of MAS and the more acute development of MIS-C.

Hyperinflammation leads to multiple organ damage and it displays various signs such as coagulopathy (low platelet and fibrinogen levels and high D-dimer levels), tissue damage/hepatitis (high LDH, aspartate aminotransferase, and alanine aminotransferase levels), cytopenias (thrombocytopenia and lymphopenia), and macrophage/hepatocyte activation (high ferritin levels). Emerging findings also vary according to the character of the underlying disease. In this study, absolute neutrophils were significantly higher and absolute lymphocytes were significantly lower in the MIS-C group compared with the MAS group ($P < 0.001$). These values were consistent with COVID-19-associated MIS-C.[1,25]

After the identification of MIS-C as a cytokine storm, pediatric rheumatologists have been involved in the treatment process because they have experience in the treatment of MAS and frequently use anti-inflammatory and immunomodulatory therapies. Widespread experience in treating MAS and other cytokine release syndromes suggests the importance of early treatment to avoid life-threatening complications in MIS-C patients.[24] In patients with MIS-C, treatment with IVIG, corticosteroids, and anti-cytokine therapies should be considered according to the patients' signs and treatment responses.[25] In our study, treatment options were used to suppress hyperinflammation according to the clinical findings of the patients in the MAS and MIS-C groups. All patients diagnosed with MIS-C initially received IVIG. Corticosteroid and anakinra were added to the treatment of patients with a lack of improvement in clinical and/or diagnostic tests, respectively. Plasmapheresis was performed in patients who did not respond to corticosteroid plus anakinra. Two patients did not respond to the treatments mentioned above, and received tocilizumab—one of them responded to this therapy but the other died from multiorgan insufficiency.

Corticosteroids were the cornerstone of the treatment in both groups. IVIG treatment was used significantly more frequently in the MIS-C group. Cyclosporine was used in the MAS group, but it was never preferred in the MIS-C group. There was no significant difference between the two groups in the use of anakinra and tocilizumab. Although all of the MIS-C patients in this study were initially admitted to the intensive care unit, there was no significant difference in exitus rates between the two groups. A single-center retrospective design, a small sample of groups, and missing data were the limitations of our study.

In conclusion, ferritin, hemoglobin, LDH, and fibrinogen, which are important determinant parameters of cytokine storm, showed more marked changes in MAS. Nevertheless, patients with MIS-C presented with more severe signs than MAS and required more intensive care follow up. MIS-C reflects a different face of cytokine storm syndrome with its emerging findings. There are no clear treatment algorithms yet, but more comprehensive treatment algorithms will be developed over time with a better understanding of the pathophysiology of MIS-C.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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ORIGINAL ARTICLE

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Association between core stability and physical function, functional performance in patients with systemic sclerosis

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Abstract

Objective: To investigate the association of core stability with physical function and functional performance in patients with systemic sclerosis (SSc).

Methods: Forty patients who met the American College of Rheumatology / European League Against Rheumatism 2013 classification criteria for SSc were included in the cross-sectional study. For evaluation of core stability, trunk muscle endurance and trunk muscle strength were assessed. Trunk extensor and trunk flexor endurance tests were used for assessment of trunk muscle endurance. Trunk muscle strength was measured with a hand-held dynamometer and modified sit-up test. To measure physical function the Health Assessment Questionnaire Disability Index (HAQ-DI) and to measure functional performance 6-minute walking test (6MWT) and sit-to-stand test (STS) were used.

Results: Patients with SSc had lower mean trunk extensor and flexor endurance test times (49.87 ± 30.81 and 32.17 ± 15.42 seconds, respectively), modified sit-up test repetition (17.42 ± 7.81) and trunk extensor and flexor muscle strength (7.48 ± 2.29 kg and 6.20 ± 1.68 kg, respectively) when compared to the reference values in healthy individuals. All measurements were used to evaluate core stability associated with HAQ-DI score, 6DMWT walking distance and STS test duration (all $P < .05$).

Conclusion: Patients with SSc have markedly reduced core stability and this negatively affects the physical function and functional performance. Therefore, this study highlights the importance of trunk muscle in patients with SSc. We suggest that not only upper-lower extremity muscles, but also trunk muscle strength and endurance should be measured and core stability exercises can be added to the training programs to maintain and/or improve physical functions and functional performance in SSc patients.

KEYWORDS

core stability, functional performance, physical function, systemic sclerosis

1 | INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune and systemic disease characterized by vasculopathy, skin thickening, tendon fibrosis, calcinosis, stiffness, arthralgia or intra-articular changes, and inflammatory or non-inflammatory myopathy. These changes, which can lead to decreased joint movement, joint contracture, weakness of the proximal muscles, reduced physical capacity and worsened quality of life, are associated with significant functional disability and psychosocial and economic burden.[1,2]

Core stability is defined as the ability to control the position and movement of the trunk above the pelvis and leg. This lets the core to procure, deliver and supervise force and motion to the terminal segment during kinetic chain activities.[3] Three physiological subsystems, passive, active and neural control, are responsible for stabilization. The vertebrae, facet articulations, intervertebral discs, spinal cord and joint capsules form the passive musculoskeletal subsystem while the muscles and tendons around the spine form the active musculoskeletal subsystem. Various force and motion transducers in the ligaments, tendons, muscles and nerve control centers also form the neural subsystem. These 3 subsystems are closely related and problems such as tissue damage, poor muscle strength or endurance and poor muscle control seen in any subsystem may cause instability.[4,5] The systemic and chronic nature of SSc, widespread sclerosis, the activity of inflammatory mediators, pain and the presence of musculoskeletal involvement including changes in the bone, joint, ligaments and muscle may burden the body structure, which may lead to changes in the subsystem of core stability. However, no previous study has, to our knowledge, assessed core stability in patients with SSc.

Trunk muscles that make up the core have an important role in performing daily activities that include sitting, standing from the chair, reaching, unexpected perturbations, walking and maintaining dynamic postural stability.[6,7] Therefore, evaluation of the core stability indirectly allows us to assess an individual performing a relevant functional move or activity.[3,8] However, the evaluation of trunk is neglected in SSc patients and the majority of studies have focused on upper and lower limb muscle weakness. Moreover, unlike limbs, the evaluation of trunk muscles is more difficult in clinical practice due to the complex muscle attachments with fascia and lack of standardized tests.[9]

Low muscle strength and mobility, pain, lung and heart disease cause deterioration of physical capacity and functional performance in SSc patients. Moreover, it has been reported in previous studies that SSc patients have decreased muscle strength and endurance in the upper and lower extremities, and this is associated with decreased physical capacity and activity, impaired functional performance and worsening quality of life.[10-13] However, the effect of core stabilization on physical functions and performance in these patients is unknown. Understanding this relationship may emphasize the burden of this disease on core stability and the importance of core stability in the continuity of daily functional activities. We hypothesized that impairment of core stability, which has not been evaluated before in this group, has negative impact on physical function and functional performance in patients with SSc. Thus, the aim of the present study was to assess the association of core stability with physical function and functional performance in patients with SSc.

2 | METHODS

2.1 | Study design and participants

The 40 patients with SSc, diagnosed by rheumatologists according to the American College of Rheumatology/European League Against Rheumatism recommendations,[14] followed at a department of rheumatology between 2018 and 2020, were included in the cross-sectional study. Patients of both genders aged between 35 and 65 years participated in the study. The exclusion criteria were the presence of orthopedic and/or neurological disorders, history of surgery for spine or lower limb, the presence of low back pain, history of a cardiopulmonary disease not related to SSc, using an assistive device for ambulation and inability to perform the tests. The patients were asked to continue their regular medical treatment and to leave it in the event of an unstable condition during the study.

The study protocol included the Helsinki Declaration essentials and was approved by the Research Ethics Committee of the Dokuz Eylul University (Approval number: 2018/28-06). All patients signed an informed consent form before their participation in the study.

2.2 | Assessments

Demographic (age, gender, body mass index [BMI]) and clinical characteristics (duration of disease, type of disease, presence of interstitial lung disease [ILD], pulmonary arterial hypertension [PAH], gastrointestinal symptoms, arthralgia and Raynaud's phenomenon), smoking status and exercise habits of patients were recorded at initial interview. Core stability, physical function and functional performance of patients were measured. At least 10 minutes of rest intervals were given between each test so that fatigue caused by the tests did not affect the results.

2.3 | Core stability assessment

Core stability components are specified as endurance, strength, flexibility, motor control and function. However, there is no consensus on definition and measurement of core stability. Therefore, we used trunk extensor and flexor endurance tests, sit-up test, trunk extensor and flexor muscle strength measurement to evaluate core stability.[8]

2.4 | Endurance tests

Assessment of the trunk extensor muscle (M. erector spinae) endurance was performed by Sorensen test. The patients were told to lie

in a prone position with the upper part of the iliac crests aligned with the edge of the table. The trunk and upper bodies are not supported, and the arms are crossed in front of the chest. The lower body (ankles, thighs, and buttocks) was fixed to the table with the help of 2 arches. The patients were told to keep their upper trunk parallel to the floor until exhaustion. The time that the patient maintained this position was recorded in seconds.[15]

Assessment of the trunk flexor muscles (M. rectus abdominis and transversus abdominis) endurance was performed by trunk flexor test. In the trunk flexor test, in the lying position, trunk flexion was supported at 60°, the knees and hips were flexed at 90°, the arms were crossed over the chest and the feet were fixed. Then, the support of the trunk was uprooted and the patients were asked to stay in this position until they felt exhausted. The test was completed when patients could not maintain this position. The time that the patient maintained this position was recorded in seconds.[8]

2.5 | Strength tests

Modified sit-up test was generally used as strength test. The modified sit-up test was started in the hook-lying position, the arms were crossed over the chest, knees were flexed at 90° and feet were fixed. To complete a full sit-up; the patients' scapulae touched the mat in the lying position and the elbows touched the knees in the sitting position. The number of correct repeats in 1 minute was recorded.[3,8]

Isometric trunk muscle strength was measured with a hand-held dynamometer (MicroFET2®, Hogan Health Industries, Inc., UT, USA). It is stated that the hand-held dynamometer is a valid and reliable method for evaluating trunk muscle strength. The patient with SSc had to create maximum isometric muscle strength against the dynamometer held by the therapist for 5 seconds, and muscle strength was recorded in kg. For the trunk extensor muscle strength, the patient was placed in the prone position. In this position, the patient was asked to bring the arms to the side and head to the midline. The dynamometer was placed at the level of the T4 spine and the patient was asked to lift his chest off the ground to create an isometric force against the dynamometer. To measure trunk flexors strength, the patient was placed in the supine position. In this position, the patient was asked to bend knees slightly, bring the arms to the side and head to the midline. The dynamometer was placed in the middle of the sternum, and the patient was told to apply isometric force against the dynamometer by lifting both scapulas from the ground.[9]

2.6 | Physical function assessment

Health Assessment Questionnaire Disability Index (HAQ-DI) was used for physical function assessment. This questionnaire includes 20 questions divided into 8 domains (dressing and grooming, arising, eating, walking, hygiene, reach, grip and other activities) related to the fine movements of the upper extremities and the motor activities of the lower limbs. Answers to each section were scored between 0 (no difficulty) and 3 (unable). HAQ-DI score of ≥1.0 is the cut-off point and represented moderate to severe functional limitation.[16]

2.7 | Physical performance assessment

In physical performance evaluation, a 6-minute walking test (6MWT) and a 5 repetition sit-to-stand test (STS) are generally used.[17]

The 6MWT measures submaximal functional performance. The test was carried out in a 30-m-long corridor. The patients were told to walk as fast as possible for 6 minutes. The total distance they walked in 6 minutes was recorded in meters. Heart rate, blood pressure and oxygen saturation values were checked for the safety of patients before and after the test.[17,18]

The STS assesses the ability to rise from a chair and sit back down as well as lower limb and body strength, power and agility. The patient sat on a back-supported chair and crossed his arms in front of his chest. The patient was asked to stand and then sit up 5 times as quickly as possible without any support from his arms. The time required for 5 repeats to sit and stand was recorded in seconds.[17,19]

2.8 | Data analysis

Sample size was calculated using G*Power program (version 3.0.10). To obtain 95% confidence interval and a power of 90% with a probability of a 2-tailed type I error of 0.05, a sample of 40 subjects were required.

Data analysis was performed using "Statistical Package for Social Sciences" Version 22.0 (SPSS Inc Chicago, IL, USA). Data distribution was evaluated by Kolmogrov-Smirnov test. Results were expressed as mean, SD and minimum-maximum values or frequencies (percentages). The Pearson correlation coefficient was used to assess the correlation between the core stability and physical function, physical performance. Coefficient of correlation was interpreted as ≥0.50 strong; those between 0.30 and 0.49 were considered moderate, and those ≤0.29 were considered weak.[20] A P value $< .05$ was considered statistically significant.

3 | RESULTS

Forty patients with SSc participated in this study. The demographic and clinic features of patients are shown in Table 1. The sample of patients was predominantly female (85%), the mean age was 50.07 ± 9.16 years and the mean BMI was 25.73 ± 3.85 kg/m^2. While 2 (5%) patients were active smokers, 9 (22.5%) patients were ex-smokers and the mean cigarette consumption was 3.37 ± 6.92 packet-years. Two (5%) patients reported that they had regular exercise habits (walking). Since the appearance of the first symptom, the mean duration of the disease has been 7.29 ± 5.19 years. For the disease subtype, 60% of patients had limited cutaneous and 40% had

TABLE 1 Demographic and clinical characteristics of patients with SSc (n = 40)

	Mean ± SD or n (%)	Min-max
Gender (male / female)	6 (15) / 34 (85)	-
Age (y)	50.07 ± 9.16	37-65
Height (cm)	162.0 ± 7.20	148.0-175.0
Weight (kg)	69.98 ± 14.71	49.7-110.0
BMI (kg/m^2)	25.73 ± 3.85	18.9-35.0
Smokers		
Active smokers	2 (5.0)	-
Former smokers	9 (22.5)	-
Smoking consumption (packet-y)	3.37 ± 6.92	0.0-20.0
Disease duration (y)	7.29 ± 5.19	2-20
Type of disease		
Limited SSc	24 (60.0)	-
Diffuse SSc	16 (40.0)	-
Pulmonary arterial hypertension	10 (25.0)	-
Interstitial lung disease	9 (22.5)	-
Gastrointestinal symptoms	18 (45.0)	-
Arthralgia	25 (62.5)	-
Digital ulcers	10 (25.0)	-
Raynaud's phenomenon	39 (97.5)	-

Abbreviations: BMI body mass index; max, maximum; min, minimum; n, number; SD, standard deviation; SSc, systemic sclerosis.

TABLE 2 Core stability, physical function and physical performance test results of patients with SSc

	Mean ± SD	Min-max
Trunk extensor endurance (s)	49.87 ± 30.81	15-100
Trunk flexor endurance (s)	32.17 ± 15.42	10-62
Sit-up test (n)	17.42 ± 7.81	4-36
Trunk extensor muscle strength (kg)	7.48 ± 2.29	2.97-13.23
Trunk flexor muscle strength (kg)	6.20 ± 1.68	2.90-10.23
HAQ-DI score	1.02 ± 0.58	0.20-2.50
6MWT walking distance (m)	380.20 ± 71.80	130-470
STS (s)	10.48 ± 2.29	6-15

Abbreviations: 6MWT, 6-minute walking test; HAQ-DI, Health Assessment Questionnaire Disability Index; max, maximum; min, minimum; n, number; SD, standard deviation; SSc, Systemic sclerosis; STS, sit-to-stand test.

diffuse cutaneous SSc. PAH and ILD were present in 25% and 22.5% of patients, respectively. Gastrointestinal symptoms were reported by 45% of patients with SSc. In our patients' group, 62.5% suffered from arthralgia and 25% had digital ulcers and 97.5% had Raynaud's phenomenon.

3.1 | Core stability, physical function and physical performance test results

Endurance tests, trunk muscle strength, physical function and physical performance tests results of patients with SSc are shown in Table 2. All the tests were performed safely and completely in all patients. The mean trunk extensor and flexor endurance tests were 49.87 ± 30.81 and 32.17 ± 15.42 seconds, respectively. The mean sit-up test was 17.42 ± 7.81 repetition, the mean trunk extensor and flexor muscle strength were 7.48 ± 2.29 and 6.20 ± 1.68 kg, respectively. The mean score of HAQ-DI was 1.02 ± 0.58 and HAQ-DI scores ≥ 1.0 were in 47.5% of the patients. The mean walking distance of 6MWT was 380.20 ± 71.80 m and the mean STS was 10.48 ± 2.29 seconds.

3.2 | Correlation results

The correlations between core stability and physical function, physical performance tests of patients with SSc are shown in Table 3. The HAQ-DI score showed moderate to strong and negative correlation with trunk extensor and flexor endurance test ($r = -.424$ $P = .006$ and $r = -.394$ $P = .012$, respectively) sit-up test ($r = -.516$ $P = .001$), trunk extensor and flexor muscle strength ($r = -.525$ $P = .001$ and $r = -.519$ $P = .001$, respectively). The 6MWT walking distance showed a strong and positive correlation with trunk extensor and flexor endurance test ($r = .525$ $P = .001$ and $r = .521$ $P = .001$, respectively) sit-up test ($r = .541$ $P \leq .001$), trunk extensor and flexor muscle strength ($r = .619$ $P \leq .001$ and $r = .585$ $P \leq .001$, respectively). The STS test showed a moderate to strong and negative correlation with trunk extensor and flexor endurance test ($r = -.805$ $P \leq .001$ and $r = -.784$ $P \leq .001$, respectively) sit-up test ($r = -.500$ $P = .001$), trunk extensor and flexor muscle strength (and $r = -.457$ $P = .003$ and $r = -.326$ $P = .040$, respectively).

4 | DISCUSSION

This is the first study to assess the core stability and its correlation with physical function and functional performance in patients with SSc. Our study showed that all parameters related to core stability deteriorated in patients with SSc compared to the reference values in healthy individuals and core stability is associated with physical function and functional performance.

"Core" is a muscular box consisting of abdominals in front, paraspinals and gluteals in the back, diaphragm as roof, and pelvic floor and hip girdle muscles at the bottom.[21] Paravertebral muscles, transversus abdominis, external oblique, internal oblique muscles forming the core have been shown to be active during endurance tests.[22,23] It is stated in some rheumatoid diseases that atrophy can be seen in these muscles and this atrophy may cause decrease in strength and endurance test scores.[24] However, we did not find

TABLE 3 Correlations between core stability and physical function, physical performance tests result of patients with SSc

	HAQ-DI score	6MWT walking distance (m)	STS
Trunk extensor endurance (s)	−0.424**	0.525***	−0.805***
Trunk flexor endurance (s)	−0.394*	0.521***	−0.784***
Sit-up test (n)	−0.516**	0.541***	−0.500***
Trunk extensor strength (kg)	−0.525***	0.619***	−0.457**
Trunk flexor strength (kg)	−0.519***	0.585***	−0.326*

Abbreviations: 6MWT, 6-minute walking test; HAQ-DI, Health Assessment Questionnaire Disability Index; n, number; SSc, systemic sclerosis; STS, sit-to-stand test.

*.01 < $P \leq .05$,

**.001 < $P \leq .01$,

***$P \leq .001$, r Pearson's correlation coefficient for patients.

any study examining the presence of atrophy in the trunk muscles or evaluating the trunk muscle morphology (ultrasound imaging), strength and endurance of SSc patients. Moreover, there is no study investigating the normative values of trunk strength and endurance in Turkish society, so we have referenced studies involving healthy individuals with similar characteristics to our population to demonstrate trunk strength and endurance change in our SSc patients. In the literature, reference values of trunk extensor and flexor muscle endurance tests in older (41-65 years), inactive, asymptomatic and healthy women are indicated as 82.5 ± 19.7 and 105.9 ± 22.7 seconds, respectively.[25] The trunk extensor and flexor endurance test results of the SSc patients in our study were lower than the stated reference values and the test results of healthy individuals in other studies.[8,25,26] In SSc, changes in the osteomyoarticular system, movement limitations and postural changes may occur due to skin stiffness resulting from excessive collagen accumulation in connective tissue, chronicity of the disease and side effects of treatment.[27] As a result, atrophy and weakness can be seen in skeletal muscles in SSc patients. Therefore, we think that the atrophy of trunk muscles, movement limitations and postural changes may cause a decrease in strength and endurance of trunk muscles in patients with SSc. Also, in these patients, it is still unclear how myopathy, skin fibrous and concomitant contractures affect muscle strength and endurance. Thus, there is a great need for studies investigating the presence of atrophy and force, endurance change in the trunk muscles and other muscle groups.

Although the daily life activities and functional levels of SSc patients are seriously affected, the studies examining the muscle strength and endurance in these patients are few and the standard measurement method is still not clear. Moreover, trunk muscle strength and endurance have not been evaluated in any research related to SSc patients. In these few studies, it is seen that an isokinetic dynamometer is frequently used in SSc, but this equipment is expensive, time-consuming and requires high motivation and compliance of the patients.[11,13,28] In the literature, we observe that isometric hand-held dynamometer and endurance tests are used most frequently for the trunk muscle strength and endurance in neurological, musculoskeletal and other rheumatoid diseases. Therefore, we chose to use an isometric hand-held dynamometer and endurance tests due to their high validity and reliability for assessment of trunk muscle strength and endurance.[8,9] The trunk muscle strength assessed by the isometric dynamometer and the modified sit-up test in patients with SSc in our study was lower than that of the healthy individuals indicated in other studies.[8,9,26] Thus, the results of our study clearly show the affected core stability in patients with SSc, similar to the findings in ankylosing spondylitis patients characterized by spinal stiffness and loss of mobility.[26]

The importance of the core is frequently emphasized in the literature, as it is a kinetic link between the upper and lower extremities that facilitates the transfer of torques and angular momentum during the execution of all body movements as part of sports and professional skills, fitness and daily life activities.[29] Although it was stated in previous studies that physical functions were affected by various factors in SSc patients,[16] we did not find any study examining the relationship between core stability and physical functions. In this study, we used HAQ-DI to evaluate the patients' physical functions, which show the degree of physical disability in daily life activities of the patients, and which correlates highly with other clinical and laboratory measurements.[30] Similar to other studies, almost half of the patients in our sample (47.5%) had HAQ-DI scores ≥1.0, a cut-off associated with high morbidity and mortality, indicating that they experienced severe functional limitations in daily activities.[16,30] Importantly, we observed that trunk muscle endurance and strength, which are the sub-evaluation parameters of core stability, were associated with HAQ-DI scores. We think that the relationship between decreased core stability and high HAQ-DI scores in SSc is clinically important as decreased trunk muscle strength and endurance may be modifiable risk factors in the impaired physical function of these patients. However, more studies are needed to support our results.

Loss of muscle strength can be observed in SSc patients due to factors such as loss of muscle mass and decrease in skeletal muscle fibers, effects of some inflammatory mediators, joint disease, synovitis and skin stiffness.[2,31,32] Also, many studies have shown that predominantly upper and lower limb muscle strength and endurance are decreased in SSc patients. In particular, lower limb muscle strength has been associated with functional performance.[10-13,32] Since 6MWT is safe, inexpensive, noninvasive and reproducible, it

is increasingly preferred to evaluate functional capacity and performance in patients with SSc.[32,33] Therefore, we used 6MWT for assessment functional performance. Yet, mostly because of the relationship of 6MWT with lower limb muscle strength, we needed an additional test, so we used 5-repeated STS with 6MWT for assessment of functional performance. The STS test is a valid and reliable method that evaluates both lower limb strength, endurance, agility and it is associated with trunk muscle strength and endurance. However, STS test has been used in only 2 studies in SSc patients according to our knowledge.[12,13,17] It was stated that both 6MWT and STS test results were lower in SSc patients than healthy individuals and reference values.[12,13,33] The 6MWT and STS test scores in our study were similar to these studies. Moreover, studies for 5-repetition STS test states that individuals who are between 60-69 years old, 11.4 seconds is acceptable as a reference value and individuals who exceed the specified time are worse than the average functional performance.[34] Although the age range of the population in our study was smaller, 5-repetition STS test scores were lower than the stated reference value. Therefore, we think that SSc disease creates a burden on physical performance regardless of age. Furthermore, it is stated that trunk muscle weakening contributes approximately 12% to the variance explained in physical performance in healthy individuals. It is also emphasized that the relationship between lower trunk muscle strength and lower physical performance capacity is independent of the lower limb muscle composition.[35] On the other hand, trunk flexor/extensor force has been associated with STS test in both healthy individuals and some neurological diseases.[36] There was a significant correlation between core stability and functional performance in SSc patients, in our study. Therefore, we think that decreased trunk strength and endurance and postural changes that occur with them in SSc may disrupt the stabilization synergy and balance between the core muscles, resulting in decreased physical functions and functional performance.

This present study has some limitations. Due to the cross-sectional planning of the study, cause-effect relationship could not exactly be given. We did not use laboratory markers and radiographic findings such as echocardiography and electromyography. Although many different tests have been used to evaluate core stability and its components, there is no consensus on this issue. Since it would take a lot of time to evaluate all the components and difficult tests to exert patients, we only measured the endurance and strength components to evaluate core stability and mostly focused on the trunk. We used the hand-held dynamometer for assessment of trunk muscle strength, but it can be affected by the examiner. Despite these limitations, to our knowledge, this is the first study to assess core stability and its relationship with physical function and functional performance in patients with SSc. In this context, we believe that the results of our study make an important contribution to the field in order to overcome the shortcomings in the literature. Further studies are needed to confirm these findings with isokinetic devices for assessment of muscle strength, to have a control group including healthy subjects and to be conducted with a large number of patients.

5 | CONCLUSIONS

In conclusion, this study results show that core stability is negatively affected in patients with SSc. As the core stability decreases the physical function and functional performance deteriorate. Given that reduced core stability has a negative impact on physical function and functional performance, we think that this study highlights the importance of trunk muscles in patients with SSc. Therefore, we suggest that assessment of general muscle strength and endurance in SSc should contain trunk muscles and not be restricted to upper and lower extremity muscles. Furthermore, core stability exercises can be added to training programs in SSc patients to maintain and/or improve physical functions, functional performance and to be more independent in daily life activities.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by HY, SÖ and AMB. The first draft of the manuscript was written by HY and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Increased malignancies in our Waikato cohort of patients with systemic sclerosis

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Abstract

Background: Systemic sclerosis (SSc) has been associated with an increased risk of malignancy (especially in the skin, lung, breast, and hematological system).

Aim: To determine the risk of malignancies in our SSc cohort.

Methods: The NZ National Cancer Registry supplied details of all malignancies recorded in patients attending the Waikato Hospital Systemic Sclerosis Clinics from 2005 to 2018. Prospectively gathered clinical data were used to look for associations between clinical variables and malignancy.

Results: Out of the 164 patients in the Waikato SSc cohort, 32 (19.5%) had developed a malignancy. The overall standardized incidence rate was found to be 2.2 (95% CI 1.4-3.4) but was higher for men (4.4, 95% CI 1.4-10.3). The absolute numbers of patients with SSc and malignancies were small and were not adequately powered to investigate the SSc subgroups. The mean age of patients with malignancy was approximately 8 years older than patients without. The most common form of malignancy was skin (14, 43.7%), followed by breast (6, 18.7%), and lymphoma (5, 15.6%).

Conclusion: This study found an increased risk of malignancy for patients within the Waikato SSc cohort. Risk was greater in male patients and the mean age of patients with malignancies was approximately 8 years older than those without malignancy.

KEYWORDS

anti-centromere, anti-polymerase III, anti-scl70, diffuse cutaneous systemic sclerosis, limited cutaneous systemic sclerosis, malignancy

1 | INTRODUCTION

Systemic sclerosis (SSc) is a rare autoimmune disease characterized by progressive inflammation, endothelial dysfunction, and fibrosis of the skin and other internal organs, which leads ultimately to organ and tissue dysfunction.[1-3]

Systemic sclerosis can be classified into two main subtypes as per LeRoy's classification that looks primarily at the extent of cutaneous involvement. Limited cutaneous systemic sclerosis (lcSSc) exhibits skin thickening distal to knees and/or elbows, while diffuse cutaneous systemic sclerosis (dcSSc) has skin thickening closer to the trunk and proximal to the knees or elbows.[4,5] Of the two, dcSSc is associated with lower survival rates because of its extensive influence on the pulmonary, cardiac, renal, and other major organ systems.[3]

Previous studies have identified a temporal association between the diagnosis of SSc and the diagnosis of malignancy.[6-11] Research suggests that patients who have SSc are at greater risks of developing skin malignancy (squamous cell carcinoma [SCC], basal cell carcinoma [BCC], and melanoma), hematological malignancies (Hodgkin's lymphoma and non-Hodgkin's lymphoma), oropharyngeal malignancies, and carcinoma of the breast and of the lung.[8-10,12-17]

The meta-analyses by Bonifazi et al[9] of the 16 pooled studies (more than 7000 patients with SSc) revealed overall increased cancer risk, notably hematological and lung malignancies with relative risk 1.75 (95% confidence interval [CI] 1.41-2.18). Similarly, another meta-analysis (of more than 6500 patients with SSc) by Onishi et al[18] revealed a pooled standardized incidence rate (SIR) for all cancers to be 1.41 (95% CI 1.18-1.68) with increased risk of lung, bladder (in women only), and hematological malignancies, non-melanoma skin cancers (notably in men), and liver cancers. However, their study did not show any difference in the SSc subtypes; likewise, studies by Hill et al[14] found that malignancy rates were similarly raised.

Given the frequent finding of breast cancer around the time of SSc onset, a biological link has been contemplated.[14] Autoimmunity and a shared genetic susceptibility have been postulated to explain this paraneoplastic phenomenon. An increased risk of cancer has been seen also in the late onset of SSc where it has been thought that aging may be associated with weakened immune surveillance and repair and hence failing to mount an anti-cancer immune response.[14]

Our aim was to determine the risk of malignancies and to test for associations between the types of malignancy, subtypes of SSc, and the antibody profiles.

2 | MATERIALS AND METHODS

2.1 | Systemic sclerosis ascertainment

The Waikato Hospital patient database was used as the primary source for extracting data. This database is based on the prospectively gathered information from the Waikato Systemic Sclerosis Cohort starting in 2005. The clinic operates in a secondary and tertiary capacity, drawing patients from neighboring regions, ie, the Waikato District Health Board (DHB), Lakes DHB, and Bay of Plenty DHB. No patient data were gathered from the private sector. Given the systemic nature of the condition and the small amount of private rheumatology provision, it is likely that this represents a very small number of cases. Both inpatients and outpatients with SSc are included in the cohort.

All patients fulfilled the 2013 American College of Rheumatology/European League Against Rheumatism classification criteria for systemic sclerosis.[19] Further classification into lcSSc, dcSSc, and scleroderma overlap syndrome (SOS) were made according to LeRoy's criteria.[20] Patients who exhibited features of other connective tissue disease overlapping with systemic sclerosis were classified as SOS.

Sources of information contributing to the database and data set for this study included clinical notes, referral letters, laboratory results, and admission histories. Where information was not available for patients from surrounding regions, clinical records were requested from their respective hospitals and practices. The date of diagnosis was defined as the date of onset of the first non-Raynaud manifestation. Although this was available for the majority of patients, when unclear, laboratory results were reviewed to assess the dates of antibody testing to serve as a proxy and approximate date of diagnosis. Patients who were documented as having early signs of SSc, or for whom clinical data were unclear on the subtype of SSc, were included in the table for interest purposes but were excluded when calculating SIRs.

2.2 | Malignancy ascertainment

The New Zealand Cancer Registry[28] is a population-based register of all primary malignant diseases diagnosed in New Zealand, excluding squamous and basal cell skin cancers. A list of National Health Indices from all patients seen in the clinic was sent to the NZ Cancer Registry, who supplied a list of all recorded malignancies from inception through to 2014. According to data collected from the National Cancer Registry, 32 of our patients had experienced at least one malignancy. This registry captures all histological diagnoses of malignancy in New Zealand, with the exception of minor skin malignancies. Further investigation into patient records was required to establish the diagnosis dates, malignancy types, and causes of death (where appropriate). Of the 32 patients, 10 (31.25%) had been diagnosed with malignancy before the diagnosis of SSc. No patients were excluded because all of the necessary data for the analysis was gathered at the initial visit.

For the detection of specific SSc antibodies (Scl-70, CENP-A, CENP-B, RP11, RP115, Fib, NOR90, Th/To, PM100, PM75, Ku, PDGFR, Ro-52) the Systemic sclerosis (Nucleoli) EUROLINE (IgG) test kit (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) was used.[21] These were checked in either the initial or first follow-up visit at the clinic. Checking these antibodies is part of routine care to assist with defining prognosis.

2.3 | Statistical analysis

The SIRs were adjusted for age and gender using the New Zealand national malignancy data from 2011—this being the mid time-point from inception of the cohort through to the study. Those who died were censored at the time of death. These were calculated from the diagnosis of SSc, as a result, the 10 patients who were diagnosed with malignancy before the diagnosis of SSc were excluded. Fisher's exact test was used to calculate the 95% CI ($P < 0.01$). For all other tests, a P value less than 0.05 was considered significant.

This research was approved by the Wellington-based, National Health and Disability Ethics Committee (18NTA116). Consent was not specifically required for analysis of this aggregated, anonymized data.

3 | RESULTS

Of the 164 patients contributing to this study (Table 1), 22 were male (13.4%) and 142 were female (86.6%). The majority of the patients identified as New Zealand Europeans (126, 76.8%), along with 13

TABLE 1 Systemic sclerosis patient characteristics

	Total cohort (n = 164)	Patients without malignancy (n = 132)	Patients with malignancy, post-SSc diagnosis (n = 22)	Patients with malignancy, pre-SSc diagnosis (n = 10)	All patients with malignancy 19.5% (n = 32)
Demographics					
Male, n (%)	22 (13.4)	16 (12.1)	5 (22.7)	1 (10)	6 (18.8)
Female, n (%)	142 (86.6)	116 (87.9)	17 (77.3)	9 (90)	26 (81.3)
Living, n (%)	137 (83.5)	116 (87.9)	13 (59.9)	8 (80)	21 (65.6)
Deceased, n (%)	27 (16.5)	16 (12.1)	9 (40.1)	2 (20)	11 (34.4)
Smoker, n (%)	70 (42.9)	57 (81.4)	8 (36.4)	5 (50)	13 (18.6)
Age (y), mean (range)	63 (22-87)	61 (22-87)	71.3 (56-85)	66.9 (50-87)	69 (49-85)
Age at SSc diagnosis (y), mean (range)	53 (20-86)	53 (20-86)	55.1 (34-70)	59.1 (44-76)	56 (34-76)
Follow up (y), mean (range)	9 (0-44)	8 (0-44)	16.1 (1-37)	8.7 (1-30)	12.5 (0-35)
Age at malignancy diagnosis (y), mean (range)	NA	NA	46.9 (18-67)	66.5 (41-80)	60 (18-80)
Time between diagnoses (y), mean (range)	NA	NA	−9.4 (0-32)	10.2 (0-26)	10 (0-32)
Ethnicity, n (%)					
NZ European	126 (76.8)	99 (75)	16 (72.7)	9 (90%)	27 (83.4)
Other European	13 (7.9)	11 (8.3)	3 (13.6)	1 (10%)	2 (6.3)
Maori	10 (6.1)	9 (6.8)	1 (4.5)	—	1 (3.1)
South East Asian	3 (1.8)	3 (2.3)	—	—	0
Asian	1 (0.6)	1 (0.8)	—	—	0
Indian	6 (3.7)	6 (4.6)	—	—	0
Tongan	1 (0.6)	1 (0.8)	—	—	0
Hispanic	1 (0.6)	1 (0.8)	—	—	0
Not stated	3 (1.8)	1 (0.8)	2 (9.0)	—	2 (6.3)
Subtype of SSc, n (%)					
Limited SSc	104 (63.4)	82 (62.1)	15 (68.2)	8 (80)	22 (68.8)
Diffuse SSc	42 (25.6)	36 (27.3)	4	1 (10)	6 (18.8)
Overlap/SOS	9 (5.5)	7 (5.3)	1 (4.5)	1 (10)	2 (6.3)
Early/unspecified	9 (5.5)	7 (5.3)	2 (9.0)	—	2 (6.3)
Serology, n (%)					
Scl-70 (n = 156)	30 (19.2)	22 (73.3)	4 (18.2)	4 (40)	8 (26.7)
CENP-A (n = 156)	66 (42.3)	53 (80.3)	9 (47.3)	5 (50)	13 (19.7)
CENP-B (n = 156)	68 (43.6)	54 (79.4)	10 (52.5)	5 (50)	14 (20.6)
RP11 (n = 154)	28 (18.8)	25 (89.3)	3 (15.8)	—	3 (10.7)
RP115 (n = 154)	31 (20.8)	28 (90.3)	3 (15.8)	—	3 (9.7)
Fib (n = 154)	2 (1.3)	2 (100)	—	—	0
NOR90 (n = 154)	3 (1.9)	3 (100)	—	—	0
Th/To (n = 154)	3 (1.9)	3 (100)	—	—	0
PM100 (n = 153)	3 (1.9)	2 (66.7)	—	1 (10)	1 (33.3)
PM75 (n = 154)	10 (6.6)	9 (90)	—	1 (10)	1 (10)
Ku (n = 154)	5 (3.3)	5 (100)	—	—	0
Ro–52 (n = 155)	39 (25.7)	33 (84.6)	5 (26.3)	1 (100)	6 (15.4)

Other European (7.9%), 10 Maori (6.1%), 6 Indian (3.7%), and 4 Asian (2.4%). The mean current age of the patients was 62 years with the mean age at SSc diagnosis being 53 years. Seventy patients were, or had been, smokers (42.94%) (ie, ever smoked at any point of time while being followed from 2005 onwards) and 27 patients (16.5%) died during the period of data collection. Using Le Roy's criteria, the cohort

TABLE 2 Malignancy types

	Total (n = 32)	SSc before malignancy (n = 22) 68.8%	Male (n = 6) 18.8%	Female (n = 26) 81.3%	Diagnosed ±5 years from SSc diagnosis (n = 12) 37.5%
Skin, n (%)	14 (43.8)	7 (50)	1 (7.1)	13 (92.9)	6 (42.9)
Melanoma	9 (28.1)	4 (44.4)	1 (11.1)	8 (88.9)	4 (44.4)
Basal cell carcinoma	3 (9.4)	2 (66.7)	0	3 (100)	1 (33.3)
Squamous cell carcinoma	2 (6.3)	1 (50)	0	2 (100)	1 (50)
Breast, n (%)	6 (18.8)	4 (66.7)	0	6 (100)	3 (50)
Lymphoma, n (%)	5 (15.6)	4 (80)	2 (40)	3 (60)	2 (40)
Colon, n (%)	2 (6.3)	1 (50)	0	2 (100)	0
Lung, n (%)	2 (6.3)	1 (50)	1 (50)	1 (50)	1 (50)
Kidney, n (%)	1 (3.1)	1 (100)	0	1 (100)	0
Ovary, n (%)	1 (3.1)	1 (100)	0	1 (100)	0
Cervix, n (%)	2 (6.3)	1 (50)	0	1 (100)	1 (50)
Prostate, n (%)	2 (6.3)	2 (100)	2 (100)	0	1 (50)
Parotid gland, n (%)	1 (3.1)	1 (100)	0	1 (100)	0
Multiple[a], n (%)	3(9.4)	NA	0	3 (100)	NA

[a]Patients with multiple malignancies (Breast/lung, Squamous cell carcinoma/basal cell carcinoma, Melanoma/squamous cell carcinoma/basal cell carcinoma).

TABLE 3 Standardized incidence rates (sex and SSc subtype distributions)

	Expected	Observed	SIR	95% CI	*P*-value
Total	9.8	22	2.24	1.40-3.39	<.001
lcSSc	6.7	15	2.25	1.26-3.71	.004
dcSSc	2.1	5	2.36	0.77-5.51	.06
Male	1.1	5	4.41	1.43-10.30	.005
Female	8.7	17	1.95	1.14-3.13	.008

Abbreviations: dcSSc, diffuse cutaneous systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; SIR, standardized incidence rates; SSC, systemic sclerosis.

was divided into subtypes consisting of 104 lcSSc (63.4%), 42 dcSSc (25.6%), 9 SOS (5.5%), and 9 early or unspecified (5.5%) (Table 1).

3.1 | Malignancies

Thirty-two patients were found to have experienced a malignancy, of whom 6 were male (18.7%) and 26 were female (81.3%). There were 22 lcSSc patients (68.8%), 6 dcSSc patients (14.3%), and 4 patients with SOS or early SSc (12.5%) (Table 1). With the exception of one Maori (3.1%), most patients with malignancies were New Zealand European (83.4%) or Other European (6.2%). The mean age of the patients with malignancy was approximately 8 years older than the mean age of the patients without malignancy (69 and 61 years, respectively) with a mean follow up of 13 years.

Less than half of the malignancy cohort were smokers (40.6%). Of the 11 (34.4%) who had died, the cause of death was felt to be related to their malignancy in 5 (45.5%). Ten patients had been diagnosed with malignancy before SSc, one man and nine women.

3.2 | Malignancy rates

The mean age at malignancy diagnosis was 60 years with the mean time between the two diagnoses being approximately 10 years. The most common malignancy (Table 2) was skin (14, 43.8%), followed by breast (6, 18.8%) and lymphoma (5, 15.6%). Of the skin malignancies there were nine malignant melanoma, three BCC, and two SCC. Three women in the cohort experienced more than one malignancy, these overlaps were between breast and lung; melanoma, BCC, and SCC; and SCC and BCC. Contrary to other studies our results did not indicate an increase in lung cancer (2, 6.3%) and neither of these cases had a history of smoking.

More than half (62.5%) of the patients who were diagnosed with malignancy were diagnosed outside ±5 years surrounding their SSc diagnosis with 10 (31.3%) patients being diagnosed with malignancy before SSc (Table 2).

Nevertheless, our results showed an increased risk of malignancy for patients with SSc compared with the national population in 2011 (Table 3), with an SIR of 2.24 (95% CI 1.4-3.4), this however did not differ significantly between the lcSSc or dcSSc subtypes (2.25 and 2.36, respectively).

Men presented the highest SIR, at 4.41, but because of the limited sample size the confidence interval was slightly wider (95% CI 1.43-10.3).

In our cohort of those who developed malignancies, three patients were on methotrexate and one was on cyclosporin for the

TABLE 4 Standardized incidence rates of Waikato cohort (New Zealand) with respect to other countries

	SIR (95% CI)				
	All	Male	Female	lcSSc	dcSSc
New Zealand Rees (2005-2018)	2.24 (1.40-3.39)	4.41 (1.43-10.30)	1.95 (1.95-3.13)	2.25 (1.26-3.71)	2.36 (0.77-5.51)
Taiwan Kuo[10] (1996-2008)	1.63 (1.31-2.01)	1.88 (1.26-2.64)	1.51 (1.15-1.97)	NA	NA
South Australia Hill[14] (1993-2001)	1.99 (1.46-2.65)	2.79 (1.59-4.53)	1.73 (1.18-2.46)	1.85 (1.23-2.68)	2.73 (1.31-5.02)
Detroit Chatterjee[13] (1973-2004)	0.91 (0.66-1.22)	1.13 (0.49-2.23)	1.01 (0.7-1.41)	0.90[a] (0.81-1.28)[a]	0.87 (0.42-1.59)
Denmark Olesen[26] (1977-2006)	1.4 (1.2-1.6)	2.0 (1.5-2.7)	1.3 (1.1-1.5)	NA	NA
Japan Hashimoto[24] (1973-2008)	1.24 (0.77-1.71)	1.40 (0.54-3.35)	1.23 (0.75-1.71)	NA	NA

Abbreviations: CI, confidence interval; dcSSc, diffuse cutaneous systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; SIR, standardized incidence rate.

[a]lcSSc and sine SSc.

co-existent autoimmune hepatitis. Another patient had been on D-penicillamine and thereafter on methotrexate for a short period of time well before the development of the malignancy. It was not possible to assess for associations between medication use and malignancy given the small numbers involved.

3.3 | Antibody profiling

Not all patients had antibody profiling (Table 1) available for analysis but results from those that did showed that anti-centromere antibody was observed most commonly within the cohort (CENP-A, 42.3% and CENP-B, 43.6%). There was not enough data to merit statistical evaluation for associations between various autoantibodies and the presence of malignancy. With only 26.7% patients testing positive for scl-70, 20% of patients testing positive for anticentromere, and around 10% of patients positive for anti-polymerase III developing a malignancy (Table 1).

4 | DISCUSSION

Our study is the first study from New Zealand to show that SSc is associated with an increased risk of malignancy when compared with the general population. This aligns with the findings from other cohorts. Although a higher risk of lung malignancy was not noted (in contrarst to other cohort studies), there was an increase in malignancies of skin, breast, and the hematological system. This may relate to the small numbers in our study.

Men were found to have malignancies twice as often as women, and no significant difference was observed in the SIRs for lcSSc compared with dcSSc. Our study is congruent with the meta-analysis of Onishi et al,[18] who also noted a higher risk of malignancy in male patients (pooled SIR 1.85, 95% CI 1.49-2.31), as well as no differences between lcSSc and dcSSc.

It is interesting to note (and not previously reported) that the age of individuals with malignancy in our cohort was a mean of 8 years older than individuals without; however, this may be a coincidence because the risk of malignancy increases with age in the general population. However, it has also been noted that the risk of malignancy is higher in older patients with late-onset SSc. These findings suggest that additional targeted malignancy screening could be beneficial for SSc patients, particularly in men and in older patients.

Compared with other cohort studies, New Zealand has the highest observed SIRs in all categories. Our results found that men had a notably higher incidence than women, which supports findings published in other studies (Table 4).

In a study of 441 patients in South Australia, New Zealand's closest neighboring country, 47 malignancy cases were identified after an SSc diagnosis had been made. Of these cases, lung malignancies were most common (12 patients), followed by breast (8 patients), and malignant melanoma (5 patients). Hematological malignancies were limited to two patients, making it the sixth most commonly observed type along with renal malignancies.

Our research was of a small group (given the low population of New Zealand), so we were not able to identify any SSc-specific antibodies or characteristics that may be responsible for the preferentially increased risk of malignancy observed in our patients. However,

the larger Australian Scleroderma Interest Group study did not detect a higher prevalence of cancer in patients with anti-RNA polymerase III antibodies compared with those who were negative for that antibody.[22] Along with this theory, other proposed explanations have included previous genetic damage, the use of immunosuppressive therapies, medications, radiation therapy, smoking or chemical factors, chronic inflammation, chromosomal abnormalities, or a mutation in the *POLR3A* gene.[14,17,23-27]

As SSc is an uncommon disorder, it is important to be aware of the limited number of patients followed in this study. However, accounting for the relatively small population of New Zealand (roughly 4.8 million) these findings are still relevant. It is also first study of its kind in New Zealand. Our computed SIR with respect to other countries are shown in Table 4.

This study has not dealt with minor skin malignancies. These are defined as lesions that were not deep (and non-metastatic) and could be managed in general practice (treated with cryotherapy, topical creams such as fluorouracil cream, imiquimod cream or ingenol mebutate gel, or by minor curettage or excision). Also, they did not require further re-excision of the lesion, and these patients did not require hospitalization for flaps or skin transplants in the areas of excision. Lastly, there was no recurrence of the lesion on the same region.

Skin malignancies that were included are those requiring skin flaps or grafts, reconstructive surgery, or hospital reassessments. It should also be noted that New Zealand has exceptionally high rates of melanoma compared with the rest of the world as the result of sun damage (approximately 4000 diagnoses a year).

The major significance of this research is that it is the first New Zealand study of its kind looking at the relationship between SSc and malignancy. It is also one of the few international studies done which attempts to include melanoma.

5 | CONCLUSION

Our data confirm the increased risk of malignancy for patients with SSc, especially in men and older patients. Our limitation was that the absolute numbers of patients with SSc were small and the study was not adequately powered to analyze the risk of malignancies depending on SSc subtype, limited or diffuse, nor with that of specific SSc-related antibodies. The results of this study nevertheless allow us to recommend increased vigilance and strongly consider cancer screening for all patients with SSc (as suggested by other international studies), particularly focused on skin, breast, and hematological system malignancies. In the future we propose a larger cohort study be done to allow further analysis into the incidence rates of specific malignancy types and to discover which characteristics and symptoms may identify patients at risk for specific malignancies.

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CONFLICT OF INTEREST

No conflict of interest has been declared by the authors.

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Comparison of relapse rates in Behçet's disease with venous involvement on different doses of azathioprine therapy, a retrospective observational study

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Abstract

Aim: The aim of this study was to investigate relapse rates in azathioprine (AZA) maintenance therapy at different doses in Behçet's disease (BD) with venous involvement.

Method: Clinical records of patients who met the diagnostic criteria of International Study Group (ISG) for BD, were diagnosed with venous involvement of BD for at least 6 months and sustained clinical remission with AZA for at least 3 months were analyzed retrospectively. The analysis cohort was divided into 2 groups based on AZA dose (Group A: ≥ 2 mg/kg/d and Group B: <2 mg/kg/d). Relapse was defined as requiring another antirheumatic/immunosuppressive drug or more than dose of 10 mg/d of prednisolone.

Results: Of 78 patients who were included into the study, there was no significant difference between the 2 groups in terms of age, gender and clinical characteristics. Mean relapse-free survival time was found to be higher in group A compared to group B (111.6 ± 11.2, 95% CI 89.5 ± 133.8 versus 51.5 ± 6.1, 95% CI 39.5 ± 63.4 months).

Conclusion: Relapse-free survival rate was less in the group receiving low-dose AZA and shows the importance of effective dose of AZA in maintenance therapy.

KEYWORDS

azathioprine, Behçet's disease, venous involvement

1 | INTRODUCTION

Behçet's disease (BD) is a multisystemic disorder characterized by mucocutaneous and ocular lesions. BD was first defined by Hulusi Behçet in 1937 with a triad of recurrent oral aphthous ulceration, genital aphthous ulceration and iridocyclitis.[1] BD is classified as a systemic vasculitis and can affect nearly every system of the body. Vascular, gastrointestinal, and neurological involvement occurs with potentially serious consequences. Vascular involvement affecting both arteries and veins of all sizes can be seen in 2.2% to 50% of patients with BD at varying rates depending on geographical regions. The rates of venous involvement resulting in superficial thrombophlebitis and deep venous thrombosis are higher than arterial thrombosis.[2]

Lower extremity vein thrombosis can be considered as its hallmark, whereas superior or inferior vena cava and cerebral venous sinuses are other common sides of the venous system. Pulmonary arteries, which resemble venous structures because of lower pressure, thinner walls, and less elasticity, are often affected.

Colchicine is the main treatment for BD patients with solely mucocutaneous involvement. Vascular or other system involvement requires more aggressive treatment with corticosteroids (CS) and immunosuppressives (IS) such as cyclophosphamide (CYC) and

azathioprine (AZA).[3] The major cause of the thrombosis in BD is the inflammation of the vessel wall which cannot be explained with thrombophilic factors. The clinical course is characterized by relapses and remissions. There is no standard IS therapy protocol and treatment strategies are usually based on clinical experience. For the induction and/ or maintenance therapy, AZA is widely used as a corticosteroid tapering IS agent in vascular BD. However, the literature data about AZA therapy in the treatment of BD is quite limited.

The purpose of this study was to investigate relapse rates in AZA maintenance therapy at different doses in venous BD.

2 | MATERIAL AND METHODS

2.1 | Patients

This retrospective study was performed 2 two different tertiary centers in Turkey (Health Sciences University, Bakirkoy Dr Sadi Konuk Training and Research Hospital, Istanbul and Health Sciences University, Numune Training and Research Hospital, Ankara). Subjects aged 18-65 years with BD fulfilling International Study Group (ISG) criteria were enrolled between June 2012 and June 2017. Demographics, disease activity, laboratory tests, treatment and follow-up data were obtained from medical records. BD patients using another antirheumatic drug with AZA, having secondary rheumatologic disease, organ failure, or acquired / hereditary thrombophilic disease were excluded from the study.

To be enrolled, patients had to have venous (deep vein thrombosis and/ or pulmonary artery thrombosis or aneurism) BD for at least 6 months and sustained clinical remission with AZA for at least 3 months. Remission was defined as the normalization of the acute phase reactants and regression and/or stabilization of clinical findings with the dose of no more than 7.5 mg/d of prednisolone at the end of the third month of induction therapy.

All patients previously had been treated with different induction treatment regimens including CS, AZA and CYC. In addition, patients had to receive stable treatment with AZA and/or colchicine without alteration in dose for at least 3 months. At each follow-up visit, information on disease activity, medications, and adverse events (AEs) were collected.

Patients with arterial involvement, another rheumatic disease, organ failure, thrombophilic disease and without longer than 6 months follow-up data were excluded. The analysis cohort (n: 78) was divided into 2 groups based on AZA dose (Group A: ≥2 mg/kg/d and Group B: <2 mg/kg/d). All subjects gave written informed consent at the time of enrollment into these studies. Institutional ethics committees approved the protocol (2017-06-37).

2.2 | Efficacy assessments

Relapse was defined as requiring another antirheumatic/ immunosuppressive drug. Remission was followed up as the absence of a new or progressing lesion. AEs requiring discontinuation of AZA were noted. AEs requiring hospitalization were defined as severe AEs.

2.3 | Statistical analysis

Data are summarized as percentages for categorical variables. Descriptive results are stated in medians. Corresponding inferential comparisons of subgroups were calculated using Kruskal-Wallis or Mann-Whitney *U* tests for numerical variables and exact χ^2 tests for nominal characteristics. A multivariate multivariable logistic regression model was used in order to predict the occurrence of disease relapses from the following set of baseline characteristics including an intercept term: age, gender, duration of disease, anticoagulant use, colchicine use. Survival analysis was performed by using Kaplan–Meier method. The starting point was accepted as when the maintenance azathioprine was given by achieving remission. Survival curves described relapse-free survival rates for both groups. For comparison of survival curves log-rank test was used and $P < .05$ was accepted as statistically significant. The software program SPSS (v. 23) was used for statistical analysis.

3 | RESULTS

We conducted a detailed file screening of 102 patients. Nine of them were excluded from the study due to insufficient follow-up data and 15 were excluded because of various exclusion criteria (1: did not receive AZA as maintenance therapy; 4: organ failure or another immunosuppressive therapy was used in combination with AZA; 1: hereditary thrombophilia; 6: treatment noncompliance during follow-up; 3: AZA maintenance duration was less than 3 months). The median follow-up period of the patients included in the study was 75 months (12-312) (Figure 1). Fifty-nine (76%) of 78 patients were randomized to group A, and 19 (24%) were in group B. The clinical characteristics of the groups formed according to the maintenance AZA dose are shown in Table 1.

There was no significant difference between the 2 groups in terms of age, gender, clinical characteristics, and use of concomitant anticoagulants. Human leukocyte antigen B51 was examined in only 5 (6%) patients and was positive in 3 of them. Pathergy test was performed on 38 patients in group A and the positivity rate was similar with group B (55% vs. 42%, $P = .256$). The most common vascular involvement in both groups was lower extremity venous involvement (76% vs. 89%, $P = .183$). Median disease duration was found to be longer in group B (60 [12-302] vs. 144 [16-312] months, $P = .006$). The rates of colchicine and corticosteroid use with AZA in maintenance treatment were lower in group A compared to group B (54% vs. 73%, $P = .054$ and 75% vs. 100%, $P = .009$ respectively).

Of the 14 patients who developed relapses, 8 were in group A and 6 were in group B (relapse rate 14% vs. 32%, respectively, $P = .036$). Except for 1 patient who relapsed with arthritis in group

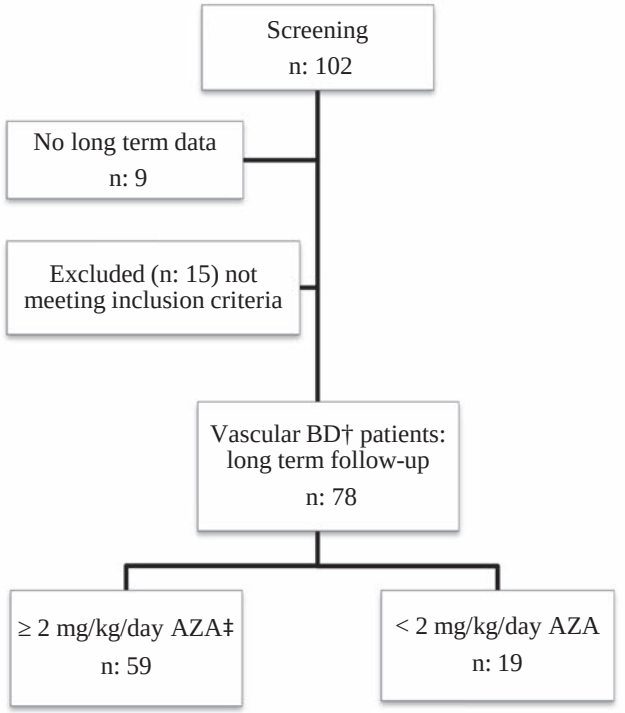

FIGURE 1 Design of the study. In total, 102 patients with Behçet's disease (BD) were screened, 78 patients indicating disease remission were divided into the 2 groups azathioprine dose ≥ 2 mg/kg/d and < 2mg/kg/d. †BD; ‡azathioprine

A, all other relapses were vascular relapse (93%). Three of these vascular relapses were in the pulmonary artery and others were in the extremity veins. One of the pulmonary artery relapses was in group A and other 2 relapses were in group B. One of the extremity vein relapses was in group A and others were in group B. Relapse-free survival curves are seen in Figure 2. Mean relapse-free survival time was found to be higher in group A compared to group B (111.6 ± 11.2, 95% CI 89.5 ± 133.8 vs. 51.5 ± 6.1, 95% CI 39.5 ± 63.4 months). In the multifactorial multivariable regression analysis performed with age, gender, duration of illness, concomitant colchicine, corticosteroid and anticoagulant use, statistical significance was not reached for the prediction of relapse (Table 2). There were no deaths during the follow-up. None of the patients had severe AEs associated with AZA on maintenance therapy. AEs were detected in 2 patients (3%) in group A, 1 patient with leukopenia and the other with hepatotoxicity.

4 | DISCUSSION

Vascular BD occupies an important place in BD's clinical spectrum. Although arterial or venous vessels of any diameter can be involved, venous involvement is much more common. Pulmonary artery involvement is accepted as venous involvement due to the anatomic structure of the pulmonary artery.[4] Vascular BD can lead to death by threatening organ functions with thrombosis and aneurysm rupture. It is crucial to initiate high doses of corticosteroids without delay. Clinical response was obtained in high-dose corticosteroid therapy in most cases. Although a strong algorithm could not be established in IS treatment, AZA and CYC are the most frequently combined drugs with corticosteroids in the initial treatment.[5] Maintenance therapy should continue for a long time after efficacy. The clinical research data on maintenance therapy, for which AZA is the first choice, is extremely limited.[6] There is only 1 randomized controlled trial (RCT) published by Yazıcı et al. and they have reported that 2.5 mg/kg/d AZA was effective in uveitis control.[7]

Saadoun et al. reported the long-term efficacy of AZA at the same dose in uveitis as real-life data.[8] In our study, AZA was preferred as maintenance therapy in all venous BD patients except 1 in 2 different centers. While the dose was mostly ≥2 mg/kg/d, it was found that some patients were given a lower dose. In this way, relapse rates were compared with the 2 groups formed. The fact that

TABLE 1 Clinical characteristics of patients with vascular Behçet's disease according to dose of AZA maintenance therapy (AZA, azathioprine)

	Group A	Group B	*P* value
Age, median (range), y	38 (19-61)	40 (23-61)	.226
Gender, male (%)	43 (73)	16 (84)	.249
Disease duration, median (range), mo	60 (12-302)	144 (16-312)	**.006**
AZA maintenance, median (range), mo	28 (7-72)	23 (3-144)	.51
Genital ulcer, n (%)	37	12	.57
Skin lesions, n (%)	32	12	.341
Pathergy positivity, n (%)	21 (55) n: 38	8 (42) n: 19	.256
Vascular involvement			
Lower extremity vein	45	17	.183
Superficial venous thrombosis	13	3	.398
Pulmonary artery	13	3	.398
Vena cava and/or hepatic vein	3	1	.681
Cranial venous sinus	2	1	.573
Other involvement n (%)			
Ocular	13	4	.602
Musculoskeletal	11	4	.526
Neurological (brain)	1	0	.756
Gastrointestinal	1	0	.756
Concomitant drug use, n (%)			
Corticosteroid	32 (54)	15 (73)	**.054**
Colchicine	44 (75)	19 (100)	**.009**
Anticoagulant	13 (22)	7 (32)	.162

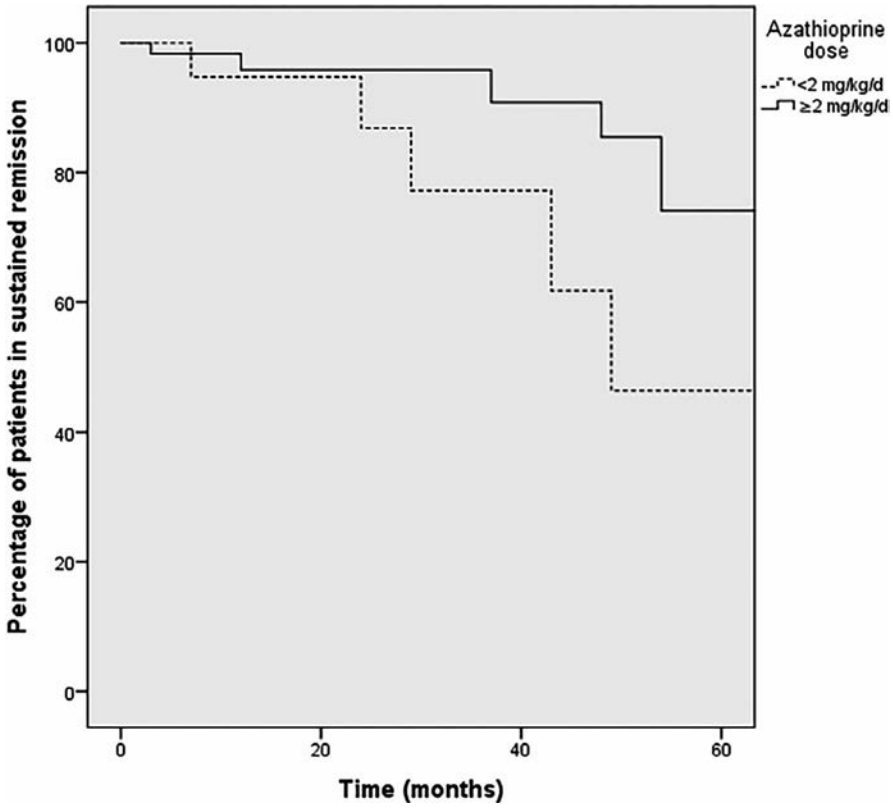

FIGURE 2 Kaplan–Meier curves for maintenance of remission. Curves indicate loss of remission over 80 mo in patients with Behçet's disease in relation to different parameters. The y-axis indicates the percentage of patients with Behçet's disease in sustained remission (100% at baseline), and the x-axis indicates time

TABLE 2 Results of multivariate logistic regression for prediction of relapse (OR, odds ratio; CI, confidence interval)

	B	Wald	*P* value	OR	OR (95% CI)
Age	-0.018	0.215	.643	0.982	0.910 to 1.060
Gender	-0.463	0.396	.529	0.630	0.149 to 2.611
Disease duration	0.013	1.489	.222	1.013	0.992 to 1.035
Corticosteroid	0.935	1.339	.247	2.546	0.523 to 12.394
Anticoagulant	-0.449	0.567	.451	0.638	0.198 to 2.054

relapse-free survival rate is less in the group receiving low-dose AZA shows the importance of effective dose of AZA in maintenance therapy. Today, with the introduction of biological treatments, especially tumor necrosis factor inhibitors to vascular BD, it is important to evaluate standard treatments at effective doses.[9]

Although there are different opinions about anticoagulant treatment, if there is no other thrombophilic cause, the clinical benefit of anticoagulant therapy has not been demonstrated.[10] In our study, it was observed that both groups were given a low dose of anticoagulant therapy. No potential effect on the relapse rate has been demonstrated. Similarly, the use of colchicine has not been shown to have an effect on the relapse rate. It is gratifying that our patient group, who received maintenance treatment with AZA for a long time, did not have severe AEs. It can be said that the rate of side effects, which requires termination of AZA, is low. Considering that it is generally preferred to be used with colchicine, colchicine seems to be used safely with AZA. There is no study in the literature evaluating the safety of concomitant AZA and colchicine use.

The most important limitation of our study is that it was designed retrospectively. Since the treatments used during remission induction are not standard in our clinical practice, we could not analyze the effect of remission induction treatment protocols on the duration of maintenance. Indeed, we would like to calculate this effect with a prospectively designed study. As a problem of retrospective analysis, we also could not calculate the duration of vascular disease. In particular, different vascular involvements reduce the power of the number of patients randomized to study. However, since vascular BD is not a common disease, it is difficult to work with large patient populations.

Another limitation is that we could not calculate the cumulative corticosteroid dose. Lack of the standard remission or clinical activity criteria in BD is a limitation not only for this study but also for other studies.[11]

As a result, long-term use of AZA is safe in vascular BD maintenance therapy. When used at an effective dose, the relapse rate decreases. It is unclear how long maintenance therapy should take and when the dose can be reduced. Further RCTs with large patient groups are needed in the treatment of vascular BD.

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Association of the genetic variants in the *endoplasmic reticulum aminopeptidase 2* gene with ankylosing spondylitis susceptibility

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Abstract

Background: Genetic polymorphisms in the endoplasmic reticulum aminopeptidase gene *ERAP2* has been attributed with the etiopathogenesis of ankylosing spondylitis (AS). Here we assessed the association of *ERAP2* gene single nucleotide polymorphisms (SNPs) with AS predisposition in Iranian patients and determined their effect on the inflammatory state of the patients.

Methods: For genotyping of rs2548538, rs2287988, and rs17408150 SNPs using a real-time allelic discrimination approach, DNA was extracted from the whole blood of 250 AS patients and 250 healthy individuals. RNA of the peripheral blood mononuclear cells was separated, cDNA was synthesized, and transcriptional levels of cytokines, including interleukin (IL)-17A, IL-23, IL-10, and transforming growth factor-β, were measured. Enzyme-linked immunosorbent assay was used to measure the serum concentration on the cytokines.

Results: Three *ERAP2* gene SNPs were not associated significantly with AS risk. Nonetheless, rs2287988 and rs17408150 SNPs showed statistically significant association with susceptibility to the disease in those AS patients who were positive for human leukocyte antigen (HLA)-B27. Transcriptional level and serum concentration of IL-17A and IL-23 were higher, but those of IL-10 were lower in both AS patients and the HLA-B27-positive patient group relative to the control group. Nevertheless, *ERAP2* gene SNPs in the HLA-B27-positive AS patients did not affect the transcription level and serum concentration of cytokines.

Conclusions: *ERAP2* gene rs2287988 and rs17408150 SNPs are associated with susceptibility to AS, but they are probably not determining the levels of IL-17A, IL-23, and IL-10 in this disease.

KEYWORDS

ankylosing spondylitis, endoplasmic reticulum aminopeptidase 2, human leukocyte antigen-B27, inflammation, single nucleotide polymorphisms

1 | INTRODUCTION

Ankylosing spondylitis (AS) is a chronic autoimmune disease and is characterized by involvement of spine and sacroiliac joints, which causes spinal deformities, increased disability, and mortality.[1] A bulk of research has suggested a significant role for genetic variations in the etiology and pathogenesis of AS,[2,3] in spite of the remarkable involvement of environmental factors as well as aberrant epigenetic regulations.[4-8] The pathogenesis of AS is complicated, and earlier research concentrated on the misfolding of the human leukocyte antigen (HLA)-B27 molecule in disease susceptibility; however, genetic studies have proposed that HLA-B27 accounts for a small part of the overall AS risk.[9] Epidemiological investigations has shown that the HLA-B27 gene is carried by almost 90% of AS patients, whereas only 1%-5% of individuals carrying the HLA-B27 gene will be affected with AS in the future.[10] These observations imply the involvement of non-HLA genes in AS risk.

Endoplasmic reticulum aminopeptidase (ERAP) 2 is an enzyme that belongs to the zinc-containing metallopeptidase family and the corresponding gene is located on the chromosome 5q15. This enzyme is found within the endoplasmic reticulum, which is involved in priming peptides during the antigen presentation pathway via the major histocompatibility complex class I.[11] In comparison to ERAP1, data are lacking about the attribution of *ERAP2* polymorphisms with AS predisposition.[4,12,13] A number of genetic polymorphisms in the *ERAP2* gene have been attributed to produce alterations in the protein structure and function. The AS-protective *ERAP2* gene single nucleotide polymorphism (SNP) rs2248374[14] alters the splicing site in the exon 10, leading to synthesis of a lengthy exon 10 transcript.[15] As a loss of enzyme polymorphism, rs2248374 causes no protein expression of ERAP2 and has been attributed with downregulation of major histocompatibility complex class I molecule levels on the cell surface.[15] Another variant is rs2549782 SNP, which confers a modulation in the specificity as well as functional velocity of the enzymatic activity of ERAP2.[14,16] *ERAP2* gene rs2549782 SNP shows a linkage disequilibrium (LD) with other *ERAP2* SNPs, including rs2548538, rs2287988, rs1056893, and rs2248374, which are marker SNPs that constitute A and B haplotypes that are associated with protein expression of ERAP2.[15] In addition, *ERAP2* gene rs17408150 leads to substitution of a T with an A at codon 669 (p.Leu-669Gln), which alters the leucine residue to glutamine, producing a significant effect on the ERAP2 enzyme function.[17]

Association of *ERAP1* SNPs with AS susceptibility in Iranian patients has already been reported in our previous studies.[18-21] Furthermore, we recently indicated the association of *ERAP2* gene SNPs with AS susceptibility in HLA-B27-positive individuals.[22] With respect to the involvement of genetic variations in the alteration of ERAP2, it seems that evaluation of such SNPs is worthwhile. Hence, this study aims to determine the associations of *ERAP2* gene rs2548538, rs2287988, and rs17408150 SNPs, for the first time to the best of our knowledge, in an Iranian AS population. Furthermore, the possible role of these SNPs was investigated in the control of inflammatory and immunomodulatory mediators in AS.

2 | MATERIALS AND METHODS

2.1 | Study participants

In this investigation, 250 individuals with AS and 250 persons as healthy controls were included (Table 1). The 1984 modified New York Criteria were used to diagnose AS.[23] Healthy controls had no background diseases or history of AS or other autoimmune diseases, neither in them nor in family members, and were matched for age and gender with the case group. AS patients were selected from individuals who were recruited to Shahid Rajaee and Emam Reza Hospitals affiliated with Alborz and Tabriz University of Medical Sciences, Iran and outpatient Rheumatology Clinics of the Tabriz University of Medical Sciences during 2015 to 2020. Approval of

TABLE 1 Baseline characteristics and clinical manifestations of AS patients and healthy controls

Characteristic	AS Patients (n = 250)	Healthy controls (n = 250)	*P* value
Age (y)	38.50 ± 8.80	37.62 ± 7.40	>.05
Female/male, n (%)	53 (21.2%)/197 (78.8%)	45 (18%)/205 (82%)	>.05
HLA-B27-positive, no (%)	202 (80.8%)	18 (7.2%)	<.0001
CRP (mg/L)	3.14 ± 2.27	1.27 ± 0.95	<.0001
Disease duration (y)	10.79 ± 7.88	–	–
BASDAI score	5.34 ± 3.57	–	–
BASFI score	3.45 ± 2.36	–	–
BASG score	4.45 ± 2.08	–	–
ASQoL score	7.74 ± 4.21	–	–

Abbreviations: ASQoL, ankylosing spondylitis quality of life; BASDAI, Bath ankylosing spondylitis disease activity index; BASFI, Bath ankylosing spondylitis functional index; BASG, Bath ankylosing spondylitis global score; CRP, C-reactive protein; HLA, human leukocyte antigen.

the study protocol was received from the local ethical review committee in Alborz University of Medical Sciences (Permission No. IR.ABZUMS.REC.1399.051). Before sampling, written informed consent forms were obtained from all AS patients and healthy individuals. All study participants were assessed for HLA-B27 positivity and the clinical condition of the AS patients was determined using the Bath AS Disease Activity Index (BASDAI), Bath AS Functional Index (BASFI), Bath AS Metrology Index (BASMI), Bath AS Global Score (BASG), and AS quality of life (ASQoL). Using venipuncture, about 10 mL of venous blood was taken from all participants.

2.2 | ERAP2 SNPs genotyping

To obtain DNA from venous blood samples, the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used based on the company's instructions. Afterwards, in order to genotype the study participants for *ERAP2* gene rs2548538, rs2287988, and rs17408150 SNPs, real-time polymerase chain reaction (PCR) was performed using TaqMan assays (Applied Biosystems, Foster City, CA, USA) and StepOnePlus Real-Time PCR system (Applied Biosystems). To perform real-time genotyping, the PCR reaction mixture comprised 5 μL TaqMan Master Mix (containing Taq DNA polymerase and dNTPs; Applied Biosystems), 0.5 μL TaqMan Genotyping Assay mix containing primers and probes (Applied Biosystems), 2 μL of genomic DNA (20 ng/μL), and distilled H_2O (final volume of 15 μL in each tube). The thermocycling settings of the PCR amplification were initial heating for 60°C for 45 seconds followed by 95°C for 10 minutes, then 40 amplification cycles at 95°C for 15 seconds and 60°C for 60 seconds, and finally 60°C for 30 seconds.

2.3 | PBMC separation, RNA isolation, and cDNA synthesis

Peripheral blood mononuclear cells (PBMCs) were isolated from the peripheral blood using Ficoll/Hypaque (Lymphodex; Inno-Train, Kronberg-im-Taunus, Germany) density-gradient centrifugation. Extraction of RNA from PBMCs was carried out using the Trizol total RNA extraction kit (GeneAll, Seoul, Korea) based on the producer's guidelines. Synthesis of the complementary DNA (cDNA) from the extracted RNA samples was conducted using the BioFact™ RT Series cDNA Synthesis Kit (Daejeon, Korea), conforming to the company's protocols.

2.4 | Quantitative real-time PCR

In order to assess the mRNA expression levels of inflammatory and immunomodulatory cytokines, including interleukin-17A (IL-17A), IL-23, IL-10, and transforming growth factor-β (TGF-β), quantitative real-time PCR was performed using the Rotor-Gene Q Real-time PCR System machine (Qiagen) and SYBR Green PCR Master Mix. The characteristics of the primers employed for real-time mRNA transcript quantification are listed in Table S1. We randomly selected 80 AS patients and 80 control individuals for mRNA expression analysis. For quantitative real-time PCR, each reaction mixture contained SYBR Green Master Mix 12.5 μL, cDNA 4.5 μL, forward and revers primer 1 μL each, and H_2O 6 μL to reach a final volume of 25 μL. The thermocycling PCR conditions were: 50°C for 2 minutes, 95°C for 10 minutes, then 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. To compute the relative mRNA transcript of target genes, the comparative C_T method, as explained by Schmittgen and Livak,[24] was used. The relative expression levels of target genes were determined through normalization in accordance with the mRNA level of the corresponding housekeeping gene (β-actin). The mathematical formula to compute the relative mRNA expression in each subject was $2^{-\Delta\Delta Ct}$.

2.5 | Serum concentration of cytokines

Like quantitative mRNA expression analyses, serum samples from the venous blood of the same individuals were used to determine the concentration of the cytokines. Hence, the enzyme linked immunosorbent assay (ELISA) technique was applied to determine the serum concentrations of IL-23, IL-17A, IL-10, and TGF-β in 80 patients and 80 controls. The optical density was determined using commercial kit (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA) and determination of the optical density in each well by an ELISA reader (Tecan Spectra, Zürich, Switzerland).

2.6 | Statistical analysis

The baseline features of the patient and control groups were determined by descriptive statistical analysis. Determination of the

associations between the *ERAP2* gene SNPs and risk of AS was analyzed using Pearson's χ^2 test as well as logistic regression, and the association level was determined through measuring the odds ratios (OR) and corresponding 95% confidence intervals (CI). Power calculation was accomplished with the method described by Chow et al[25] The LD for SNP pairs, the haplotypic analysis, and examination of the genotype distribution of SNPs in the control group to meet the Hardy-Weinberg equilibrium were carried out using the SHEsis online tool.[26] Adjusting of the *P* values was carried out by Benjamini-Hochberg method. Testing for the normal distribution of scale data (mRNA expression, serum concentration, and clinical indexes) was accomplished with the Kolmogorov-Smirnov test. To approximate the significance of differences in the mRNA expression and serum concentration of the cytokines between study groups, the mean comparisons were made by the non-parametric Mann-Whitney *U* test. Furthermore, the Kruskal-Wallis test was used to perform mean comparison of data among patients with three genotypes for SNPs. Data presentations were made through average ± standard deviation for scale data or by percentage for nominal data. Data analysis was accomplished using SPSS software v.25.0 (IBM, Armonk, NY, USA) and graphing of data in bar charts was conducted using GraphPad PRISM software (version 8.00; GraphPad Inc., San Diego, CA, USA).

3 | RESULTS

3.1 | Baseline features of the participants

The baseline and laboratory indexes of the study population are listed with details in the Table 1. The male/female distributions of the study participants in the AS and control groups were 53 (21.2%)/197 (78.8%) and 45 (18%)/205 (82%), respectively. The ages of the AS and control groups were 38.50 ± 8.80 and 37.62 ± 7.40, respectively. Matching of the two study groups for age ($P > 0.05$) and sex ($P > 0.05$) was observed. HLA-B27 was positive in 202 (80.8%) AS patients and 18 (7.2%) healthy controls ($P < 0.0001$). Moreover, C-reactive protein level in the case group (3.14 ± 2.27) was significantly higher ($P < 0.0001$) relative to the control group (1.27 ± 0.95).

TABLE 2 Allele and genotype frequencies of *ERAP2* gene rs2548538, rs2287988, and rs17408150 SNPs in AS patients and healthy controls and corresponding association analyses

SNP	Allele / genotype	AS (n = 250) n (%)	Control (n = 250) n (%)	OR (95% CI)	*P*	Adjusted *P*[a]	Power (1–*β*)
rs2548538	T vs A	186 (37.2)	197 (39.4)	0.91 (0.70-1.17)	.47	–	0.11
	A (Reference)	314 (62.8)	303 (60.6)	–	–	–	
	TT vs AA	32 (12.8)	38 (15.2)	0.79 (0.46-1.38)	.42	0.81	0.12
	TA vs AA	122 (48.8)	121 (48.4)	0.95 (0.65-1.39)	.81	0.81	0.05
	TT vs TA + AA	32 (12.8)	38 (15.2)	0.81 (0.49-1.35)	.43	0.81	0.12
	TT + TA vs AA	154 (61.6)	159 (63.6)	0.91 (0.63-1.31)	.64	0.81	0.07
	AA (Reference)	96 (38.4)	91 (36.4)	–	–	–	
HWE			*P* = .830				
rs2287988	G vs A	217 (43.4)	192 (38.4)	1.23 (0.95-1.58)	.11	–	0.36
	A (Reference)	283 (56.6)	308 (61.6)	–	–	–	
	GG vs AA	47 (18.8)	34 (13.6)	1.58 (0.93-2.71)	.66	0.66	0.35
	GA vs AA	123 (49.2)	124 (49.6)	1.47 (0.77-1.68)	.50	0.66	0.05
	GG vs GA + AA	47 (18.8)	34 (13.6)	0.80 (0.90-2.37)	.12	0.48	0.35
	GG + GA vs AA	170 (68.0)	158 (63.2)	1.23 (0.85-1.79)	.25	0.50	0.20
	AA (Reference)	80 (32.0)	92 (36.8)	–	–	–	
HWE			*P* = .443				
rs17408150	A vs T	242 (48.4)	220 (44.0)	1.98 (0.93-1.53)	.16	–	0.28
	T (Reference)	258 (51.6)	280 (56.0)	–	–	–	
	AA vs TT	49 (19.6)	43 (17.2)	1.45 (0.85-2.49)	.17	0.20	0.10
	AT vs TT	144 (57.6)	134 (53.6)	1.37 (0.90-2.09)	.14	0.20	0.14
	AA vs AT + TT	49 (19.6)	43 (17.2)	1.31 (0.86-1.99)	.20	0.20	0.10
	AA + AT vs TT	193 (77.2)	177 (70.8)	1.39 (0.93-2.08)	.10	0.20	0.37
	TT (Reference)	57 (22.8)	73 (29.2)	–	–	–	
HWE			*P* = .165				

Abbreviations: 95% CI, 95% confidence interval; AS, ankylosing spondylitis; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; SNP, single nucleotide polymorphism.

[a]False discovery rate correction for multiple comparisons by Benjamini-Hochberg.

TABLE 3 Allele and genotype frequencies of *ERAP2* gene rs2548538, rs2287988, and rs17408150 SNPs in HLA-B27 positive AS patients and healthy controls

SNP	Allele /genotype	HLA-B27-positive AS (n = 202) n (%)	Control (n = 250) n (%)	OR (95% CI)	*P*	Adjusted Pa
rs2548538	T vs A	167 (41.3)	197 (39.4)	1.08 (0.82-1.41)	.465	–
	A (Reference)	237 (58.7)	303 (60.6)	–	–	–
	TT vs AA	27 (13.3)	38 (15.2)	1.04 (0.57-1.88)	.877	0.87
	TA vs AA	113 (55.9)	121 (48.4)	1.37 (0.90-2.07)	.134	0.40
	TT vs TA + AA	27 (13.3)	38 (15.2)	0.86 (0.50-1.46)	.574	0.76
	TT + TA vs AA	140 (69.3)	159 (63.6)	1.29 (0.87-1.91)	.201	0.40
	AA (Reference)	62 (30.7)	91 (36.4)	–	–	–
HWE			*P* = .830			
rs2287988	G vs A	185 (45.7)	192 (38.4)	1.35 (1.03-1.76)	.023	–
	A (Reference)	219 (54.2)	308 (61.6)	–	–	–
	GG vs AA	41 (20.3)	34 (13.6)	1.91 (1.09-3.35)	.022	0.08
	GA vs AA	103 (50.9)	124 (49.6)	1.31 (0.86-2.00)	.191	0.19
	GG vs GA + AA	41 (20.3)	34 (13.6)	1.61 (0.98-2.66)	.055	0.09
	GG + GA vs AA	144 (71.3)	158 (63.2)	1.45 (0.97-2.15)	.071	0.09
	AA (Reference)	58 (28.7)	92 (36.8)	–	–	–
HWE			*P* = .443			
rs17408150	A vs T	211 (52.2)	220 (44.0)	1.39 (1.06-1.81)	0.013	–
	T (Reference)	193 (47.3)	280 (56.0)	–	–	–
	AA vs TT	44 (21.8)	43 (17.2)	2.13 (1.19-3.82)	.010	0.01
	AT vs TT	123 (60.9)	134 (53.6)	1.91 (1.19-3.06)	.007	0.01
	AA vs AT + TT	44 (21.8)	43 (17.2)	1.34 (0.83-2.14)	.220	0.22
	AA + AT vs TT	167 (82.7)	177 (70.8)	1.96 (1.24-3.10)	.003	0.01
	TT (Reference)	35 (17.3)	73 (29.2)	–	–	–
			P = .165			

Bold values represent statistically significant comparisons.

Abbreviations: 95% CI, 95% confidence interval; AS, ankylosing spondylitis; HLA-B27, human leukocyte antigen-B27; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; SNP, single nucleotide polymorphism.

[a]False discovery rate correction for multiple comparisons by Benjamini-Hochberg.

TABLE 4 Haplotype association of *ERAP2* gene rs2548538, rs2287988, and rs17408150 SNPs according to Haploview

	Block 1 haplotypes			Frequencies			
Row	rs2548538	rs2287988	rs17408150	Haplotype frequency[a] (250 AS patients) n (%)	Haplotype frequency (250 control participants) n (%)	OR (95% CI)	*P*
1	A	A	A	74 (14.8)	65 (13.7)	2.13 (1.25-4.31)	.014
2	A	A	T	36 (7.2)	13 (2.7)	2.85 (1.50-5.40)	.0083
3	A	G	A	227 (45.4)	303 (60)	0.86 (0.47-0.82)	.005
4	A	G	T	59 (311.9)	78 (15.7)	0.73 (0.51-1.05)	.433
5	T	A	T	39 (7.8)	77 (15.4)	0.57 (0.31-0.75)	.004
6	T	G	T	49 (9.9)	41 (9.1)	1.07 (0.77-2.88)	.517

Abbreviations: 95% confidence interval; AS, ankylosing spondylitis; odds ratio, 95% CI; OR.

[a]Frequencies <0.03 were excluded.

3.2 | Association test of ERAP2 polymorphisms

The frequency distribution pattern of the genotypes for *ERAP2* gene rs2548538 (P = 0.830), rs2287988 (P = 0.443), and rs17408150 (P = 0.165) SNPs in the healthy control group adhered to the Hardy-Weinberg equilibrium. In the total population analysis, results revealed that none of the three SNPs was significantly associated with risk of AS (Table 2). Notwithstanding this, when the HLA-B27-positive AS patients and the control group were compared, rs2287988 and rs17408150 SNPs showed statistically significant association with AS risk (Table 3).

For rs2287988, there was significant association in the minor G allele and GG genotype. The G allele was represented in 45.7% of the HLA-B27-positive AS patients and in 38.4% of the healthy controls (OR 1.35, 95% CI 1.03-1.76, P = 0.023). The GG genotype was frequently presented in the HLA-B27-positive AS patients relative to controls (20.3% vs 13.6%), and the difference was statistically significant (OR 1.91, 95% CI 1.09-3.35, P = 0.022).

In the case of rs17408150, the C allele had a significant association with higher risk of AS in the HLA-B27-positive patients (OR 1.39, 95% CI 1.06-1.81, P = 0.013). The AA genotype was more represented in the HLA-B27-positive AS group (21.8%) than the controls (17.2%); hence the AA genotype was associated significantly with increased AS risk (OR 2.13, 95% CI 1.19-3.82, P = 0.010). The distribution of the heterozygous AT genotype was statistically significantly different between HLA-B27-positive AS cases and the control group (OR 1.91, 95% CI 1.19-3.06, P = 0.007). The dominant model of AA + AT vs TT for rs17408150 SNP had a significant association with the higher AS risk (OR 1.96, 95% CI 1.24-3.10, P = 0.003) in the AS group with positive HLA-B27.

3.3 | Frequency of the haplotype

Regarding the haplotypic analysis (rs2548538 A/T, rs2287988 A/G, and rs17408150 A/T), four haplotypes had significant

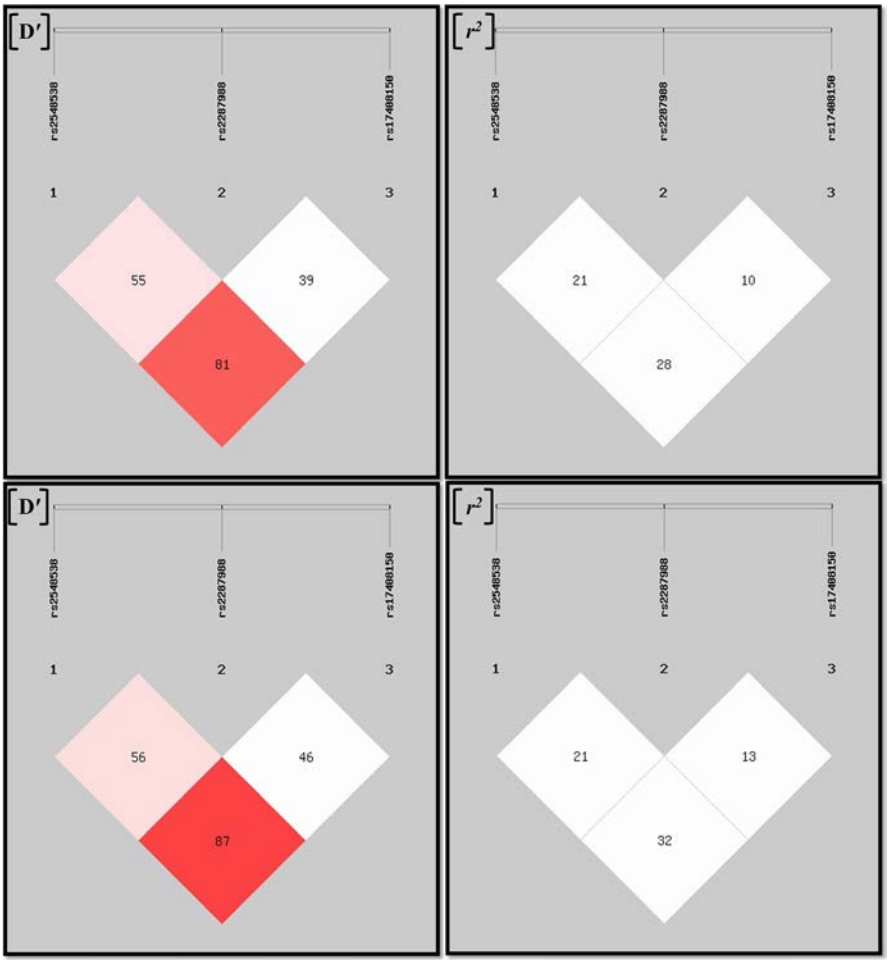
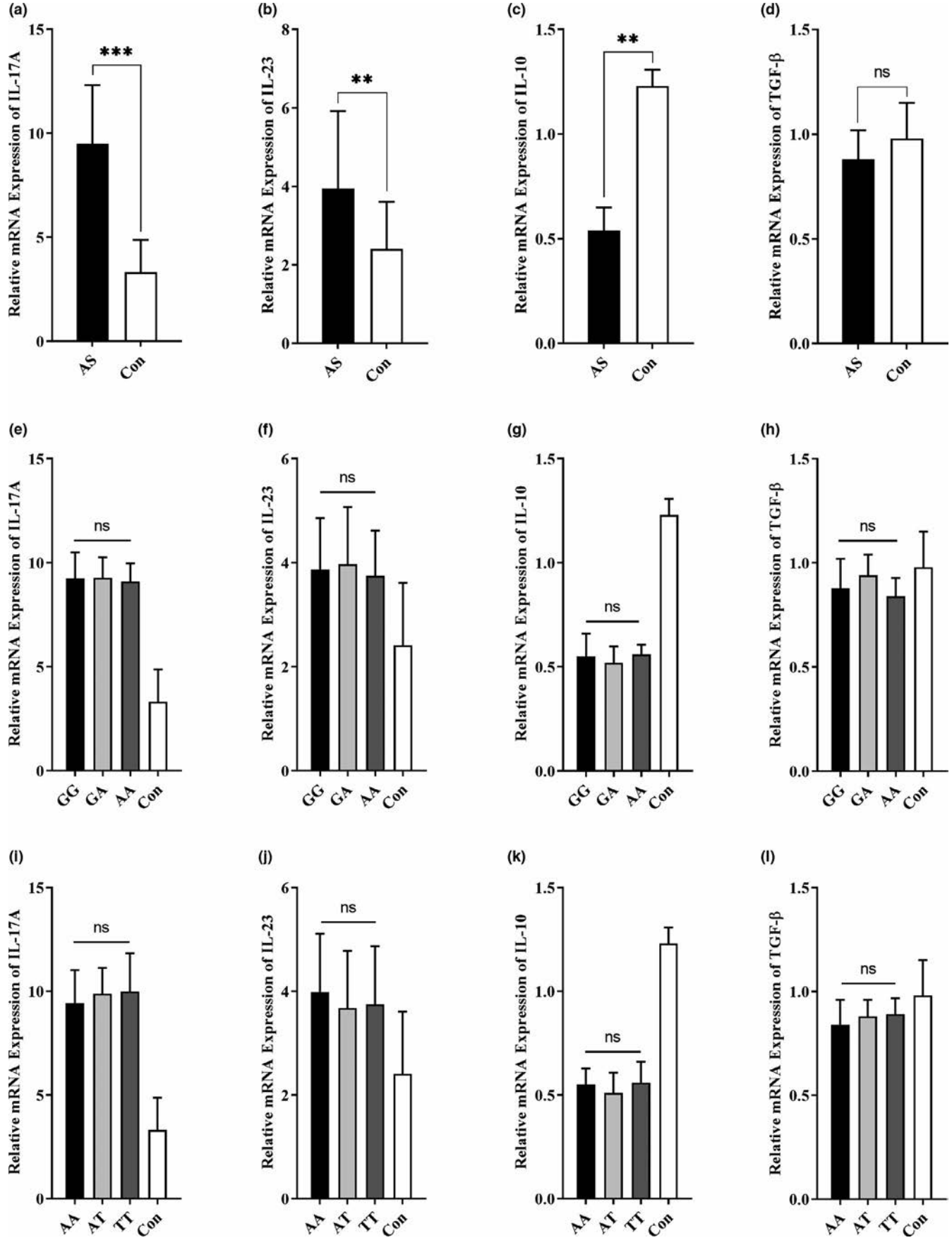


FIGURE 1 The linkage disequilibrium (LD) block structure under the *ERAP2* gene rs2548538, rs2287988, and rs17408150 single nucleotide polymorphisms (SNPs). Two upper captures present the LD scores in the total population, whereas the AS patients positive for HLA-B27 and all healthy controls included in the analysis are illustrated in the lower captures. Inside each block, the scores from 0% to 100% imply the value of D' or r^2 for SNP pairs. As the scores increase, the possibility of two SNP pairs being inherited simultaneously is higher

FIGURE 2 Bar charts show the relative mRNA amounts of interleukin-17A (IL-17A), IL-23, IL-10, and transforming growth factor-β (TGF-β) in the study groups (A-D). The comparison of the relative mRNA levels of cytokines in the ankylosing spondylitis (AS) patients with three genotypes for rs2287988 single nucleotide polymorphism (SNP) (E-H). Relative transcription level of the cytokines in the AS patients harboring three different genotypes for rs17408150 SNP (I-L). The mean comparisons were conducted by statistical tests of Mann-Whitney's U or Kruskal-Wallis test (**P < 0.01, ***P < 0.001)


(a)
Relative mRNA Expression of IL-17A
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(b)
Relative mRNA Expression of IL-23
8
6
4
2
0
**
AS
Con
(c)
Relative mRNA Expression of IL-10
1.5
1.0
0.5
0.0
**
AS
Con
(d)
Relative mRNA Expression of TGF-β
1.5
1.0
0.5
0.0
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AS
Con
(e)
Relative mRNA Expression of IL-17A
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5
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GG
GA
AA
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(f)
Relative mRNA Expression of IL-23
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GG
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AA
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(g)
Relative mRNA Expression of IL-10
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AA
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(h)
Relative mRNA Expression of TGF-β
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Relative mRNA Expression of IL-17A
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(k)
Relative mRNA Expression of IL-10
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0.0
ns
AA
AT
TT
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(l)
Relative mRNA Expression of TGF-β
1.5
1.0
0.5
0.0
ns
AA
AT
TT
Con

associations with AS risk. The AAA (OR 2.13, 95% CI 1.25-4.31, $P = 0.014$) and AAT (OR 2.85, 95% CI 1.50-05.40, $P = 0.0083$) haplotypes were associated with increased AS risk, but the AGA (OR 0.86, 95% CI 0.47-0.82, $P = 0.005$) and TAT (OR 0.57, 95% CI 0.31-0.75, $P = 0.004$) haplotypes were associated with decreased risk of AS (Table 4).

3.4 | Linkage disequilibrium test

The structure of SNP pairs in the LD block according to the SNP sequence of rs2548538, rs2287988, and rs17408150 are shown in Figure 1. The D' value between rs2548538 and rs2287988 SNPs was 55% and between rs2548538 and rs17408150 SNPs was 81%. Nonetheless, a partially stronger linkage was detected when only the HLA-B27-positive patients were included in the analysis ($D' = 56\%$ and 87%, respectively, for rs2548538-rs2287988 and rs2548538-rs17408150). However, the r^2 values indicated no remarkable LD between SNP pairs.

3.5 | mRNA expression of cytokines

The mRNA transcription level of IL-17A (fold change [FC] 2.85, $P = 0.0001$, Figure 2A) and IL-23 (FC = 1.63, $P = 0.0084$, Figure 2B) had upregulated levels in the PBMCs obtained from AS patients in relation to healthy participants. However, there was significant underexpression of the mRNA of IL-10 (FC 0.43, $P = 0.0024$, Figure 2C) in the PBMCs obtained from AS patients relative to the healthy participants. The mRNA expression of TGF-β was lower, but non-significantly, in the case group relative to the control group (FC 0.89, $P > 0.05$, Figure 2D). The mRNA expression of all four cytokines had no significant difference among AS patients with three genotypes for rs2287988 and rs17408150 SNPs (Figure 2 and Table S2).

Transcription levels of IL-17A (FC 3.00, $P = 0.0001$, Figure 3A) and IL-23 (FC 1.70, $P = 0.0084$, Figure 3B) had upregulated levels in the PBMCs obtained from AS patients positive for the HLA-B27 gene relative to healthy participants. Conversely, the transcript level of IL-10 (FC 0.41, $P = 0.001$, Figure 3C) was significantly lower in the PBMCs obtained from AS patients positive for HLA-B27 relative to healthy participants. The mRNA expression of TGF-β had no significant difference between HLA-B27-positive AS patients and the normal controls (FC 0.82, $P > 0.05$, Figure 3D). The mRNA expression of all cytokines had no significant difference among the AS patients positive for HLA-B27 carrying three different genotypes for rs2287988 and rs17408150 SNPs (Figure 3 and Table S2).

3.6 | Serum concentration of cytokines

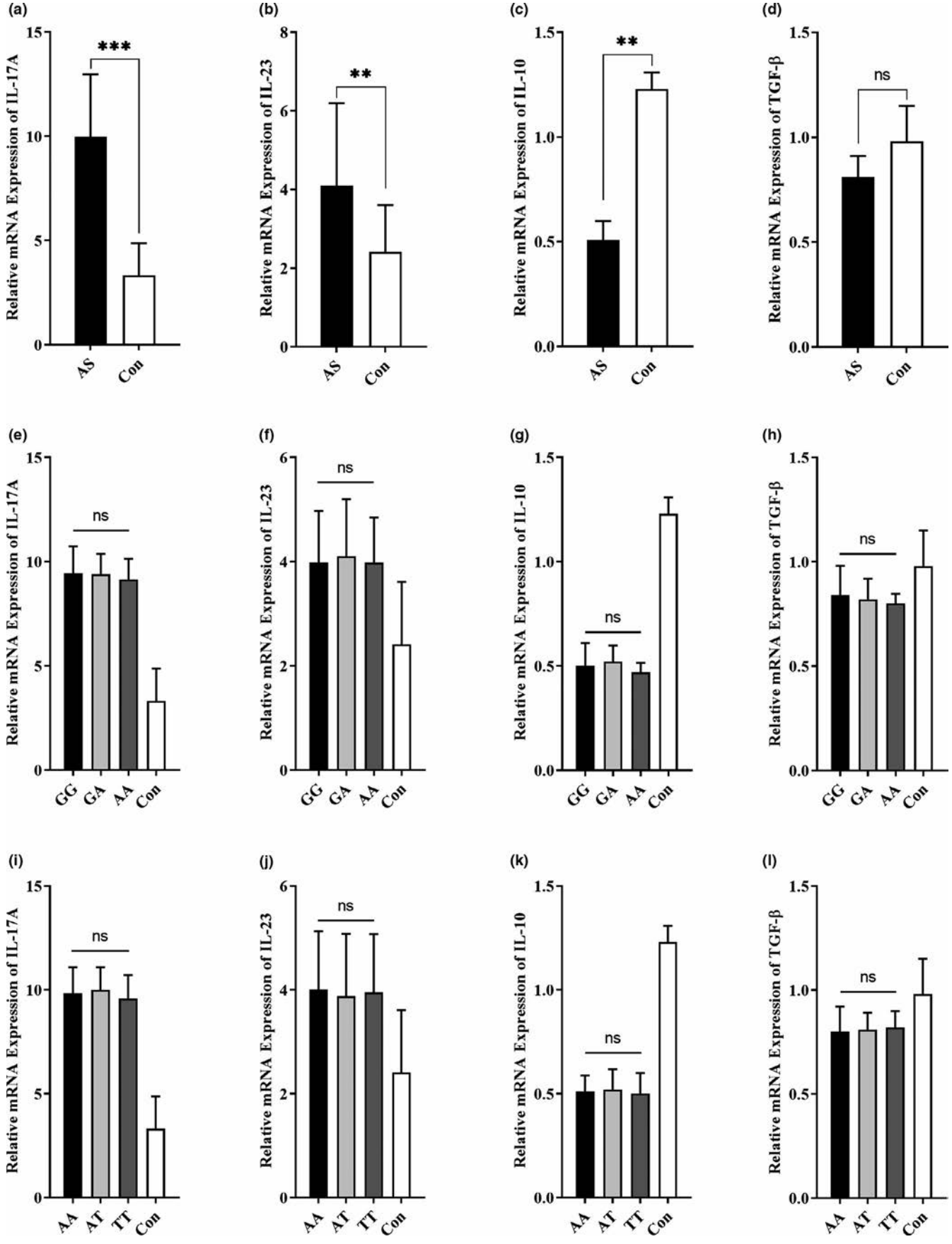
The serum concentrations of IL-17A (46.50 ± 13.10 vs 19.56 ± 6.48, $P = 0.0001$, Figure 4A) and IL-23 (388.9 ± 38.78 vs 200.2 ± 24.88, $P = 0.0001$, Figure 4B) were increased in the AS group relative to the normal control group. However, the serum concentration of IL-10 was significantly lower in the AS group compared with the control group (1.89 ± 0.18 vs 3.68 ± 1.10, $P = 0.0015$, Figure 4C). The difference in the serum concentration of TGF-β was not statistically significant between AS group and the control group (20.4 ± 4.51 vs 23.4 ± 4.21, $P > 0.05$, Figure 4D). The serum concentrations of all cytokines had no significant difference among the AS cases positive for HLA-B27 and harboring three different genotypes for rs2287988 and rs17408150 SNPs (Figure 4 and Table S3).

The serum concentrations of IL-17A (49.11 ± 14.21 vs 19.56 ± 6.48, $P = 0.0001$, Figure 5A) and IL-23 (395.4 ± 39.7 vs 200.2 ± 24.88, $P = 0.0001$, Figure 5B) were higher in the AS patients positive for HLA-B27 relative to normal controls. In contrast, the serum concentration of IL-10 was significantly lower in the HLA-B27-positive AS group compared with the control group (1.75 ± 0.14 vs 3.68 ± 1.10, $P = 0.0017$, Figure 5C). However, the difference in the serum concentration of TGF-β was not statistically significant between HLA-B27-positive AS cases and the control group (19.7 ± 4.47 vs 23.4 ± 4.21, $P > 0.05$, Figure 5D). The serum concentration of all cytokines had no significant difference among the AS cases positive for HLA-B27 carrying three different genotypes for rs2287988 and rs17408150 SNPs (Figure 5 and Table S3).

3.7 | Association of the genotypes and clinical manifestations

The *ERAP2* gene rs2548538, rs2287988, and rs17408150 polymorphisms were investigated in association with clinical features of AS patients. It was observed that none of the characteristics of the AS patients, including CRP, disease duration, BASDAI, BASFI, BASG, and ASQoL scores, had significant associations with *ERAP2* gene polymorphisms (Table 5). Moreover, analysis of the association of the *ERAP2* gene rs2548538, rs2287988, and rs17408150 SNPs with the mentioned clinical manifestations in HLA-B27-positive AS patients resulted in no significant association.

FIGURE 3 Bar charts show the relative transcriptional amounts of interleukin-17A (IL-17A), IL-23, IL-10, and transforming growth factor-β (TGF-β) mRNAs in the ankylosing spondylitis (AS) patients positive for HLA-B27 and healthy controls (A-D). The comparison of the relative mRNA amounts of cytokines in the AS patients positive for HLA-B27 harboring three different genotypes for rs2287988 single nucleotide polymorphism (SNP) (E-H). Relative transcription level of the cytokines in the AS patients positive for HLA-B27 harboring three different genotypes for rs17408150 SNP (I-L). The mean comparisons were carried out using the statistical tests of Mann-Whitney's *U* or Kruskal-Wallis (**$P < 0.01$, ***$P < 0.001$)


(a)
Relative mRNA Expression of IL-17A
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AS
Con
(b)
Relative mRNA Expression of IL-23
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AS
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(c)
Relative mRNA Expression of IL-10
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AS
Con
(d)
Relative mRNA Expression of TGF-β
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(e)
Relative mRNA Expression of IL-17A
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(f)
Relative mRNA Expression of IL-23
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(g)
Relative mRNA Expression of IL-10
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(h)
Relative mRNA Expression of TGF-β
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(i)
Relative mRNA Expression of IL-17A
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Relative mRNA Expression of IL-23
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Relative mRNA Expression of IL-10
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(l)
Relative mRNA Expression of TGF-β
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AT
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Con

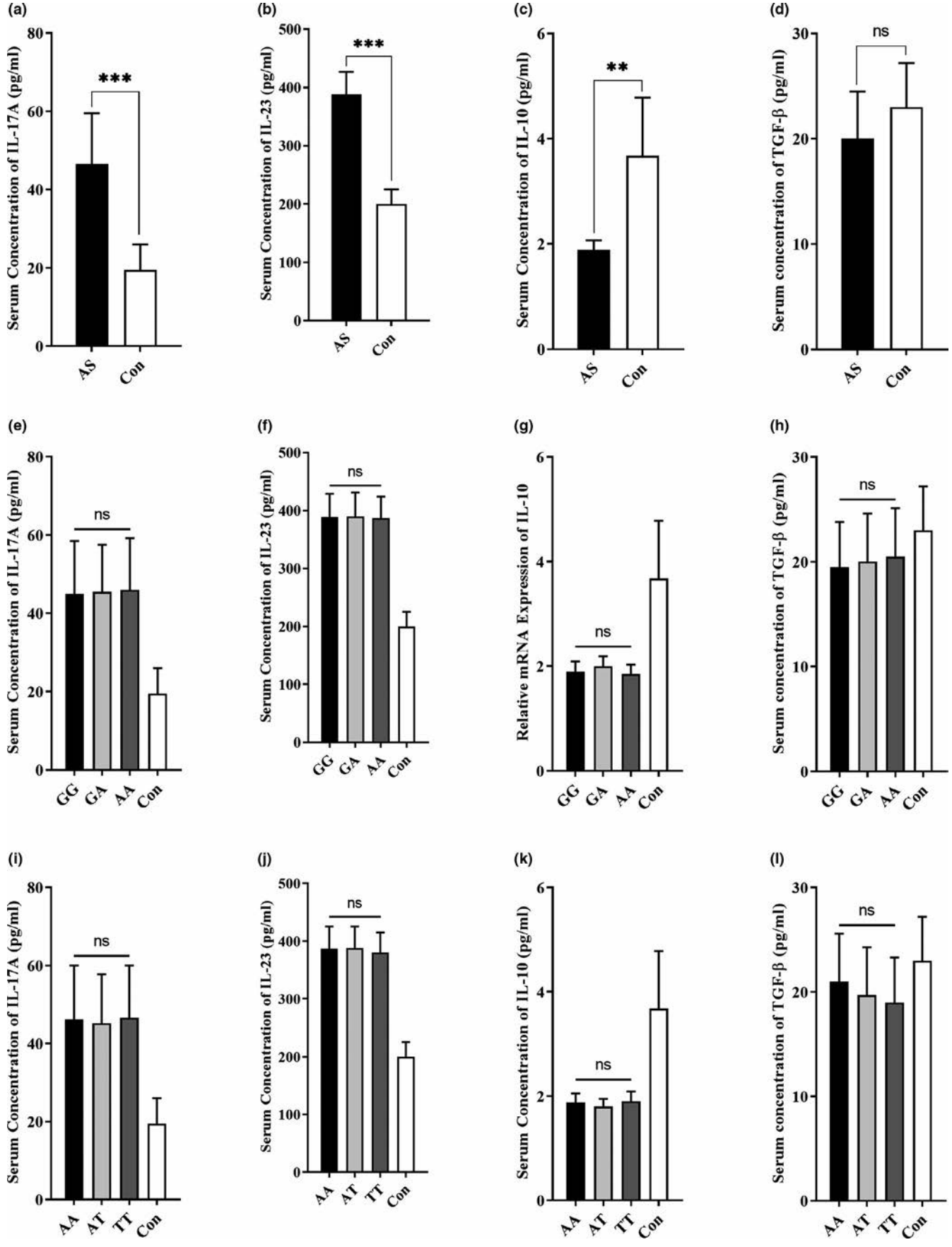

(a)
Serum Concentration of IL-17A (pg/ml)
***
AS
Con
(b)
Serum Concentration of IL-23 (pg/ml)
***
AS
Con
(c)
Serum Concentration of IL-10 (pg/ml)
**
AS
Con
(d)
Serum concentration of TGF-β (pg/ml)
ns
AS
Con
(e)
Serum Concentration of IL-17A (pg/ml)
ns
GG
GA
AA
Con
(f)
Serum Concentration of IL-23 (pg/ml)
ns
GG
GA
AA
Con
(g)
Relative mRNA Expression of IL-10
ns
GG
GA
AA
Con
(h)
Serum concentration of TGF-β (pg/ml)
ns
GG
GA
AA
Con
(i)
Serum Concentration of IL-17A (pg/ml)
ns
AA
AT
TT
Con
(j)
Serum Concentration of IL-23 (pg/ml)
ns
AA
AT
TT
Con
(k)
Serum Concentration of IL-10 (pg/ml)
ns
AA
AT
TT
Con
(l)
Serum concentration of TGF-β (pg/ml)
ns
AA
AT
TT
Con

FIGURE 4 Bar charts depict the serum levels of interleukin-17A (IL-17A), IL-23, IL-10, and transforming growth factor-β (TGF-β) in the ankylosing spondylitis (AS) patients and normal controls (A-D). The comparison of the serum levels of cytokines in the AS patients harboring three different genotypes for rs2287988 single nucleotide polymorphism (SNP) (E-H). Serum concentrations of the cytokines in the AS patients with three genotypes for rs17408150 SNP (I-L). The mean comparisons were done using statistical tests of Mann-Whitney's *U* or Kruskal-Wallis (**$P < 0.01$, ***$P < 0.001$)

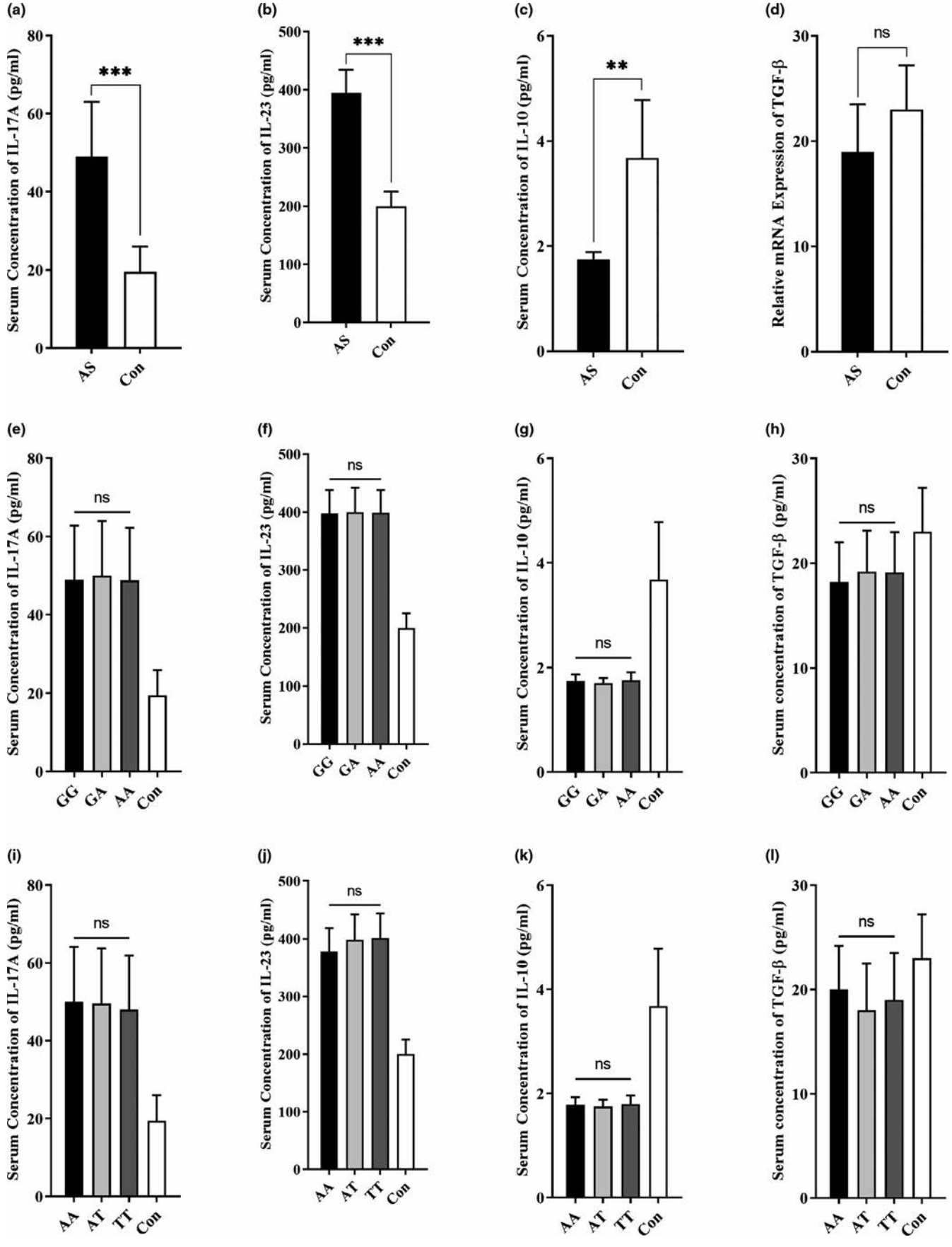
4 | DISCUSSION

To date, large-scale analyses have found over 60 genetic loci for AS risk, even though most investigations have assigned a large proportion of genetic-related risk of AS to the HLA-B27 gene.[3,27] Investigations on the SNPs of the *ERAP2* gene in the context of AS risk are scarce. Herein, the second survey by our group aims to investigate the associations of *ERAP2* gene polymorphisms with the risk of AS in an Iranian population. Additionally, the possible alteration of inflammatory and immunomodulatory cytokines by the *ERAP2* gene rs2548538, rs2287988, and rs17408150 polymorphisms was investigated. However, it should be noted that we did not identify associations between SNPs and AS risk, and the power scores of the calculated *P* values for ORs were below 0.80. Our calculations indicated that in order to reach a statistical power of 0.80 in rs2548538 SNP (for example), we would have to include a further 7000 participants in the groups studied. This implies the inclusion of further individuals in future studies.

Association of the *ERAP2* gene with AS was detected after identification of *ERAP1* gene association with AS.[12] Both *ERAP1* and *ERAP2* genes are found on chromosome 5; they are structured in an inverse direction and share a common intergenic sequence.[28] Studies have found a haplotype in association with AS that is constituted with both *ERAP1* and *ERAP2* polymorphisms.[29,30] In contrast, *ERAP2* polymorphisms generate two main haplotypes, namely haplotype A and B; haplotype A results in protein expression of ERAP2, whereas haplotype B causes no protein expression of ERAP2. The prevalence of both haplotypes A and B has been reported to be similar (approximately 50%), and therefore about 25% of individuals are haplotype B homozygous and have no expression of *ERAP2*.[15] Lack of *ERAP2* expression in haplotype B is because of the *ERAP2* gene rs2248374 polymorphism that influences the RNA stability.[15] It has been reported that there is a strong LD between haplotypes A and B of *ERAP2* SNPs, which can be involved in the immune evasion of trophoblasts.[31] Lack of *ERAP2* expression has been proposed to be protective in AS and other inflammatory disorders.[32] Compared with *ERAP1* polymorphisms, there are few data on the association of *ERAP2* gene variations with AS disease. Our analyses revealed no association of *ERAP2* gene rs2548538, rs2287988, and rs17408150 SNPs with AS risk in an Iranian population. However, we found that, although AAA (OR 2.13) and AAT (OR 2.85) haplotypes were associated with higher AS risk, the AGA (OR 0.86) and TAT (OR 0.57,) haplotypes were associated with lower risk of AS. In addition, there was an LD between rs2548538 and rs2287988 SNPs and between rs2548538 and rs17408150 SNPs. Interestingly, a partially stronger linkage was detected when only the HLA-B27-positive AS group was included in the analysis.

According to studies, the association of ERAP2 with AS seems to be independent of HLA-B27.[14,33] ERAP2 has been reported to impress directly the B*2705 peptidome, removing a number of ligands containing N-terminal basic residues, leading to promoted levels of nonamers by the increased activity of ERAP1.[34] Based on the level of ERAP1 trimming activity, the influences of ERAP2 on the B27 peptidome could be altered.[35] A study indicated that the *ERAP2* gene rs2248374 SNP was particularly associated with risk of psoriatic arthritis in individuals who are negative for HLA-B27.[36] We observed that *ERAP2* gene rs2287988 and rs17408150 polymorphisms were associated with increased risk of the disease in AS patients positive for HLA-B27. In addition, we previously reported association between *ERAP2* gene rs2910686 SNP and AS risk in patients positive for HLA-B27.[22] Further evidence is required to conclusively determine the *ERAP2* involvement in collaboration with HLA-B27 in AS pathogenesis.

The overall effect of ERAP2 on the conformation of HLA-B27 has not been fully determined. A study reported that ERAP2 expression did not significantly impress the expression of the folded as well as unfolded HLA-B27 molecules, markers of endoplasmic reticulum stress, and the expression of proinflammatory cytokines.[37] In contrast, a study demonstrated that lack of ERAP2 triggered upregulation of free heavy chain (FHC)-B27 molecule and promotion of the unfolded protein response pathway in patients.[38] The FHC-B27 molecules have been reported to stimulate an unfolded protein response in antigen-presenting cells like macrophages, which may result in increased generation and release of IL-23.[39] Antigen-presenting cells presenting FHC-B27 molecules can promote the differentiation and expansion of T helper 17 (Th17) cells. Cells presenting FHC-B27 molecules can trigger the release of IL-17A from Th17 cells. IL-23 produced by antigen-presenting cells may bind to the IL-23 receptor on the Th17 cells, resulting in further production of IL-17.[40,41] Our previous investigation indicated that the transcriptional levels and serum concentrations of IL-17A, IL-23, tumor necrosis factor-α, and interferon-γ were not different among AS subjects positive for HLA-B27 carrying different genotypes of the *ERAP2* gene rs2910686 polymorphism (which had an association with higher AS risk in the AS group positive for HLA-B27).[22] As such, the current study indicated that transcriptional levels as well as serum concentrations of IL-17A, IL-23, IL-10, and TGF-β were not significantly different among AS patientts positive for HLA-B27 who harbor different genotypes of the AS-associating rs2287988 and rs17408150 SNPs of the *ERAP2* gene. In addition, no association was found between clinical manifestations of the AS patients and *ERAP2* gene polymorphisms. Taken together, it appears that although HLA-B27 affects the association of *ERAP2* gene rs2287988 and rs17408150


(a)
Serum Concentration of IL-17A (pg/ml)
***
AS
Con
(b)
Serum Concentration of IL-23 (pg/ml)
***
AS
Con
(c)
Serum Concentration of IL-10 (pg/ml)
**
AS
Con
(d)
Relative mRNA Expression of TGF-β
ns
AS
Con
(e)
Serum Concentration of IL-17A (pg/ml)
ns
GG
GA
AA
Con
(f)
Serum Concentration of IL-23 (pg/ml)
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(g)
Serum Concentration of IL-10 (pg/ml)
ns
GG
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Con
(h)
Serum concentration of TGF-β (pg/ml)
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AA
Con
(i)
Serum Concentration of IL-17A (pg/ml)
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TT
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(j)
Serum Concentration of IL-23 (pg/ml)
ns
AA
AT
TT
Con
(k)
Serum Concentration of IL-10 (pg/ml)
ns
AA
AT
TT
Con
(l)
Serum concentration of TGF-β (pg/ml)
ns
AA
AT
TT
Con

FIGURE 5 Bar graphs show the levels of IL-17A, IL-23, IL-10, and transforming growth factor-β (TGF-β) in the serum of the ankylosing spondylitis (AS) patients positive for HLA-B27 and normal controls (A-D). The comparison of the serum levels of cytokines in the AS patients positive for HLA-B27 harboring three different genotypes for rs2287988 single nucleotide polymorphisms (SNP) (E-H). Serum concentrations of the cytokines in the AS patients positive for HLA-B27harboring three different genotypes for rs17408150 SNP (I-L). The mean comparisons were made using the statistical tests of Mann-Whitney's *U* or Kruskal-Wallis (**$P < 0.01$, ***$P < 0.001$)

TABLE 5 Association of *ERAP2* gene rs2548538, rs2287988, and rs17408150 SNPs with clinical manifestations of AS patients (n = 250)

Characteristic	rs2548538 (TT)	rs2548538 (TA)	rs2548538 (AA)	*P* value
Disease duration (y)	10.37 ± 2.91	10.71 ± 3.63	10.55 ± 3.05	.388
CRP (mg/L)	3.25 ± 3.10	3.11 ± 3.41	3.44 ± 3.75	.655
BASDAI score	5.21 ± 3.44	4.46 ± 2.88	4.46 ± 2.43	.266
BASFI score	3.11 ± 2.49	3.56 ± 2.16	3.64 ± 2.15	.385
BASG score	4.05 ± 3.11	5.28 ± 2.43	4.55 ± 2.76	.455
ASQoL score	6.89 ± 3.28	7.80 ± 4.15	7.86 ± 3.54	.390
	rs2287988 (GG)	rs2287988 (GA)	rs2287988 (AA)	P value
Disease duration (y)	9.88 ± 4.14	10.74 ± 3.75	10.22 ± 4.69	.256
CRP (mg/L)	3.15 ± 2.57	3.62 ± 2.41	3.11 ± 2.50	.399
BASDAI score	4.66 ± 2.19	4.11 ± 2.45	5.74 ± 2.48	.313
BASFI score	3.85 ± 2.08	3.18 ± 2.39	3.44 ± 2.76	.746
BASG score	4.57 ± 2.30	4.61 ± 2.20	5.18 ± 2.47	.287
ASQoL score	7.18 ± 3.65	6.98 ± 3.27	7.55 ± 5.40	.818
	rs17408150 (AA)	rs17408150 (AT)	rs17408150 (TT)	P value
Disease duration (y)	10.24 ± 3.79	9.70 ± 3.87	10.47 ± 4.08	.213
CRP (mg/L)	3.28 ± 2.23	3.07 ± 2.63	4.54 ± 2.75	.711
BASDAI score	5.41 ± 2.16	4.12 ± 2.44	4.85 ± 2.11	.570
BASFI score	3.25 ± 2.61	2.82 ± 2.85	3.35 ± 2.84	.597
BASG score	4.11 ± 2.38	5.14 ± 2.42	5.71 ± 2.38	.423
ASQoL score	7.55 ± 4.82	7.35 ± 5.61	6.47 ± 2.63	.646

Abbreviations: AS, ankylosing spondylitis; ASQoL, ankylosing spondylitis quality of life; BASDAI, Bath ankylosing spondylitis disease activity index; BASFI, Bath ankylosing spondylitis functional index; BASG, Bath ankylosing spondylitis global score; CRP, C-reactive protein.

SNPs with AS risk, these *ERAP2* polymorphisms might not be involved in the alteration of HLA-B27 and, hence, the inflammatory settings in the AS disease.

5 | CONCLUSION

For the first time, we have evaluated the association between *ERAP2* gene rs2548538, rs2287988, and rs17408150 SNPs and the risk of AS in Iranian patients. The results indicated that although *ERAP2* gene SNPs had no association with AS risk, rs2287988 and rs17408150 SNPs had an association with promoted AS risk in the patients positive for HLA-B27. Nonetheless, these SNPs were not associated with the transcriptional levels or serum concentrations of the inflammatory or immunomodulatory cytokines. Although we tried to evaluate the involvement of *ERAP2* polymorphisms in AS susceptibility in two studies, further evaluations of this gene, particularly in haplotype analyses, need to be carried out to clarify the bona fide involvement of *ERAP2* SNPs in the etiology and pathogenesis of AS.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to report.

AUTHOR CONTRIBUTIONS

ME performed the experiments, participated in manuscript preparation, and read the manuscript critically. FE performed the statistical analysis, participated in manuscript preparation, and read the manuscript critically. ST contributed in performing the experiments, participated in manuscript preparation, and read the manuscript critically. FSM introduced the patients, participated in manuscript preparation, and read the manuscript critically. SS produced the graphical illustrations, participated in manuscript preparation, and read the manuscript critically. AG-S, MH, SA, FB, FJ-N, and JGN participated in manuscript preparation and read the manuscript critically. HM developed the main idea, obtained the

financial support, participated in manuscript preparation, and read the manuscript critically.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Approval of the study protocol was received from the local ethical review committee in Alborz University of Medical Sciences (Permission No. IR.ABZUMS.REC.1399.051) and written informed consent forms were supplied by all participants.

RESEARCH INVOLVING HUMAN SUBJECTS AND/OR ANIMALS

Research carried out here was in compliance with the Helsinki Declaration. The protocol of this study was approved by the Human Research Ethics Committee from the Alborz University of Medical Sciences, Karaj, Iran (Permission No. IR.ABZUMS.REC.1399.051). Written informed consent forms were obtained from patients and healthy controls before blood taking.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Utility of magnetic resonance imaging in Crohn's associated sacroiliitis: A cross-sectional study

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Abstract

Objective: Prevalence of sacroiliitis in Crohn's disease (CD) is variable depending on defining criteria. This study utilized standardized sacroiliac joint (SIJ) magnetic resonance imaging (MRI) to identify sacroiliitis in CD patients and its association with clinical and serological markers.

Methods: Consecutive adult subjects with CD prospectively enrolled from an inflammatory bowel disease clinic underwent SIJ MRI. Data collected included CD duration, history of joint/back pain, human leukocyte antigen-B27 status, Bath Ankylosing Spondylitis Metrology Index (BASMI), Bath Ankylosing Spondylitis Disease Activity Index, Harvey Bradshaw Index (HBI) for activity of CD, Ankylosing Spondylitis Disease Activity Score, and various serologic markers of inflammation. Three blinded readers reviewed MRIs for active and structural lesions according to the Spondyloarthritis Research Consortium of Canada modules.

Results: Thirty-three CD patients were enrolled: 76% female, 80% White, median age 36.4 years (interquartile range 27.2-49.0), moderate CD activity (mean HBI 8.8 ± SD 4.5). Nineteen subjects (58%) reported any back pain, 13 of whom had inflammatory back pain. Four subjects (12%) showed sacroiliitis using global approach and 6 (18%) met Assessment of SpondyloArthritis international Society MRI criteria of sacroiliitis. Older age (mean 51.2 ± SD 12.5 vs. 37.2 ± 14; $P = .04$), history of dactylitis (50.0%

vs. 3.4%, $P = .03$) and worse BASMI (4.1 ± 0.7 vs. 2.4 ± 0.8, $P \leq .001$) were associated with MRI sacroiliitis; no serologic measure was associated.

Conclusion: There were 12%-18% of CD patients who had MRI evidence of sacroiliitis, which was not associated with back pain, CD activity or serologic measures. This data suggests that MRI is a useful modality to identify subclinical sacroiliitis in CD patients.

KEYWORDS

back pain, Crohn's disease, cytokines, magnetic resonance imaging, sacroiliitis

1 | INTRODUCTION

Inflammatory back pain (IBP) affects 5%-6% of the United States (US) population[1] and it is the most common clinical manifestation of axial spondyloarthritis (SpA). However, prevalence of axial SpA in the US is estimated to be much lower than that of IBP (~1%),[2] suggesting IBP lacks specificity for axial SpA. It is crucial to differentiate IBP due to axial SpA from other causes of chronic low back pain as they have different treatments and prognoses. However, diagnosis of axial SpA can be fraught with challenges[2] and can be particularly difficult in the setting of a co-existing systemic inflammatory disease such as Crohn's disease (CD), which affects 0.2% of US adults.[3] In a systematic literature review, the clinical arm of Assessment of SpondyloArthritis international Society (ASAS) classification criteria for axial SpA showed high specificity (94%) but low sensitivity (23%)[4,5] and sensitivity can be lower, particularly in human leukocyte antigen (HLA)-B27 negative Crohn's patients.[6] Standard radiographs similarly have low sensitivity, and lead to delayed diagnosis, since the transition from non-radiographic axial SpA (nr-axSpA) to radiographic axial SpA (r-axSpA) can take up to 10 years.[7,8]

Disconnect between sacroiliac joint (SIJ) pathology and musculoskeletal complaints in patients with CD is underscored by the fact that radiographic evidence of sacroiliitis is reported in up to 27% of CD patients without current symptoms of back pain.[9,10] These structural changes on X-ray suggest a history of undiagnosed inflammation, which may have been amenable to intervention. Information regarding rate of progression from nr-axial SpA to radiographic SpA in CD specifically is sparse. Since CD often affects younger patients, undertreating SIJ inflammation, and missing the opportunity to mitigate disease progression could have a significant impact on health-related quality of life (HR-QoL) and disability during prime wage-earning and child-rearing years.[11]

Incorporation of magnetic resonance imaging (MRI) to assess axial SpA allows early recognition of axial SpA in CD, especially when patients do not present with classic IBP symptoms or are HLA-B27 negative.[12] However, the ASAS classification criteria of active sacroiliitis are mainly based on bone marrow edema (BME), which puts less weight on structural lesions occurring when BME is absent.[5] This is relevant as structural lesions can be present on MRI in the absence of changes on SIJ radiographs,[13] and in the absence of BME, and may reflect damage from previous inflammatory SIJ disease. There is limited information in the literature evaluating the association of SIJ abnormalities on MRI with back pain in patients with CD, and little data on associations between MRI evidence of SIJ disease and bowel disease activity and biomarkers of systemic inflammation.

Key Messages

1. 12%-18% of prospectively enrolled Crohn's disease patients had evidence of sacroiliitis on contemporary standardized MRI evaluation of sacroiliac joints
2. Sacroiliitis in Crohn's disease had no correlation with inflammatory back pain and other clinical or serological markers of Crohn's disease
3. MRI is a useful tool to recognize axial spondyloarthritis in CD patients, presence of which may impact therapeutic decision making in these patients.

Therefore, the primary aims for this cross-sectional study in a data-deficient area were to: (a) explore the prevalence of MRI evidence of sacroiliitis in CD patients; (b) evaluate the relationship of MRI features of sacroiliitis in CD patients with and without back pain; (c) determine if clinical features of sacroiliitis are associated with MRI evidence of sacroiliitis in CD patients; (d) determine if clinical biomarkers in CD are associated with MRI evidence of sacroiliitis; and (e) explore association of MRI evidence of SIJ inflammation with peripheral blood cytokines.

2 | MATERIAL AND METHODS

2.1 | Study subjects

This is a cross-sectional study of consecutive subjects prospectively identified and enrolled from an outpatient clinic of Jill Roberts Center for Inflammatory Bowel Disease (IBD) at a tertiary care academic medical center. Subjects between 18 and 65 were enrolled from April 2016 through May 2017 (Figure S1). All subjects met clinical, pathological or radiological criteria for CD.[14-16] Patients with ulcerative colitis, indeterminate colitis, other inflammatory arthritis (eg, rheumatoid arthritis, systemic lupus erythematosus, psoriatic or reactive arthritis),

co-existent autoimmune diseases (eg, celiac disease, Behçet's disease) or skin psoriasis were excluded. All subjects were either biologic naïve or had been off systemic biologics >6 months prior to enrollment and could remain on non-biologic CD therapy (eg, methotrexate, sulfasalazine, azathioprine or 6-mercaptopurine). Patients could also be on vedolizumab, an antagonist of α4β7 integrin in the intestinal epithelium which has no established efficacy in extra-intestinal manifestations of CD. Other exclusions included malignancy less than 5 years in remission (except for non-melanomatous skin cancer) or having a contraindication to MRI.

2.2 | Ethics committee

Study was conducted according to Good Clinical Practice guideline and was approved by Institutional Review Boards of Hospital for Special Surgery and Weill Cornell Medicine.

2.3 | Clinical and serologic assessment

After obtaining informed consent, a detailed history was elicited. Subjects reporting back pain were further classified as having IBP if they met ASAS criteria,[17] that is, they fulfilled 4 out of following 5 back pain parameters: onset of symptoms <40 years of age, insidious onset of pain, nocturnal pain, improvement with exercise and no improvement with rest. Subjects with IBP were further classified as having axial SpA according to European Spondylarthropathy Study Group criteria (ESSG)[18] based on IBP plus the underlying CD diagnosis. One investigator (FM) performed a 66-68 joint count, entheses exam (lateral epicondyles of humerus, medial condyles of femur and Achilles tendons), obtained Bath Ankylosing Spondylitis Metrology Index (BASMI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Ankylosing Spondylitis Disease Activity Index (ASDAS), and a Harvey Bradshaw index (HBI), a validated measure of CD activity.[19] Peripheral blood was collected for measurement of C-reactive protein (CRP) and cytokine analysis (interleukin [IL]-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12/23, IL-17A, IL-17F, IL-21, IL-22, γ-interferon and tumor necrosis factor [TNF]-α). CRP value was used to calculate ASDAS-CRP (Ankylosing Spondylitis Disease Activity Index-CRP), a validated measure of axial disease activity.[20] Cytokine concentrations in serum were determined using a Legendplex human Th cytokine 13-plex panel kit (BioLegend) according to the manufacturer's instructions. Data were acquired with a BD LSRFortessa flow cytometer (BD Biosciences) and analyzed using the BioLegend's LEGENDplex Data Analysis Software.

2.4 | MRI protocol

MRI was performed with 1.5 Tesla clinical imaging units (GE Healthcare, Waukesha, WI, USA) using phased-array coils. Sequences were acquired in a semicoronal plane tilted parallel to the long axis of the SIJ with 3-mm section thickness and 34 slices acquired. Sequences were as follows: T1-weighted spin echo (T1; time to recovery [TR] 500-600 milliseconds, time to echo [TE] 12 milliseconds) and short tau inversion recovery (STIR) fast spin echo (TR 4000-5000 milliseconds, time to inversion 150 milliseconds, effective TE 15-20 milliseconds). All subjects underwent T1 and STIR sequence MRI of SIJ.

2.5 | Evaluation of SIJ MRI

The semicoronal images were independently read and scored by 2 expert rheumatologists (SJP, UW) and 1 newly trained rheumatologist reader (GK), blinded to any clinical information. SIJ MRIs were evaluated and scored for presence of BME and structural lesions (erosion, fat metaplasia, backfill and ankylosis) using a validated scoring method originally derived from the Spondyloarthritis Research Consortium of Canada (SPARCC) SIJ module.[21,22] SIJ MRI was considered "positive" for presence of sacroiliitis if it met global evaluation, based on the reader's overall evaluation of presence or absence of sacroiliitis by taking into account the contextual signature of both active and structural SIJ lesions.[23] In addition, we tested whether the following 3 MRI criteria for sacroiliitis were met: (a) ASAS definition of active sacroiliitis;[24] (b) SPondyloarthritis Caught Early (SPACE) proposal,[25] based on presence of erosions and fat metaplasia; and (c) Morpho proposal,[26] based on presence of BME and/or erosion. For analysis, MRI positivity for sacroiliitis was defined based on majority-of-readers agreement (≥2 out of 3 readers).

2.6 | Standardized lesion definitions on SIJ MRI

Standardized lesion definitions illustrated by a set of annotated reference images were applied.[21,27] SIJ lesions were scored binarily as being present or absent per joint quadrant for BME, fat metaplasia and erosion (range 0-8 lesions per MRI slice), or per joint half for backfill and ankylosis (range 0-4 lesions per MRI slice). SI joint quadrants were generated by virtual lines subdividing each SIJ into an upper and lower half on the iliac and sacral side. BME was defined as an increase in bone marrow signal in the subchondral bone on STIR images, fat metaplasia as a focal increased signal in bone marrow on T1SE images. For both lesion types, the center of the sacrum at the same craniocaudal level was used as the primary reference for normal bone marrow signal. Erosion was determined as full-thickness loss of dark appearance of either iliac or sacral cortical bone of the SIJ and change in normal bright appearance of adjacent bone marrow on T1SE images. Normal iliac or sacral marrow on the same slice at the same craniocaudal level served as reference signal. Backfill was defined as bright signal on T1SE sequence within an erosion cavity, demarcated from adjacent bone marrow by an irregular band of dark signal reflecting sclerosis at the border of the original erosion. Ankylosis was defined as bright signal on T1SE images extending across the SIJ.

2.7 | Statistical analysis

Descriptive analyses were conducted for all baseline variables and are presented as means or medians for continuous variables and percentages for categorical variables. Differences between groups were quantified using the independent *t* test (normally distributed data), Mann-Whitney test (non-normally distributed data) and Chi-square test when appropriate. Mean (SD) and median (interquartile range [IQR]) of SIJ quadrants/halves affected by a given lesion were computed over 3 readers pooled. The frequency of affected SIJ quadrants/halves on subject level and the frequency of MRI evidence for sacroiliitis according to 4 pre-defined MRI classification criteria were described as concordantly reported by the majority (≥2/3) of readers to enhance specificity. We also calculated the proportion of subjects where all 3 readers agreed that a given SIJ lesion is absent. Agreement between readers for granular SIJ lesions (ie, BME, erosion, ankyloses, fat metaplasia and backfill) was calculated by intraclass correlation coefficient (ICC), two-way random effects, single measure, absolute agreement definition, for all readers together and for the 3 possible reader pairs separately.[28] ICC values of <0.50, <0.75, ≤0.90, and >0.90 were considered to reflect poor, moderate, good, and excellent reproducibility, respectively.[29] Furthermore, kappa (κ) statistics were utilized to determine agreement between readers for dichotomous outcomes of 4 definitions of MRI positivity.[30] Kappa agreement was categorized according to Landis and Koch:[31] <0 = no agreement, 0.00-0.20 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial and 0.81-1.00 = perfect. SIJ MRI of 71 patients from an unrelated axial SpA inception cohort[23] (24 subjects with active ankylosing spondylitis, 23 subjects with both IBD and ankylosing spondylitis and 24 subjects with non-specific back pain) served to determine reader agreement in a calibration sample with a relatively high frequency of the 5 SIJ lesions under consideration. SPSS Statistics, Version 25.0 (IBM, Armonk, NY, USA) was used to perform statistical analysis.

3 | RESULTS

Thirty-three subjects with CD were enrolled. Subjects were 76% female, 80% White, had a median age of 36.4 years (IQR 27.2-49.0) with moderate CD activity (mean HBI 8.8 ± SD 4.5). Fifty-five percent were noted to have peripheral arthritis on exam at the time of MRI. Nineteen subjects (58%) had back pain, 13 (39%) of whom met ASAS criteria for IBP. Only 1 subject (3%) was HLA-B27 positive. Subjects with and without any back pain were similar in terms of duration and activity of CD (Table 1). However, more subjects with any back pain were noted to have evidence of peripheral SpA at the time of assessment compared to subjects without back pain (74% vs. 29%; $P = .015$). Subjects with any back pain also had worse BASMI scores (2.94 ± 0.86 vs. 2.11 ± 0.90; $P = .01$), worse BASDAI scores and (5.33 ± 1.83 vs. 2.39 ± 1.62, $P < .001$) and worse ASDAS-CRP (3.15 ± 0.97 vs. 1.66 ± 0.78, $P < .01$; Table 1).

TABLE 1 Clinical characteristics and SIJ MRI finding in CD subjects stratified by presence of back pain

Variable	Any back pain (N = 19, 57.6%)	No back pain (N = 14, 42.4%)	*P* value
Patient			
Age	42.4 ± 13.3	34.1 ± 12.6	.080
Female gender	13/19 (68.4%)	12/14 (85.7%)	.416
Tobacco use	5/18 (27.8%)	1/14 (7.1%)	.196
Axial SpA (ESSG)[a]	13/19 (68%)	—	—
Peripheral arthritis (current)	14/19 (73.7%)	4/14 (28.6%)	**.015**
Peripheral arthritis (history)	16/19 (84.2%)	6/14 (42.9%)	**.024**
BASDAI	5.3 ± 1.8	2.4 ± 1.6	**<.001**
BASMI	2.9 ± 0.9	2.1 ± 0.9	**.011**
ASDAS-CRP	3.1 ± 0.9	1.6 ± 0.8	**<.001**
CRP	1.6 ± 2.0	1.7 ± 3.3	.930
Crohn's disease			
Duration of disease	11.9 ± 6.7	14.2 ± 10.5	.476
Vedolizumab use	3/19 (15.8%)	4/13 (30.8%)	.401
Past history of biologic use	11/19 (57.9%)	8/13 (61.5%)	.837
Surgery related to CD	10/18 (55.6%)	9/13 (69.2%)	.484
CD activity (HBI score)	9.1 ± 4.9	6.9 ± 3.7	.183
MRI positivity			
Global assessment positive	4/19 (21.1%) (6.1%-45.6%)[b]	0/14 (0%) (0.0%-23.1%)[b]	.119
Alternative MRI definitions of positivity			
ASAS positive	4/19 (21.1%) (6.1%-45.6%)[b]	2/14 (14.3%) (1.8%-42.8%)[b]	.999
SPACE positive	0/19 (0%) (0.0%-17.6%)[b]	0/14 (0%) (0.0%-23.1%)[b]	N/A
Morpho positive	5/19 (26.3%) (9.1%-51.2%)[b]	1/14 (7.1%) (0.2%-33.9%)[b]	.209

Abbreviations: ASAS, Assessment of SpondyloArthritis international Society; ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score C-reactive protein; BASDAI, Bath Ankylosing Spondylitis Activity Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; CD, Crohn's disease; CRP, C-reactive protein; ESSG, European Spondyloarthropathy Study Group; HBI, Harvey Bradshaw Index; SpA, spondyloarthritis; SPACE, SPondyloArthritis Caught Early.

Bold values correspond with a *P* value <0.05, i.e. statistically significant.

[a]Modified ESSG: ASAS criteria was utilized to define IBP. Original ESSG Axial SpA[17] utilized Calin criteria.[41]

[b]95% CI.

Twelve percent (4/33) of all CD subjects showed evidence of sacroiliitis on MRI based on global assessment (Figures 1 and 2). All 4 of these subjects reported back pain at the time of MRI and

constitute 21% of CD subjects reporting back pain (4/19). None of the subjects without any back pain showed MRI sacroiliitis according to global assessment (21% vs. 0%, $P = .12$) (Table 1). Among the CD subjects with any back pain, 21% (4/19) showed BME meeting ASAS definition of active sacroiliitis and 26% (5/19) met Morpho proposal (presence of ≥ 3 quadrants of BME and/or ≥ 2 erosions). Of subjects without back pain, 14% (2/14) and 7% (1/14) met ASAS and Morpho criteria respectively. None of the patients in either group met SPACE proposal for MRI positivity (Table 1). IBP was present in 100% (4/4) of those with global MRI positivity and in 52% (15/29) of those without global MRI positivity ($P = .12$) (Table 2).

CD subjects with global MRI positivity were older (51.2 ± 12.5 vs. 37.2 ± 12.9; $P = .04$), even after controlling for CD duration (data not shown). These Crohn's subjects also had worse BASMI scores (4.1 ± 0.7 vs. 2.4 ± 0.8; $P \leq .001$) and reported history of dactylitis (50% vs. 3.4%, $P = .03$) Presence of HLA-B27, peripheral arthritis (either current or previous history), duration of CD, CD activity, history of biologic use in the past, current treatment of CD with vedolizumab were not associated with MRI positivity (Table 2). In addition, there were no statistically significant associations of any serum cytokines and MRI evidence of sacroiliitis (Table 3).

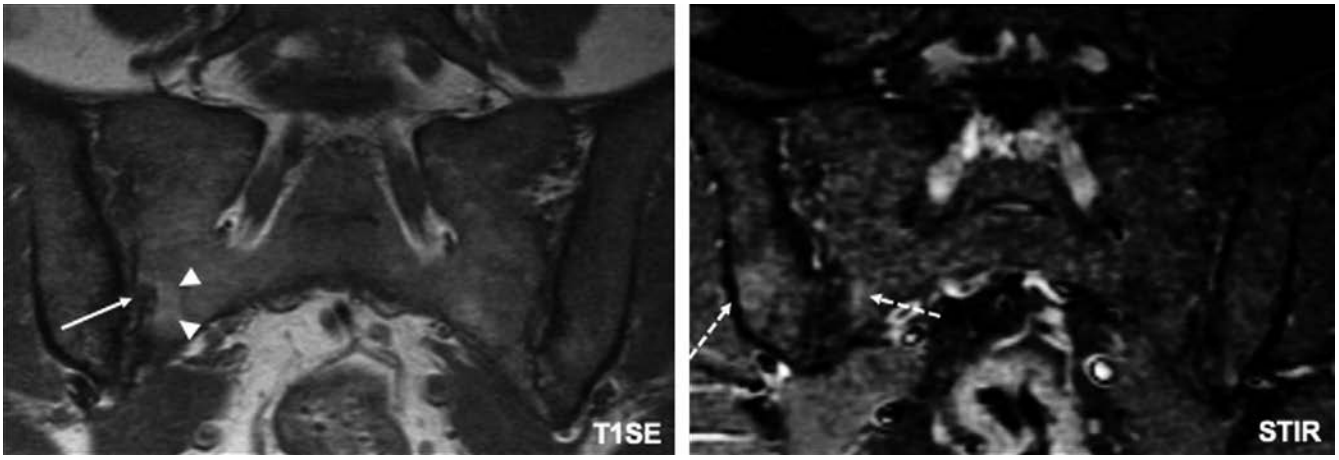

FIGURE 1 Active erosive sacroiliitis: 36-year old (human leukocyte antigen-B27 not available) female with Crohn's disease (CD) for 12 y, moderate CD activity, remote history of peripheral arthritis (absent at the time of magnetic resonance imaging) and inflammatory back pain. The T1SE sequence (left panel) shows a sacral erosion (arrow) with perifocal fat metaplasia (arrowheads) in the right distal sacroiliac joint. The structural lesions are surrounded by bone marrow edema (broken arrows) of both sacrum and ilium (short tau inversion recovery scan, right panel)

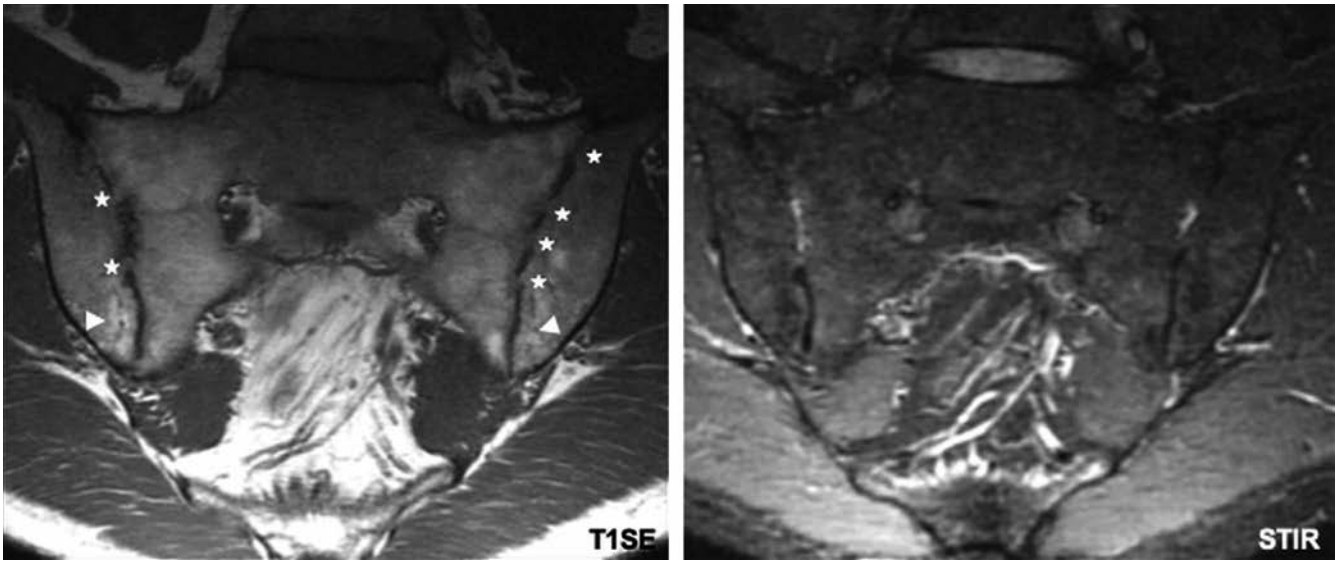

FIGURE 2 Incipient ankylosis: 36-year old human leukocyte antigen-B27 negative male with Crohn's disease (CD) for 13 y, moderate CD activity, peripheral arthritis on exam and inflammatory back pain. Multiple small bony bridges (asterisks) are visible in both sacroiliac joints on the T1SE sequence (left panel) together with fat metaplasia (arrowheads) in the distal ilium bilaterally. Bone marrow edema is absent on short tau inversion recovery slices (right panel)

TABLE 2 Clinical characteristics of CD subjects stratified by global MRI positivity

Variable	Global MRI Positive (N = 4, 12.1%)	Global MRI Negative (N = 29, 87.9%)	*P* value
Demographics			
Age	51.2 ± 12.5	37.2 ± 12.9	**.04**
Female gender	2/4 (50.0%)	23/29 (79.3%)	.24
Tobacco use	2/4 (50.0%)	4/28 (14.3%)	.15
ASAS clinical arm variables			
Inflammatory back pain	4/4 (100.0%)	15/29 (51.7%)	.12
Peripheral arthritis (current)	3/4 (75.0%)	15/29 (51.7%)	.60
Peripheral arthritis (history)	4/4 (100.0%)	18/29 (62.1%)	.28
Current enthesitis	2/8 (25.0%)	8/29 (27.6%)	.99
History of uveitis	1/4 (25.0%)	3/29 (10.3%)	.42
History of dactylitis	2/4 (50.0%)	1/29 (3.4%)	**.03**
HLA-B27[a]	0/4 (0.0%)	1/29 (3.4%)	.99
CRP	2.6 ± 3.0	1.5 ± 2.6	.43
SpA related variables			
BASDAI	4.3 ± 1.7	4.2 ± 2.4	.92
BASMI	4.1 ± 0.7	2.4 ± 0.8	**<.001**
ASDAS-CRP	3.3 ± 0.4	2.5 ± 1.2	.19
Crohn's disease variables			
Duration of disease	17.9 ± 3.8	12.2 ± 8.7	.20
HBI score	7.8 ± 4.9	8.3 ± 4.5	.83
Vedolizumab use	0/4 (0.0%)	7/28 (25.0%)	.55
Prior history of biologic use	3/4 (75.0%)	16/28 (57.1%)	.63
Surgery related to CD	3/4 (75.0%)	16/28 (57.1%)	.99

Abbreviations: ASAS, Assessment of SpondyloArthritis international Society; ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score C-reactive protein; BASDAI, Bath Ankylosing Spondylitis Activity Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; CD, Crohn's disease; CRP, C-reactive protein; HBI, Harvey Bradshaw Index; HLA-B27, Human Leukocyte Antigen B-27.

Bold values correspond with a *P* value <0.05, i.e. statistically significant.

[a]HLA-B27 missing in 2 subjects.

TABLE 3 Cytokine levels of CD subjects stratified by ASAS MRI positivity

Variable	ASAS MRI Positive (N = 6, 18%)	ASAS MRI Negative (N = 25, 82%)	*P* value
Cytokine			
Interleukin 2	4.7 ± 2.0	5.6 ± 7.8	.79
Interleukin 4	3.5 ± 1.6	5.7 ± 4.6	.33
Interleukin 5	3.7 ± 0.0	3.8 ± 0.1	.25
Interleukin 6	6.2 ± 7.2	5.1 ± 6.9	.71
Interleukin 9	2.4 ± 1.5	1.9 ± 0.9	.29
Interleukin 10	31.0 ± 59.3	6.5 ± 1.8	.36
Interleukin 13	8.9 ± 6.3	8.9 ± 4.4	.93
Interleukin 12-23	214.0 ± 159.5	259.1 ± 227.7	.65
Interleukin 17A	25.3 ± 13.1	21.1 ± 10.5	.40
Interleukin 17F	4.8 ± 2.2	3.8 ± 3.5	.49
Interleukin 21	19.8 ± 36.2	40.0 ± 43.2	.30
Interleukin 22	15.2 ± 7.1	16.7 ± 11.2	.75
Interferon-γ	75.7 ± 31.0	79.0 ± 28.8	.80
TNF-α	19.5 ± 15.2	35.4 ± 94.1	.68

Abbreviations: ASAS, Assessment of SpondyloArthritis International Society; CD, Crohn's disease; TNF, tumor necrosis factor.

BME was the most frequently observed MRI lesion seen in all CD subjects with mean ± SD number of SIJ quadrants affected being 0.86 ± 1.45 (Figure 1B). Up to 30% of subjects showed BME in at least 1 quadrant with 21% showing BME in ≥ 2 SIJ quadrants while 3% had ≥ 6 SIJ quadrants with BME. Ankylosis was the next most frequent MRI lesion with a mean ± SD number of affected SIJ halves as 1.02 ± 3.45 (Figure 2A). Fat metaplasia was noted in up to 6% of subjects affecting a mean of 0.98 ± 3.55 SIJ quadrants (Figures 1A and 2A). Erosion and backfill were least frequent (3% of subjects) (Table 4). The proportion of subjects where all 3 readers agreed on absence of a given lesion type on SIJ MRI, was 58%, 88%, 97%, 88% and 91% for BME, erosion, backfill, fat metaplasia and ankylosis respectively.

In a comparison cohort of patients with incipient axial SpA, which showed abundant SIJ lesion frequency, agreement for granular SIJ lesions (mean ICC, 95% CI) between all readers was consistent with previous reports: excellent for ankylosis (0.94, 0.91-96), good for BME (0.89, 0.85-0.92) and erosion (0.85, 0.79-0.90) and moderate for backfill (0.69, 0.57-0.78) and fat metaplasia (0.62, 0.44-0.75) (Table S1). There was a remarkably consistent concordance between the 2 experienced (R1 and R2) and the newly trained reader (R3) (Table S1). In this cohort, agreement for dichotomous MRI outcomes, mean κ was substantial with an average κ of 0.72 for global, 0.64 for ASAS, 0.80 Morpho and 0.72 for SPACE. Concordance between the 2 experienced (R1 and R2) and newly trained reader (R3) remained consistent and κ values remained substantial for global (R3 and R1: 0.66, and R3 and R2: 0.77), Morpho (R3 and R1: 0.79 and R3 and R2 0.80) and SPACE (R3 and R1: 0.78 and R3 and R2: 0.66) while it was moderate to substantial for ASAS (R3 and R1: 0.53 and R3 and R2: 0.69) in the axial SpA inception cohort.. In the 33 Crohn's subjects with low lesion frequency as outlined above, agreement (mean ICC, 95% CI) was poor for identification of BME (0.43, 0.21-0.63), erosion (0.17, −0.03-0.41) and backfill (0.30, 0.09-0.53) and moderate for fat metaplasia (0.51, 0.30-0.70) and ankylosis (0.65, 0.47-0.79). Agreement (κ) was moderate in detection of MRI positivity in all criteria (0.42 for Morpho, 0.45 for ASAS, 0.49 for SPACE and 0.51 for global) (Table S2).

TABLE 4 Frequency of 5 MRI lesion types based on affected sacroiliac joint quadrants

SIJ quadrants affected	BME	Erosion	Fat metaplasia	Backfill	Ankylosis
Mean ± SD	0.86 ± 1.45	0.05 ± 0.15	0.98 ± 3.55	0.04 ± 0.23	1.02 ± 3.45
Median (IQR)	0 (0-1.3)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
n (%) with ≥ 1 quadrants	10 (30)	1 (3)	2 (6)	1 (3)	2 (6)
n (%) with ≥ 2 quadrants	7 (21)	0 (0)	1 (3)	0 (0)	2 (6)
n (%) with ≥ 3 quadrants	5 (15)	0 (0)	1 (3)	0 (0)	2 (6)
n (%) with ≥ 4 quadrants	2 (6)	0 (0)	1 (3)	0 (0)	2 (6)
n (%) with ≥ 5 quadrants	2 (6)	0 (0)	1 (3)	0 (0)	2 (6)
n (%) with ≥ 6 quadrants	1 (3)	0 (0)	1 (3)	0 (0)	2 (6)
n (%) with ≥ 7 quadrants	0 (0)	0 (0)	1 (3)	0 (0)	2 (6)

Note: Mean (SD) and median (interquartile range) of SIJ quadrants: pooled over 3 readers. n (%) with ≥ 1 to ≥ 7 SIJ quadrants: number of subjects (percentage) having ≥ 1 to ≥ 7 SIJ quadrants affected by a given lesion type as concordantly reported by ≥ 2/3 readers.

Abbreviation: BME, bone marrow edema; SIJ, sacroiliac joint.

4 | DISCUSSION

This cross-sectional study in a poorly understood subtype of SpA investigated the prevalence of sacroiliitis using 4 different MRI proposals in a sample of prospectively enrolled subjects with CD. We also evaluated the relationship of MRI changes with clinical and serological markers of disease activity. Our major findings were as follows: (a) 12% of patients with CD had MRI evidence of sacroiliitis, based on global assessment and 18% met ASAS definition; (b) patient-reported overall and IBP had no statistically significant relationship with MRI evidence of sacroiliitis in these subjects with CD; (c) older age, and poor spinal mobility on physical exam and prior history of dactylitis were associated with MRI evidence of sacroiliitis; (d) presence of peripheral arthritis, CD activity or serum cytokine levels were not associated with MRI evidence of sacroiliitis. Importantly, whether or not subjects reported IBP had no statistically significant association with sacroiliitis on MRI. Given that about 1 out of 6 of unselected Crohn's patients had MRI evidence of sacroiliitis, these findings suggest that MRI could be an important tool for identifying sacroiliitis in Crohn's patients, regardless of type of back pain if present.

How best to define sacroiliitis on MRI remains controversial, and which types of MRI lesions are most relevant in different populations is unclear. We explored a number of different imaging proposals to score the MRIs for sacroiliitis.[24-26] One prior study from the UK reported much higher prevalence of MRI defined sacroiliitis (39%) in CD patients, which could be in part explained by higher prevalence of HLA-B27 positivity in their cohort, as well as differences in MRI methodology and interpretation.[32] Moreover, CD patients in our cohort were either biologic naïve or off systemic biologics for at least 6 months. These data suggest that MRI may be a useful tool to identify sacroiliitis in CD patients, currently not on systemic biologics and may benefit from the addition of therapies with proven efficacy in treatment of axial SpA.

We did not observe a statistically significant association between patient-reported back pain and MRI evidence of sacroiliitis. In addition, we found that 7% and 14% of CD patients without any back pain had MRI evidence of sacroiliitis based on ASAS and Morpho proposals respectively. Both these criteria rely on presence of BME, which has been reported to be present in 25%-33% of healthy asymptomatic subjects, challenging specificity of this finding.[33] In our cohort, CD subjects without any back pain at the time of MRI did not have MRI evidence of sacroiliitis when assessed globally. Global evaluation takes into account the contextual information by concomitant presence of active and structural SIJ lesions.[26] This approach mirrors strategies utilized by radiologists to determine presence of sacroiliitis in clinical practice. Our inability to detect a statistically significant association with back pain and global MRI positivity is most likely due to small sample size and needs to be explored in a large sample.

Our study showed older age was associated with MRI positivity (meeting any 1 of 4 MRI proposals as above) and this relationship was not influenced by the duration of CD. Subjects with MRI positivity also had higher BASMI scores, reflecting worse spinal mobility on physical exam. This could serve as an easy clinical exam in CD patients prior to obtaining MRI. Prior history of dactylitis was also associated with MRI positivity but having history of other SpA features in the clinical arm of ASAS classification criteria (ie, presence of IBP, peripheral arthritis, uveitis, enthesitis, HLA-B27) was also not associated with MRI positivity in our cohort. Furthermore, our findings confirm previous studies which found clinical factors related to

CD, such as CD activity measures, duration of disease or surgical history have no association with sacroiliitis on imaging.[34,35] This lack of correlation with clinical disease activity and MRI evidence of SIJ inflammation underscores the potential importance of utilizing MRI for early identification of patients at risk for sacroiliitis, irrespective of type of back pain.

The IL-23/IL-17 axis plays a key role in pathogenesis of axial SpA and could explain the gut-joint axis in Crohn's-associated axial SpA as there is overexpression of IL-23 in the terminal ileum of CD patients,[36] association of IL-23R single nucleotide polymorphism with both AS[37] and IBD[38] and clinical efficacy of blocking IL-12/23p40 subunit in CD.[38,39] TNF-α is another major cytokine in pathogenesis of both CD and SpA. Our results are similar to previous studies which found no difference in serum cytokine levels between subjects with and without MRI evidence of sacroiliitis.[40] However, we recognize that this study is underpowered to show how tissue-specific differences regulate inflammatory disease.

Our study has several limitations. Our sample size is small, hence lack of association with clinical parameters will need to be examined in a larger cohort. However, our sample size is comparable to a previously published study which included 44 subjects.[32] We also examined MRI evidence of sacroiliitis in a group of CD patients with varying levels and duration of disease activity, which may have also hampered our ability to identify associations. The cross-sectional design precluded evaluating the longitudinal effect of BME on disease progression. Longitudinal follow-up with SIJ MRI at intervals will be important to better understand the prospective effect of subclinical sacroiliitis in CD patients.

Our agreement analysis ranged from moderate to excellent for granular SIJ lesions and substantial for various criteria of MRI positivity in the external cohort enriched for all types of SIJ lesions, suggesting major reader concordance consistent with the literature, yet low agreement in the Crohn's patients, who displayed a very low frequency across all lesion types. This gap in MRI reader concordance is most likely explained by low lesion frequency and variability,[41] replicating an observation from a previous report in healthy athletes.[33]

The SIJ MRI findings in our study provide useful preliminary insights into a poorly understood subset of SpA patients. In conclusion, we found that 12%-18% of CD subjects had MRI evidence of sacroiliitis and there was an association with older age, history of dactylitis and higher BASMI scores. Other clinical and serological markers of CD and SpA were not associated with MRI evidence of sacroiliitis, which emphasizes the potential role of SIJ MRI in identifying CD patients with subclinical sacroiliitis. This is clinically important, as early institution of axial SpA therapy might modify radiographic progression.[42] Lack of concordance between CD disease activity and SpA symptoms suggests different therapeutic approaches are warranted for different clinical presentations of CD-associated SpA, especially since a number of therapeutic options approved for management of axial SpA, such as nonsteroidal anti-inflammatory drugs and IL-17 blockers, can actually cause CD to flare.[43] Therefore, given the prevalence of MRI evidence of sacroiliitis in CD, careful choice of therapy will be paramount, as well as the need for development of new targeted therapies, which could simultaneously target pathways responsible for both the bowel and axial manifestations of CD.

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CONFLICT OF INTEREST

The authors in this manuscript declare no relevant financial conflict of interest.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Identification of serum interleukin-13 and interleukin-13 receptor subunit expressions: Rheumatoid arthritis–associated interstitial lung disease

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Abstract

Aim of the work: To identify the role of serum IL-13, and its receptor subunit expressions as a serologic marker of rheumatoid arthritis (RA)-associated ILD (RA-ILD).

Patients and Methods: Fifty RA patients with ILD and 50 RA patients without ILD were examined, in addition to 50 controls. Disease Activity Score in 28 joints (DAS-28), the Health Assessment Questionnaire (HAQ), and medication history were evaluated. ESR, CRP, RF, Anti-CCP, Serum Krebs von den Lungen-6 (KL-6), surfactant protein D (SP-D) levels, Interleukin 13 and its receptors (IL-13 Rα1 and L-13 Rα2), and mRNA relative expression levels in peripheral blood mononuclear cells (PBMCs) were measured. High-resolution computed tomography (HRCT) scores were used with all RA patients with interstitial lung disease.

Results: Mean age, percent of male affection, duration of the disease, DAS28 and MHAQ were significantly higher in the RA–ILD group than in the RA–no ILD group. ESR, CRP, RF, anti-CCP, serum KL-6, SP-D, IL-13 levels, IL-13 Rα1and IL-13 Rα2 mRNA expressions were significantly increased in RA patients compared to controls; in addition, their levels were significantly higher in the RA–ILD group than in the RA–no ILD group. Serum IL-13 levels and IL-13 Rα1and IL-13 Rα2 were positively correlated with RF, Anti-CCP, KL-6, SP-D, and the HRCT score ($P < .001$).

Conclusions: Serum IL-13 and its receptor subunit expressions are useful biomarkers which can be used in detecting severity of the interstitial lung disease in RA patients.

KEYWORDS

IL-13 Rα1and IL-13 Rα2 mRNA expression, RA-associated ILD, serum IL-13 levels

1 | INTRODUCTION

Rheumatoid arthritis (RA) is the most common systemic autoimmune disease, affecting 1-2% of adults.[1,2] The primary targets of RA are the joints and the synovium. However, extraarticular manifestations of cutaneous nodules, ocular, vascular/cardiac disease, and pulmonary complications can lead to significant morbidity and an excess mortality.[3,4] Insights in the past several years underscored the epidemiologic impact of clinically/functionally significant RA-associated ILD (RA-ILD), and identified factors contributing to the pathogenesis of this potentially devastating complication of RA. Despite advances, the complexity of RA-ILD and lack of reliable predictors highlight a

need for improved biomarker development to guide the application and timing of therapeutic agents as immunomodulators or newly-studied antifibrotic agents.[5,6]

Interleukin 13 (IL-13), mainly secreted by Th2 cells, is a proinflammatory cytokine that[7,8] was shown to play an important role in inflammatory and fibrotic diseases, such as asthma, idiopathic pulmonary fibrosis, systemic sclerosis, and pulmonary granulomatous disease.[9,10] IL-13 binds to two structurally and functionally distinct receptors: IL-13 receptor α1 (IL-13Rα1) and IL-13 receptor α2 (IL-13Rα2). Both are overexpressed in malignant glioma and other cancer cell types.[11-14]

IL-13, was found to be increased in the blood and bronchoalveolar lavage of patients with Idiopathic pulmonary fibrosis. It also promotes pulmonary fibrosis in fluorescein isothiocyanate– and radiation-induced lung fibrosis models. Mechanistically, IL-13 differentiates human lung fibroblast to myofibroblast through a c-Jun N-terminal kinases dependent pathway.[15]

Our aim was to identify the role of serum IL 13, and its receptor subunit expressions (IL-13Rα1& IL-13 Rα2) as serologic markers of RA-associated ILD (RA-ILD).

2 | SUBJECTS AND METHODS

2.1 | Study population

Fifty RA patients with interstitial lung disease (RA-ILD) and fifty RA patients without ILD (RA–no ILD) were selected from the inpatient and outpatient clinics of the Rheumatology Department, Faculty of Medicine, Tanta University. The patients were collected from May 2020 through September 2020. Fifty healthy volunteers were included as a control group for laboratory investigations.

All patients with rheumatoid arthritis (RA) were diagnosed according to the American College of Rheumatology (ACR) and European League against Rheumatism (EULAR) 2010 criteria for a diagnosis of RA.[16] ILD was defined according to Bongartz et al.[17] Definitive ILD was used when the diagnosis was made by a pulmonologist, in the presence of two of the following three criteria: ILD observed in chest radiograph or chest CT, restrictive pattern observed on pulmonary function test (<80% predicted forced expiratory volume in 1 [FEV1], forced vital capacity [FVC], total lung capacity [TLC], diffusing capacity for carbon monoxide [DLCO]: >80% predicted FEV1/FVC), and bronchoscopy or surgical lung biopsy compatible with ILD.

Abnormally high resolution computed tomography (HRCT) scan showing at least one of the following features: (a) reticulation and fibrosis, (b) traction bronchiectasis, (c) honeycombing, or (d) ground glass opacification. [18] Patients with chronic chest disorders as asthma, obstructive pulmonary disease and history of active or treated tuberculosis, or congestive heart failure were excluded.

The study was approved by the Scientific and Ethics committees of Tanta University Hospital, Tanta, Egypt. (Approval code 33813). Written informed consent was obtained from the RA patients.

2.2 | Patients and methods

2.2.1 | Clinical assessment

Demographic data, clinical features including respiratory parameters (cough and dyspnea), disease activity for 28 joint indices score (DAS-28),[19] Health Assessment Questionnaire (HAQ),[20] and medication history were evaluated.

2.2.2 | Laboratory assessments

Blood sampling

After 12 hours of overnight fasting, 7 mL of venous blood samples were collected from patients and controls. A portion of blood was collected in a dry sterile centrifuge tube, allowed to clot at room temperature, centrifuged at 3500 *g* for 10 minutes, as the serum was frozen at −80°C until analysis. Another portion of blood collected in heparinized tubes was stored at −80°C until preparation of peripheral blood mononuclear cells.

Biochemical assays

1. Erythrocyte sedimentation rate (ESR in mm/h) was determined with the Westergren method.
2. Serum C-reactive protein (CRP in mg/L), was quantified by using ELISA Kits (Cat. No.KA0238, Novus Biologicals, USA) as per the manufacturer's instructions.
3. Rheumatoid factor (RF), was measured using commercial ELISA kits supplied by (Cat.No.RFG31-K01, Eagle Bioscience, New Hamshire, USA).
4. Anti-Cyclic Citrullinated peptide antibodies (anti-CCP), was measured using commercial ELISA plates coated with second generation citrullinated peptides (CCP2) (FCCP600; Axis-Shield Diagnostics, Dundee, UK). The assay was used per the manufacturer's instructions, with a value >5 IU/mL considered positive.
5. Serum Krebs von den Lungen-6 (KL-6) and surfactant protein D (SP-D) levels. They were measured using commercially available ELISA kits supplied by (Kamiya Biomedical Company, Tukwila, WA, USA and Cusabio Systems, Houston, TX, USA, respectively) according to the manufacturer's protocols.
6. IL-13 assay. Serum concentrations of IL-13 were quantitatively assayed with the ELISA kit (Cat. No. 39-8242-65; Invitrogen Corporation (Camarillo, CA, USA) according to the manufacturer's protocol.
7. Interleukin 13 receptor alpha 1 and 2 (IL-13 Rα I and L-13 Rα2) mRNA relative expression levels in peripheral blood mononuclear cells (PBMCs).
   a. Preparation of Peripheral blood mononuclear cells (PBMCs): PBMCs were prepared by density gradient centrifugation using Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). Briefly, heparinised blood was carefully layered on Ficoll, and PBMC were harvested from the white interphase after centrifugation for 30 minutes at 400 g, at room temperature and washed

with phosphate buffered saline (PBS). The PBMCs samples were stored at −80°C till the samples were further processed for RNA isolation.

b. RNA extraction, cDNA synthesis, and real-time PCR:
   (i) *RNA extraction*: Total RNA was extracted from PBMCs using Gene JET RNA Purification Kit (Thermo Scientific, # K0731, USA) according to the manufacturer protocol. Total RNA concentration and purity were determined by measuring OD260 and OD260/280 ratio, respectively, on a NanoDrop spectrophotometer (NanoDrop Technologies, Inc Wilmington, USA), RNA was then stored at −80°C.
   (ii) *cDNA synthesis*: was performed using the RevertAid H Minus First Strand cDNA Synthesis kit (Cat#K1632, Thermo Scientific Fermentas, St. Leon-Ro, Germany) according to the manufacturer's instructions. Ten μL of random hexamer primers (Roche, Mannheim, Germany) were added to 21 μL of RNA which was denatured for 5 minutes in the thermal cycler (Biometra, USA). The RNA-primer mixture was cooled to 4°C. The cDNA master mix was prepared (5 μL of first strand buffer, 10 mM of dNTPs,1 μL of RNase inhibitor, 1 μL of reverse transcriptase Superscript™ II-RT enzyme and 10 μL of DEPC treated water) according to the kit protocol and was added to each sample. The total volume of the cDNA master mix was 19 μL for each sample. This was added to 31 μL RNA-primer mixture resulting in a reaction volume of 50 μL, which was then incubated in the programmed thermal cycler 1 hour at 37°C, followed by inactivation of enzymes at 95°C for 10 minutes, and finally cooled at 4°C. The RNA was reverse transcribed into cDNA which was then stored at −20°C
   (iii) *Real-time quantitative PCR*: This cDNA was used as a template to determine the relative expression of the human Interleukin-13 Receptor α1 (IL-13RA1) and IL13RA2 genes using StepOnePlus real-time PCR system (Applied Biosystem, USA). Primer sequences specific for human IL-13RA1 (NCBI GenBank Nucleotide accession # NM_001560.2), IL-13RA2 (NCBI GenBank Nucleotide accession # NM_000640.2) and β-actin (NCBI GenBank Nucleotide accession # NM_001101.4) were designed using Primer3 software (http://bioinfo.ut.ee/primer3/) [21] as follows: IL-13RA1, (Forward) 5′-CTCTGGAGTAATTGGAGCCAAGA-3′ and (Reverse)5′-TGCGACGATGACTGGAACAA-3′; IL-13RA2, (Forward) 5′-TTGCGTAAGCCAAACACCTA-3′ and (Reverse) 5′-TGAACATTTGGCCATGACTG-3. β-actin, (Forward) 5′-CTCTTCCAGCCTTCCTTCCT-3′ and (Reverse) 5′-AGCACTGTGTTGGCGTACAG-3′. β-actin was used as a reference to calculate fold change in target gene expression. A 25-μL PCR mix was prepared by adding 12.5 μL of 2X Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, # K0221, USA), 2 μL of cDNA template, 1 μL forward primer, 1 μL reverse primer, and 8. 5 μL of nuclease free water. The thermal cycling conditions were as follows: Initial denaturation at 95°C for 10 minutes was followed by 40 cycles with denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds. Productions of the expected amplification fragments without unanticipated products and primers were confirmed by melting curve analysis. The determination of the relative levels of gene expression was performed using the comparative cycle threshold (△△Ct(method and normalized to the reference gene β-actin.[22]

2.2.3 | Radiographic examination

HRCT (Aquilion 16, Canon Medical Systems, Irvine, CA, USA) of the chest without contrast was performed for RA patients with interstitial lung disease (RA-ILD). Moreover, HRCT scanning with 1-2-mm–thickness cuts was performed at the end of an inspiration. The distribution of interstitial lung abnormalities (ILA) based on septal lines, reticulation, traction bronchiectasis, cyst formation, and/or ground-glass attenuation: this is where 0 = no ILD, 1 = indeterminate ILD (focal or unilateral ground-glass attenuation, focal or unilateral reticulation, or patchy ground-glass abnormality involving <5% of the lung), 2 = mild/early ILD (changes affecting >5% of any lobar region with nondependent ground-glass or reticular abnormalities, diffuse centrilobular nodularity, nonemphysematous cysts, honeycombing, or traction bronchiectasis), 3 = advanced ILD (bilateral fibrosis in multiple lobes associated with honeycombing and traction bronchiectasis in a subpleural distribution).[23]

2.3 | Statistical analysis

Data were analyzed using SPSS software (version 11, SPSS Inc, Chicago, Illinois). Baseline characteristics are presented as mean ± standard deviation for the continuous variables, and as frequency and percentage for the discrete ones. Comparisons between groups were conducted using ANOVA. Tukey's post hoc test was used for multiple comparison. Correlation between variables was examined using the Pearson's correlation coefficient. Multiple linear regression analysis was performed to investigate the independent association of several parameters with IL-13.

3 | RESULTS

One hundred RA patients are included in this study: cases are classified as fifty RA-associated with ILD (RA-ILD) and fifty RA without ILD (RA–no ILD). Demographic, clinical, and laboratory data for patients and controls are summarized in Table 1.

3.1 | Characteristics of RA patients

Mean age, percent of male affection, duration of disease, disease activity (DAS28) and disability scores (MHAQ) in the RA –ILD group

TABLE 1 Demographic data, clinical, and laboratory parameters in RA patients

	RA-ILD (n = 50)	RA–no ILD (n = 50)	Controls (n = 50)
Demographic parameters			
Age, y	67.27 ± 10.87*,**	55.76 ± 8.43	54.66 ± 7.32
Female, no. (%)	35 (70)*,**	47 (94)	45 (90)
Smoker, no. (%)	5 (10)	3 (6)	2 (4)
Clinical parameters			
RA duration (y)	16.5 ± 11.6*	10.5 ± 6.4	
DAS28	4.89 ± 1.68*	3.64 ± 1.16	
HAQ	1.19 ± 0.73*	0.93 ± 0.63	
Respiratory parameters, no (%)			
Cough	8 (16)	0 (0)	
Dyspnea	12 (24)	0 (0)	
Laboratory parameters (mean ± SD)			
ESR, (mm/h)	45.5 ± 26.6*,**	30.3 ± 22.4	10.20 ± 3.49
CRP, (mg/dL)	4.6 ± 4.3*,**	3.5 ± 3.3	3.8 ± 1.85
RF, (IU/mL)	194.76 ± 160.23*,**	150.63 ± 128.61	6 ± 1.4
Anti-CCP (units/mL)	290.22 ± 220.01*,**	187.25 ± 165.98	10 ± 4.4
IL-13 (pg/mL)	43.83 ± 5.76*,**	18.31 ± 3.48	6.54 ± 1.12
KL-6 (units/mL)	1458 ± 1070*,**	640 ± 487	333 ± 294
SP-D (ng/mL)	353 ± 219*,**	161 ± 143	40 ± 51
Current medication use, no. (%)			
Prednisone	30 (60)	4 (8)	
Methotrexate	43 (86)	45 (90)	
Leflunomide	12 (24)	10 (20)	
Anti-TNF	3 (6)	2 (4)	

Note: Values are expressed as Mean ± SD or n (%).

Abbreviations: Anti-CCP, anti-cyclic citrullinated peptide; Anti-TNF, anti-tumor necrosis factor; CRP, C-reactive protein; DAS28; disease activity for 28 joint indices score; ESR, Erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; IL-13, interleukin 13; KL-6, Krebs von den Lungen-6; RF, Rheumatoid factor; SP-D, Surfactant protein D.

*Significance $P < .05$ comparison between RA-ILD and RA-no ILD.

**Significance $P < .05$ comparison between RA-ILD and controls.

were significantly higher than the RA–no ILD group. There is no significant difference between the RA –ILD group and the RA–no ILD group, regarding type of treatment used (Table 1).

3.2 | Respiratory symptoms and signs

Twenty out of fifty (40%) RA-ILD patients showed respiratory symptoms, including post-activity dyspnea (24%,12/50), followed by cough (16%, 8/50). However, among the 50 RA- no ILD group cases, no respiratory symptoms were recorded (Table 1).

3.3 | Laboratory and radiological findings

In this study, acute phase reactants (ESR, CRP), RF, anti-CCP, serum KL-6, SP-D, and IL-13 levels were significantly increased in RA patients vs. controls. Their levels were significantly higher in the RA–ILD group than in the RA–no ILD group (Table 1).

Regarding IL-13 Rα1 and IL-13 Rα2 mRNA relative expression: our data revealed that IL-13 Rα1and IL-13 Rα2 mRNA expression (assessed by qRTPCR) in peripheral blood mononuclear cells of RA patients were significantly increased compared to healthy control subjects ($P < .001$). Moreover, the RA–ILD group exhibited a significant increase in IL-13 Rα1and IL-13 Rα2 mRNA expression compared to the RA–no ILD group ($P < .001$). These data are illustrated in (Figures 1 and 2).

Regarding HRCT scanning, forty out of fifty patients (80%) of the RA–ILD group revealed an interstitial lung abnormality (ILA) score of 2, while ten patients (20%) of the RA–ILD group revealed an ILA score of 3 (Figure 3).

We analyzed the correlation of IL-13 with clinical and radiographic parameters, (Table 2) and saw that serum IL-13 levels and relative IL-13 mRNA expression (IL-13 Rα1and IL-13 Rα2) were

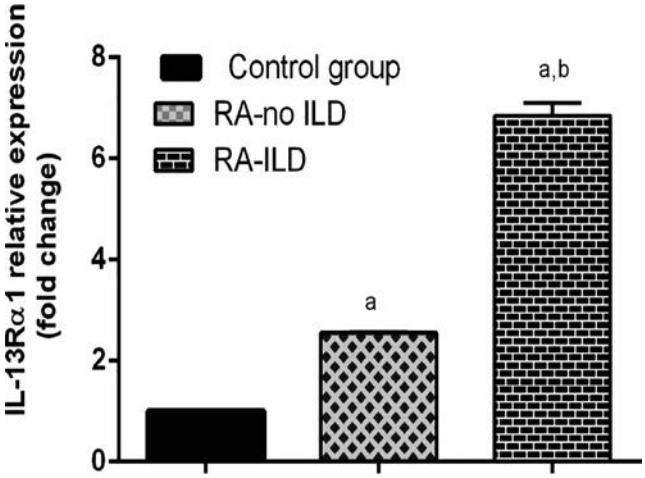

FIGURE 1 Interleukin 13 receptor alpha 1 (IL-13 Rα1) mRNA relative expression levels. Values are expressed as mean ± SD. *P was considered significant at <.05. a Significance vs. control group, b Significance vs. RA-no ILD patients using One way ANOVA followed by Tukey's post hoc test for multiple comparison

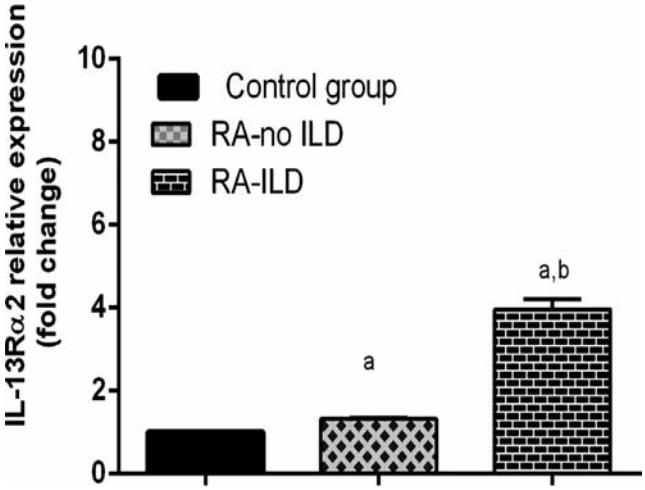

FIGURE 2 Interleukin 13 receptor alpha 2 (IL-13 Rα 2) mRNA relative expression levels. Values are expressed as mean ± SD. *P was considered significant at <.05. a Significance vs. control group, b Significance vs. RA-no ILD patients using One way ANOVA followed by Tukey's post hoc test for multiple comparison

positively correlated with RF, Anti-CCP, KL-6, SP-D, and HRCT score ($P < .001$). Multiple linear regressions for relative expression levels of IL-13 revealed statistically significant correlations with independent factors: Anti-CCP ($\beta = 0.395$, $P < .001$), KL-6 ($\beta = 0.389$, $P < .001$) and SP-D ($\beta = 0.345$, $P < .001$).

4 | DISCUSSION

Pulmonary involvement is a common extra-articular manifestation of RA, which includes pleural disease, pulmonary nodules, interstitial lung disease (ILD), and airway disease. ILD is a common but serious form of lung involvement in RA, in which radiographic changes and PFT may precede symptoms by years. Once clinically apparent, ILD is associated with significant mortality.[24]

IL-13Rα1 and IL-13Rα2 are two receptors for IL-13, which are also a fibrogenic cytokine. As a transmembrane receptor, IL-13Rα1 can combine with IL-4Rα to form stable complexes[25]: they activate the JAK/STAT signaling pathway to induce TGF-β generation, eventually leading to fibrosis. Moreover, IL-13Rs are expressed on many different hematopoietic and non-hematopoietic cells within the airways, while IL-13Rs are found on ciliated respiratory epithelial cells, smooth muscle cells in bronchial tissue, and submucosal glands in nasal mucosa.[26]

RA patients with ILD were of an older age, with male predominance, and longer disease duration than RA patients without ILD. These results coincided with those of Zou et al,[18] who evaluated 110 RA patients and concluded that older age was closely related to ILD. Moreover, Mori et al[27] studied 189 RA patients and found that a longer disease duration was a risk factor for pulmonary involvement.

Our study revealed a significant difference between patients with ILD and without ILD, regarding parameters of disease activity (DAS28, ESR, CRP). These results agree with Bongartz et al[17] who evaluated 582 RA patients and reported that the risk of developing ILD was higher in those with more severe and active RA. In contrast, Saracoglu et al[28] found no relationship between pulmonary changes seen in HRCT, or clinical and laboratory disease activity parameters. These contrasting results can be attributed to differences in disease type (established or early), or in the study population.

In the present study, RF and anti-CCP levels were significantly higher in the RA-ILD group than the RA-no ILD group. This is supported by Bongartz et al[29] who proposed that the lung is a production site for citrullinated proteins via peptidylarginine deiminases. Some of these proteins may trigger immune responses in the lung and result in high antibody titers to citrullinated protein antigens. It is not clear whether these antibodies contribute to the initiation of pulmonary disease in RA patients or whether they reflect ongoing pulmonary injury. Yet, Alison et al[30] found that symptomatic lung disease and RF positivity preceding the development of clinically apparent articular RA is not a novel finding, only that the lack of specificity of RF for RA makes it difficult to support mechanistic arguments for the initiation of RA-related immunity in the lung, on the basis of RF positivity alone. In contrast, the high specificity of anti-CCP antibodies for RA (>97%), especially with concomitant RF positivity, suggests that anti-CCP and RF positivity in patients with ILD, but no clinically apparent articular RA, is related to RA-specific immunologic dysregulation. Alexiou et al[31] reported an association of high levels of serum anti-CCP Abs with pulmonary fibrosis in Greek RA patients. Aubart et al[32] using multivariate logistic regression analysis, observed that increased levels of anti-CCP Abs are associated with co-occurrence of pulmonary diseases, such as ILD, bronchiectasis, and rheumatoid nodules in French RA patients.

Our data revealed increased serum levels, as well as IL-13 Rα1and IL-13 Rα2 mRNA expression in RA patients (both with and without ILD groups), compared to healthy control subjects. Furthermore, RA

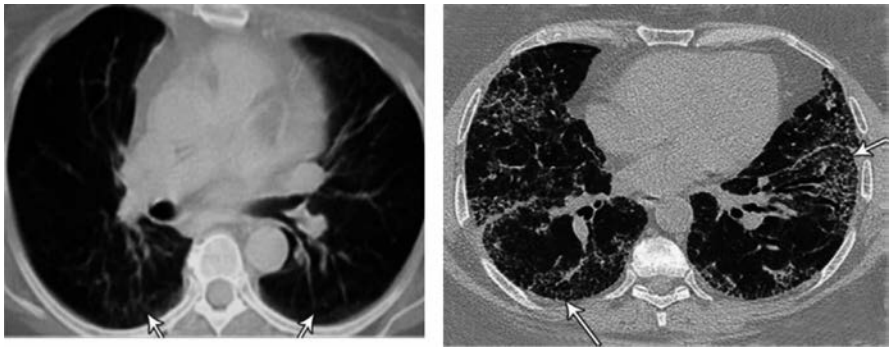

FIGURE 3 HRCT scans obtained at the level of the lower lobes of the Lungs in RA patient, showing reticulation and ground glass & honeycombing appearance in both lungs which are consistent with interstitial lung diseases

TABLE 2 Correlation between serum IL-13 levels and relative IL-13 mRNA expression with clinical and radiographic parameters

	Serum IL-13		IL-13 Rα1		IL-13 Rα2	
Variables	***r***	***P***	***R***	***P***	***r***	***P***
Age	.212	NS	.012	NS	.112	NS
Duration (y)	.140	NS	.170	NS	.113	NS
DAS28	.063	NS	.076	NS	.098	NS
HAQ	.055	NS	.034	NS	.098	NS
ESR, (mm/h)	.121	NS	.198	NS	.172	NS
CRP, (mg/dL)	.131	NS	.145	NS	.194	NS
RF, (IU/mL)	.512	<.001*	.598	<.001*	.556	<.001*
Anti-CCP (units/mL)	.541	<.001*	.543	<.001*	.550	<.001*
KL-6 (units/mL)	.444	<.001*	.544	<.001*	.498	<.001*
SP-D (ng/mL)	.660	<.001*	.560	<.001*	.645	<.001*
HRCT score	.505	<.001*	.665	<.001*	.573	<.001*

Abbreviations: Anti-CCP, anti-cyclic citrullinated peptide; CRP, C-reactive protein; DAS28, disease activity for 28 joint indices score; ESR, Erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; HRCT, High-resolution computed tomography scores; KL-6, Krebs von den Lungen-6; RF, Rheumatoid factor; SP-D, Surfactant protein D.

*Pearson's correlation coefficient.

patients with ILD exhibited a significant increase in IL-13, Rα1and IL-13 Rα2 mRNA expression compared to RA patients with no ILD. Moreover, serum IL-13 levels and relative IL-13 mRNA expression were positively correlated with RF, Anti-CCP, KL-6, SP-D and the HRCT score.

The biological functions of IL-13 include human B-cell growth, immunoglobulin class that switches to IgE, upregulation of CD23 and MHC cell II on human B cells, downregulation of proinflammatory cytokines, and increased expression of vascular-cell adhesion molecule 1 on endothelial cells. Thus, it could be involved in the pathogenesis of lung fibrosis in RA patients with ILD. This agrees with Xiang-Tong et al,[33] who supplied a relationship between IL-13Rα2 and TGF-β in IL-13-induced human fetal lung fibroblast-1 (HFL-1) fibrosis model in vitro, investigating the role of IL-13Rα2 in HFL-1 lung fibrosis model with exogenous IL-13 stimulation, their study found that under high concentrations of the IL-13 in HFL-1 cell line, IL-13Rα2 did not exhibit its TGF-β-related signaling transduction pattern, which might be attributed to the stimulation of sIL-13Rα. Jakubzick et al[34] showed that idiopathic interstitial pneumonia (IIP) fibroblasts can be targeted, as they reflect the expression of IL-4 and IL-13 receptor subunits. IL13-PE targeted and killed fibroblasts of IIP patients, but this immunotoxin had a minimal effect on proliferation of normal fibroblast lines, which either lacked or expressed low levels of IL-4 and IL-13 receptor subunits.

Based on multiple case reports and reviews, there was an increased pulmonary toxicity induced by the biologics and especially the anti-TNF therapy, alone or in combination with methotrexate. For methotrexate, leflunomide, and anti-TNF therapy, the estimated prevalence of induced ILD is around 1%. For comparison, estimates of the prevalence of ILD in RA range widely between 1% and 58%.[35,36] Indeed, the vast majority of RA patients who develop new pulmonary symptoms while receiving disease-modifying anti-rheumatic drugs (DMARDs) or biologics do not have a drug-induced reaction. Respiratory tract infections are the most frequently reported complication in RA patients in general and in those treated with biologic agents in particular.[37] It is difficult to differentiate drug-induced toxicity from RA-related ILD, as the clinical, radiological, and histopathological findings are nonspecific and overlap. The combination of different agents, such as anti-TNF therapy and methotrexate, increases the problem. This agrees with Wolfe and Saravanan et al, suggesting that pneumonitis occurring denovo or the exacerbation

of pre-existing ILD in RA may be due to modification of the disease process in the lung, rather than a direct toxic effect.[38]

As described by Camille and Boulos,[39] DMARDs or biologics may induce pneumonitis or worsen RA-related pre-existing ILD. Nonetheless, identifying a causal relationship between RA therapy and ILD-induced toxicity is difficult, partly because it is a rare condition, but in this study, the percentage of DMARDs and biological disease-modifying anti-rheumatic drugs was almost equal in both groups.

5 | CONCLUSIONS

Serum IL-13 and its receptor subunit expressions are useful biomarkers which can be used in detecting severity of the interstitial lung disease in RA patients.

6 | LIMITATIONS OF THE STUDY

The present study has a small number of RA -ILD patients.

ACKNOWLEDGMENTS

No specific acknowledgment.

CONFLICT OF INTEREST

No conflict of interest.

AUTHOR CONTRIBUTIONS

Marwa aboelhawa: Conceptualization, design of the work& Methodology. Amal elbarbary: supervision& analysis, interpretation of data. Doaa Wassem; data collection and original draft preparation. Manal shawky: Writing- Reviewing and Editing. Hanaa Hibishy: Software, and investigation& share in writing. Reham Elkolaly: Software, and investigation& share in writing.

ETHICAL APPROVAL

Written Informed consents were obtained from all the participants before entering the study. The study was approved by the research ethics committee of the Tanta University, faculty of medicine. Approval code 33813.

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ORIGINAL ARTICLE

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MiR-223-3p and miR-22-3p inhibit monosodium urate-induced gouty inflammation by targeting NLRP3

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Abstract

Background: MicroRNAs (miRNAs) have been shown to play a crucial role in inflammation regulation; however, their relationship with inflammation in acute gouty arthritis has not been fully elucidated. Herein, we conducted a study to explore the regulatory roles of miR-223-3p and miR-22-3p in gouty-associated inflammation.

Methods: In vitro and in vivo experiments were conducted to examine the molecular mechanisms of miRNA regulation in gouty inflammation. Dual-luciferase reporter assay was used to verify the direct target of miR-223-3p and miR-22-3p.

Results: We found that miR-223-3p and miR-22-3p interacted with the 3′ untranslated region segment of NLRP3 (nucleotide-binding domain leucine-rich repeat [NLR] and pyrin domain containing receptor 3) and inhibited its expression. A decreased expression of miR-223-3p and miR-22-3p was observed in both mice air pouch synovium and phorbol myristrate acetate-treated THP-1 cells stimulated with monosodium urate ($P < .05$). Compared with the negative control group, NLRP3 expression at the transcript and protein level in miR-223-3p and miR-22-3p overexpression group significantly decreased after 6 hours of monosodium urate treatment in vivo and in vitro ($P < .05$). The results of the dual-luciferase reporter assay demonstrated that miR-223-3p and miR-22-3p directly targeted NLRP3.

Conclusion: The findings of the present study show that miR-223-3p and miR-22-3p can reduce the inflammatory effects of gout by inhibiting the expression of NLRP3.

KEYWORDS

gouty inflammation, miR-223-3p, miR-22-3p, monosodium urate, NLRP3

1 | INTRODUCTION

Gout is a metabolic and inflammatory disease associated with hyperuricemia and deposition of monosodium urate (MSU) crystals in tissues.[1] NLRP3 (nucleotide-binding domain leucine-rich repeat [NLR] and pyrin domain containing receptor 3) inflammasome plays a pivotal role in the occurrence of gouty inflammation.[2] MSU crystals promote the release of mitochondrial reactive oxygen species (ROS) into the cytoplasm and the activation and assembly of the NLRP3 inflammasome. The adaptor protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) on the activated NLRP3 inflammasome can recruit and activate the

pro-inflammatory protease Caspase-1, thereby promoting the cleavage and maturation of pro-interleukin (IL)-1β into biologically active IL-1β.[3] The release of IL-1β is key to gout inflammation and causes vasodilation and rapid recruitment of neutrophils to the crystal deposition site, thereby driving the occurrence of acute inflammation. The current treatment of acute gouty arthritis is mainly based on non-steroidal anti-inflammatory drugs, colchicine, and glucocorticoids, which exhibit adverse effects.[4] Finding a specific inhibitor for NLRP3 inflammasome and then alleviating the release of gout inflammatory factors will be a new direction for treating gouty arthritis in the acute phase.[5]

MicroRNA (miRNA) is a type of noncoding RNA found in eukaryotes with a length of 18-25 nucleotides. Mature miRNAs participate in the assembly of RNA induced silencing complex, and they target and recognize messenger RNA (mRNA) through complementary base pairing. Also, they guide the degradation of the silencing complex and prevent the translation of the corresponding target mRNA.[6] miRNA expression patterns are highly specific and conservative; thus, different disease types have different expression patterns.[7] At present, there are few studies on the regulation and response mechanisms of miRNAs in gouty inflammation. Therefore, there is a need to study the effect of targeted regulation of gouty arthritis-specific miRNAs on inflammation.

Our previous study found that the expression of miR-223-3p and miR-22-3p were significantly down-regulated in patients with acute gouty arthritis compared with hyperuricemia patients.[8] Based on the above findings, we hypothesized that miR-223-3p and miR-22-3p bind to the 3'-UTR (untranslated region) segment of NLRP3 and accelerates mRNA degradation, thereby mediating the translation of NLRP3 protein. Subsequently, this reduces the release of IL-1β in inflammatory cells and regulates the inflammation of acute gouty arthritis.

2 | MATERIALS AND METHODS

2.1 | Mice

Thirty male Balb/c mice weighing 19-23 g (8 weeks old; SPF grade; Certificate Number SCXK [Jing] 2016-0006) were purchased from the Weitong Lihua Laboratory Animal Center (Beijing, China).[9,10] All mice were bred under defined conditions with 55% humidity and a temperature of 25℃ at the SPF animal experiment center of the Science and Education Building of Qingdao University Hospital. All the animal experiments were performed in strict adherence to the principles of laboratory animal management of Qingdao University.

2.2 | Cells

THP-1 cells were obtained from the Procell Life Science & Technology Co. Ltd. and cultured on RPMI-1640 medium (Hyclone) supplemented with 10% fetal bovine serum (Hyclone) and 1% penicillin/streptomycin (Beijing Suolabao Biotech). The THP-1 cells were differentiated into macrophages by stimulation with phorbol myristrate acetate (PMA) (MedChemExpress) at various concentrations (10, 20, 50, 100, 200, 400 ng/mL). Three time points of 24, 48, and 72 hours were selected to observe the cell status. Subsequently, we determined the optimal concentration for PMA stimulation and observation time and constructed a THP-1 inflammatory cell model.

2.3 | MSU crystals preparation

Briefly, 1 g of uric acid (Sigma) was diluted in 200 mL deionized water by heating under a water bath at 60°C. The pH of the solution was then adjusted to 8.9 using NaOH (0.5 mol/L).[11] Uric acid precipitate was removed from the solution using a filter paper. The solution was stored at room temperature for 24 hours to allow the MSU crystals to precipitate out. The MSU precipitate was washed with absolute ethanol thrice, sterilized, and stored for use in subsequent experiments (MSU was dissolved in phosphate-buffered solution [PBS] at 50 mg/mL for in vitro and in vivo experiments).

2.4 | Western blot

Cell or tissue samples were lysed using RIPA high-efficiency tissue cell lysate (Beijing Suolabao Biotech) and 1% phenylmethylsulfonyl fluoride protease inhibitor (Beijing Suolabao Biotech). Bicinchoninic acid (BCA) kit (Thermo Fisher) was used to determine the protein concentrations. The protein samples were then separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes. The PVDF membranes were blocked with 5% skimmed milk containing Tris-buffered saline/Tween 20 (TBST) at room temperature for 1 hour, then incubated overnight with NLRP3 antibody (1:1000; Cell Signaling Technologies) at 4°C. Next, the membranes were washed thrice with TBST and incubated with a second antibody (anti-rabbit immunoglobulin G, 1:5000; Biyuntian Biotechnology Co.) for 1 hour at room temperature. The bands were visualized using an imaging device connected to an imaging developer (Millipore). Beta-actin was used as an internal reference protein.

2.5 | RNA extraction, reverse transcription, and quantitative polymerase chain reaction (qPCR) analysis

Trizol reagent (Sigma) was used to extract total RNA from the tissues and cells.[12] U6 snRNA and 18sRNA were used for internal reference of miRNA and mRNA, respectively. The mRNA primers, miRNA mimics, miRNA inhibitor, miRNA agomir, and negative control were constructed and synthesized by Shanghai Jima Biotechnology Co., Ltd. Reverse transcription and PCR amplification of purified mRNA were performed using reverse transcription kit (Takara) and real-time PCR

kit (Takara) as per the manufacturer's instructions. Details of the primers used are shown in Table 1.

2.6 | Enzyme-linked immunosorbent assay (ELISA)

The level of IL-1β secretion in the air pouch on the back of mice and on the surface of THP-1 cells was measured using ELISA kit (Elabscience) according to the manufacturer's instructions.

2.7 | The air pouch mice model

The mice model used in this study was previously described by Pessler et al.[9] and the dosage schedule and route of administration were according to Ma et al.[10] The air pouch mice model was established in a sterile environment. Briefly, the mice were divided into the miR-223-3p, miR-22-3p agomir, and negative control groups. On day 0, the air pouch was created by subcutaneous injection of 3 mL of sterile air on the center of the mice's backs. On day 2, 200 μL of agomir (1 nmol/mouse) was injected into the subcutaneous pouch. The mice in the negative control group were injected with an equal volume of sterile PBS. On day 3, 2 mL of sterile air was added to re-inflate the air pouch. The procedure performed on day 2 was repeated on day 4. On day 6, the MSU-induced air pouch mice model was created by subcutaneous injection of 2 mg MSU crystals dissolved in 0.5 mL sterile PBS. Six hours after MSU injection, the mice were anesthetized with chloral hydrate and sacrificed. The mouse air pouch was dissected, the air pouch synovial epithelial tissue and the air pouch fluids were separated and analyzed (Figure 1A).

TABLE 1 Primer sequences used for reverse transcription polymerase chain reaction

Samples	Primer name	Sequence 5′–3′
Balb/c mice	NLRP3-forward	GCGGACTGTCCCATCAATGC
	NLRP3-reverse	AGCAGCTCACCAACCACAGT
	IL1β-forward	CTCACAAGCAGAGCACAAGC
	IL1β-reverse	AGCTGTCTGCTCATTCACGA
	18srRNA-forward	GGCCGTTCTTAGTTGGTGGAGCG
	18srRNA-reverse	CTGAACGCCACTTGTCCCTC
THP-1 cell	NLRP3-forward	GCACCTGTTGTGCAATCTGAA
	NLRP3-reverse	TCCTGACAACATGCTGATGTGA
	IL1β-forward	TCCAGGGACAGGATATGGAG
	IL1β-reverse	TCTTTCAACACGCAGGACAG
	18srRNA-forward	GTAACCCGTTGAACCCCATT
	18srRNA-reverse	CCATCCAATCGGTAGTAGCG
miRNA	mir-223-3p-forward	GAAGTTCGTCCTGTCAGTTTGTC
	mir-223-3p-reverse	TATGGTTGTTCTCGTCTCTGTGTC
	mir-22-3p-forward	TATAGTAGAAAGCTGCCAGTTGAAG
	mir-22-3p-reverse	TATGGTTGTTCTGCTCTCTGTGTC
	U6-forward	CAGCACATATACTAAAATTGGAACG
	U6-reverse	ACGAATTTGCGTGTCATCC
	mir-223-3p mimic/agomir	Sense: UGUCAGUUUGUCAAAUACCCCA
		Antisense: GGGUAUUUGACAAACUGACAUU
	mir-22-3p mimic/agomir	Sense: AAGCUGCCAGUUGAAGAACUGU
		Antisense: AGUUCUUCAACUGGCAGCUUUU
	Negative control mimic/agomir	Sense: UUCUCCGAACGUGUCACGUTT
		Antisense: ACGUGACACGUUCGGAGAATT
	mir-223-3p inhibitor	UGGGGUAUUUGACAAACUGACA
	mir-22-3p inhibitor	ACAGUUCUUCAACUGGCAGCUU
	Negative control inhibitor	CAGUACUUUUGUGUAGUACAA

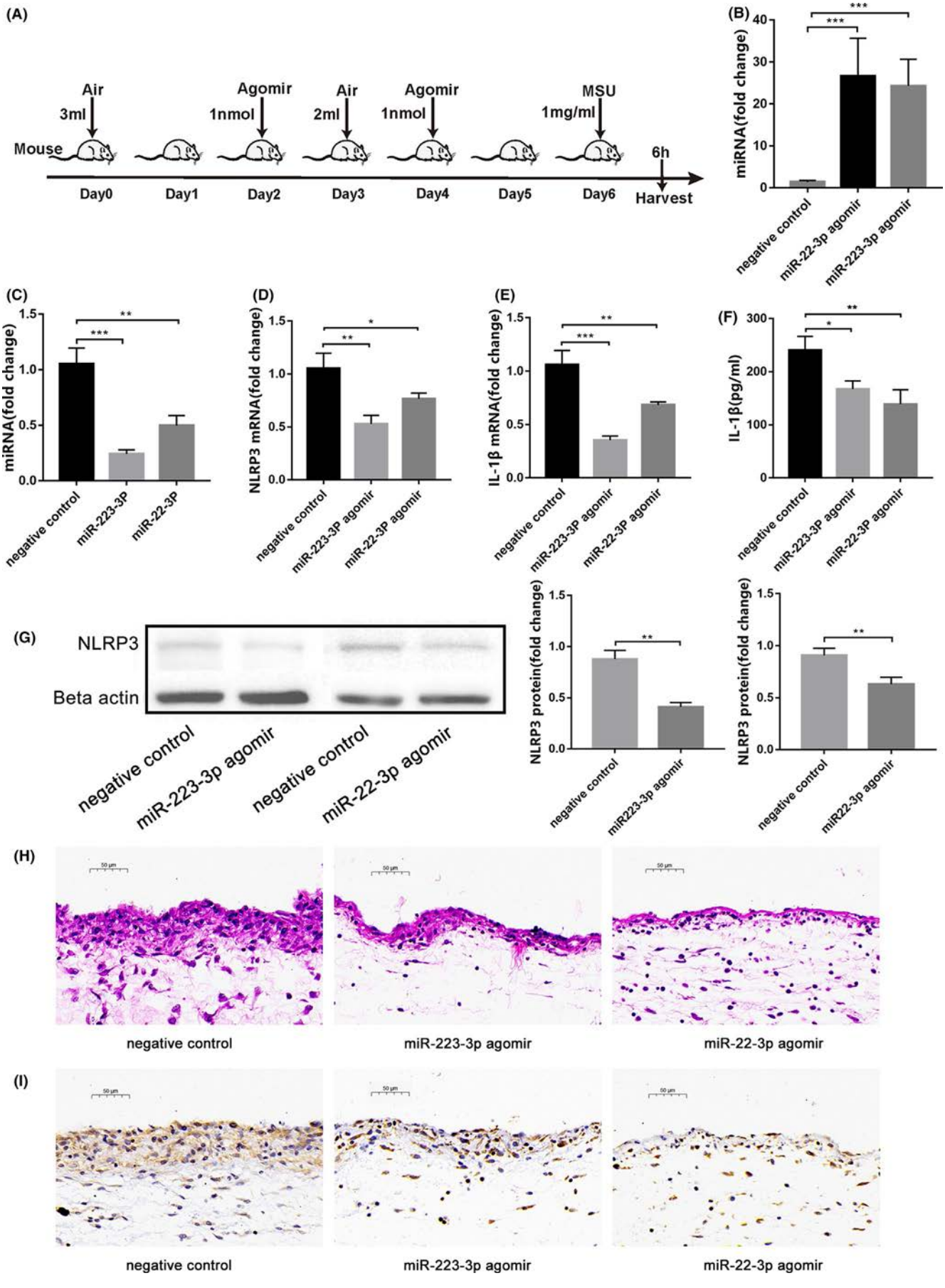

(A)
Air
3ml
Mouse
Agomir
1nmol
Air
2ml
Agomir
1nmol
MSU
1mg/ml
6h
Harvest
Day0
Day1
Day2
Day3
Day4
Day5
Day6
(B)
miRNA(fold change)
negative control
miR-22-3p agomir
miR-223-3p agomir
(C)
miRNA(fold change)
negative control
miR-223-3P
miR-22-3P
(D)
NLRP3 mRNA(fold change)
negative control
miR-223-3P agomir
miR-22-3P agomir
(E)
IL-1β mRNA(fold change)
negative control
miR-223-3P agomir
miR-22-3P agomir
(F)
IL-1β(pg/ml)
negative control
miR-223-3P agomir
miR-22-3P agomir
(G)
NLRP3
Beta actin
negative control
miR-223-3p agomir
negative control
miR-22-3p agomir
NLRP3 protein(fold change)
negative control
miR223-3p agomir
NLRP3 protein(fold change)
negative control
miR22-3p agomir
(H)
negative control
miR-223-3p agomir
miR-22-3p agomir
(I)
negative control
miR-223-3p agomir
miR-22-3p agomir

FIGURE 1 In vivo experimental analysis of mir-223-3p and mir-22-3p on gout inflammation. A: Construction of the mice air pouch inflammation model. B: Transfection effect of miR-223-3p and miR-22-3p in the mice air pouch. C: Expression difference of miR-223-3p and miR-22-3p in the mice air pouch synovium stimulated by monosodium urate (MSU) for 6 h. D-F: Expression difference of NLRP3 (nucleotide-binding domain leucine-rich repeat [NLR] and pyrin domain containing receptor 3) and interleukin (IL)-1β and the secretion level of IL-1β in the mice air pouch fluid stimulated by MSU for 6 h. G: Protein expression difference of NLRP3 in the mice air pouch synovium tissues stimulated by MSU for 6 h, and the bar chart of protein expression difference was calculated by Image J software through band gray analysis. H-I: Immunohistochemical staining of hematoxylin and eosin (HE) and NLRP3 in the synovial epithelium of the mice air pouch stimulated by MSU for 6 h. Note: *<.05; **<.01; ***<.001

2.8 | Hematoxylin-eosin (HE) and immunohistochemical staining

The air pouch synovial epithelial tissues were fixed with 10% formalin, dehydrated by absolute ethanol, and embedded in paraffin for tissue sectioning. Xylene was added to the slices for dewaxing and then washed with alcohol and double-distilled water. Sections were used for HE staining, dehydrated, and mounted for preservation. The sections were repaired in hot citric acid buffer (pH 6.0) for immunohistochemical staining, blocked with 3% bovine serum albumin, and incubated with the NLRP3 antibody (1:600; Beijing Suolabao Biotech) overnight at 4°C. Finally, chromogenic agent diaminobenzidine (DAB) was performed, and hematoxylin was used for nuclei counterstaining. The positive cell nucleus of immunohistochemical staining was dyed brown–yellow.

2.9 | Cell Counting Kit-8 (CCK-8) assay

The activity of PMA-treated THP-1 cells and the optimal stimulating concentration of MSU were assessed using CCK-8 (MedChemExpress) according to the manufacturer's instructions.

2.10 | THP-1 cell transfection

The differentiated THP-1 cells were seeded onto a 24-well culture plate at a concentration of 5×10^5 cells/mL, and 100 μL of fresh 1640 medium supplemented with 10% fetal bovine serum (FBS) was added to each well. Subsequently, 100 μL of serum-free 1640 medium and 375 ng miRNA were aliquoted into 1.5 mL Eppendorf tubes to ensure that the final concentration of the transfected miRNA was 50 nmol/L. Next, 6 μL of HiperFect transfection reagent (Qiagen, Hilden, Germany) were added to the solution, mixed, and left to stand for 10 minutes at room temperature. The solution was then transferred into a 24-well cell culture plate and incubated in a 37°C, 5% CO_2 incubator. After 6 hours of culture, 300 μL of fresh 1640 medium containing 10% FBS was added to each well, and the plates were further incubated for 48 hours.

2.11 | Dual-luciferase reporter assay

The miR-223-3 and miR-22-3p targeted NLRP3 locus was predicted using Target scanhuman 7.2 (http://www.targetscan.org/vert_72/) and miRDB (http://mirdb.org/). After constructing miR-223-3p and miR-22-3p pmirGLO dual-fluorescein plasmids (Gemma Gene), miRNA mimic and pmirGLO were co-transfected into the differentiated THP-1 cells using Lipofectamine 3000 (ThermoFisher) transfection reagent, according to the manufacturer's instructions. Luciferase reporter assay was performed using the Dual-luciferase Reporter Assay System (Progema).

2.12 | Statistical methods

All statistical analyses were performed using GraphPad Prism 7.0. The differences between 2 groups were analyzed using a *t* test, whereas the differences between multiple groups were analyzed using a one-way analysis of variance (ANOVA). All experiments were performed in triplicate and repeated thrice. Unless otherwise specified, $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Expression of miRNAs are decreased in MSU-induced air pouch synovium

miR-223-3p and miR-22-3p agomir were injected into the air pouch mice model. Both miR-223-3p and miR-22-3p were successfully transfected into the mice air pouch (Figure 1B). According to the results, the levels of miR-223-3p and miR-22-3p in the air pouch of both groups decreased significantly after MSU-induced inflammation (Figure 1C).

3.2 | The miRNAs improve inflammation in air pouch tissues

Total RNA and protein were extracted from the mice air pouch synovium following MSU stimulation for 6 hours. The mRNA expressions of NLRP3 and IL-1β in the miR-223-3p and miR-22-3p groups were lower than in the negative control group based on real-time PCR analysis (Figure 1D-E). The mice air pouch fluid was collected simultaneously from all the 3 groups for ELISA analysis to examine IL-1β levels. The secretion of IL-1β in the miR-223-3p and miR-22-3p agomir groups was significantly lower than in the negative control group (Figure 1F). Western blot assay of the NLRP3 protein demonstrated that NLRP3 protein expression in both miR-223-3p and miR-22-3p groups was substantially lower than in the negative control

group (Figure 1G; $P < .05$). HE staining of the sections from air pouch synovium tissues of each group revealed that leukocyte chemotaxis of the air pouch epithelium in the miR-223-3p and miR-22-3p agomir groups was significantly lower than in the negative control group under a microscope (Figure 1H). Furthermore, immunohistochemical analysis of NLRP3 demonstrated that the positive expression of NLRP3 in the miR-223-3p and miR-22-3p agomir groups was significantly lower than in the negative control group (Figure 1I).

3.3 | The activity of PMA-treated THP-1 cells and the optimal stimulating concentration of MSU

When the PMA stimulation concentration was set at 100 ng/mL and observed at 72 hours, THP-1 cells were induced with better differentiation from the suspension state. CCK-8 cell viability assay revealed that THP-1 cell viability varied significantly at 100 μg/mL MSU (Figure 2A; $P < .05$). The viability of THP-1 cells was inhibited with an increase in MSU concentration. Therefore, 100 μg/mL MSU was selected as the stimulation concentration of THP-1 cells. By observing the dynamics of NLRP3 expression during the inflammatory stimulation of the THP-1 cells, we found that the expression of NLRP3 in THP-1 macrophages peaked after MSU stimulation at 6 hours, then decreased gradually, and reached the lowest level 48 hours later (Figure 2B).

3.4 | Expression levels of miRNAs are decreased in inflammatory model of differentiated THP-1 cells

THP-1 macrophages were transfected into the cells of the miRNA mimic, inhibitor, and negative control groups. The expression levels of miR-223-3p and miR-22-3p in the mimic group were significantly increased in the THP-1 cells, whereas the expression was inhibited in the inhibitor group (Figure 2C; $P < .05$). We also investigated the expression levels of miR-223-3p and miR-22-3p in THP-1 cells under MSU inflammatory condition and revealed that both intracellular expressions were significantly lower than in the control group (Figure 2D; $P < .05$).

3.5 | The miRNAs improve inflammation in differentiated THP-1 cells

In the differentiated THP-1 cells stimulated by MSU, the mRNA expressions of NLRP3 and IL-1β in the miR-223-3p and miR-22-3p mimic group were lower than in the negative control group, whereas the opposite trend was observed in the inhibitor group (Figure 2E; $P < .05$). The results of ELISA analysis demonstrated that IL-1β secretion in the miR-223-3p and miR-22-3p mimic group was also significantly lower than in the negative control group (Figure 2F; $P < .05$). Western blot assay revealed that the protein expression of NLRP3 in the miR-223-3p and miRNA22-3p mimic groups was markedly lower than that in the negative control group, whereas that in the miR-223-3p and miRNA22-3p inhibitor group was significantly higher than in the negative control group (Figure 2G; $P < .05$).

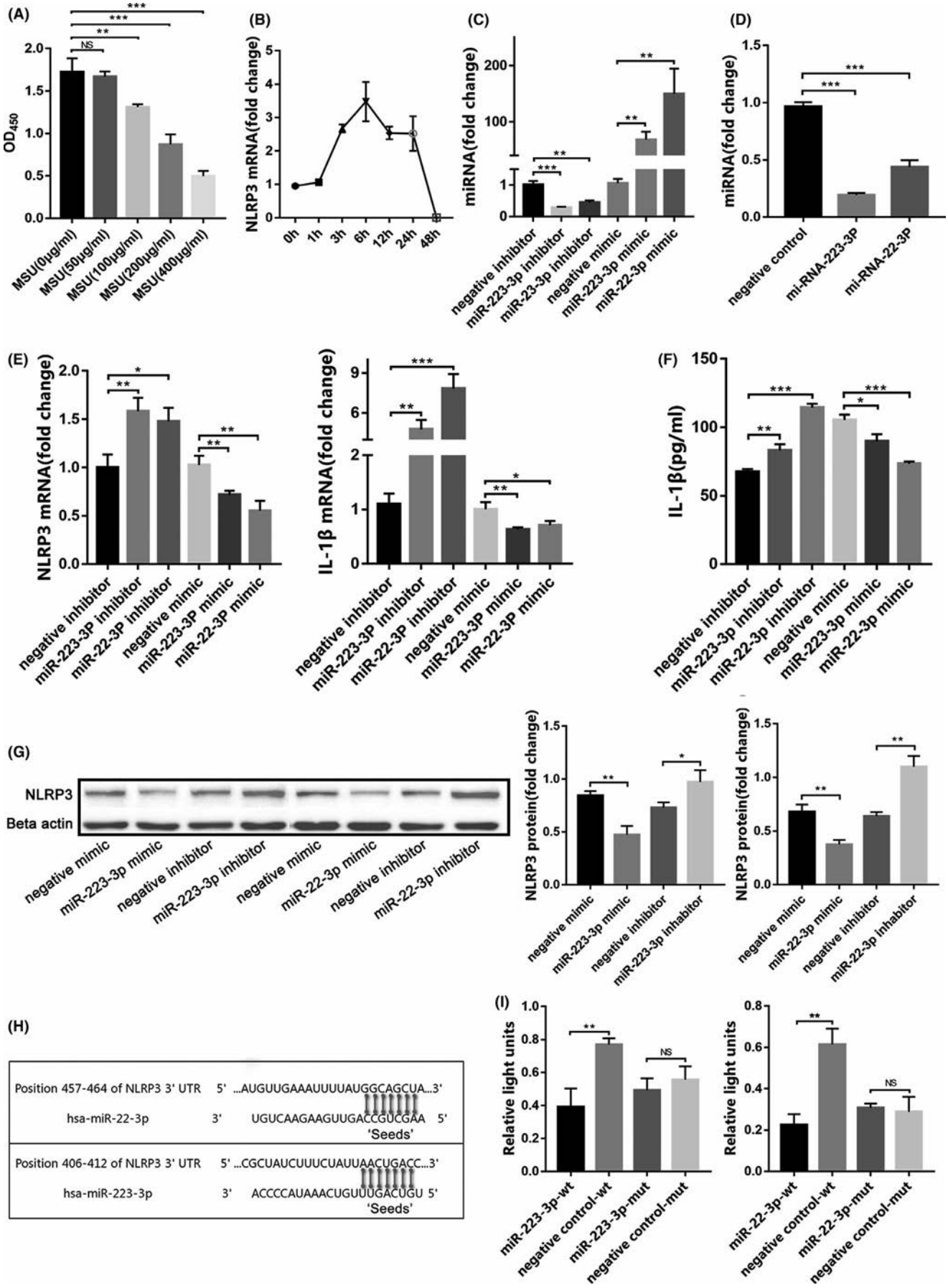
3.6 | Both miR-223-3p and miR-22-3p regulate NLRP3 by binding to its 3'-UTR region

TargetScan software was used to explore the interaction targets of both miRNAs with the gout-related inflammatory factor NLRP3, and a mutant site "Seed" was established (Figure 2H). The results were analyzed using the Dual-luciferase Reporter Assay System of Promega. Both miR-223-3p and miR-22-3p in the wild-type group inhibited the expression of 3'-UTR of NLRP3 gene in pmirGLO plasmid, leading to decreased expression of firefly luciferase, relative to the negative control group (as revealed by the attenuated ratio of luminescence). In the mutant group, the expression and relative luminescence value of firefly luciferase were the same as in the negative control group (Figure 2I; $P < .05$).

4 | DISCUSSION

Gout is a common form of metabolic arthritis mainly caused by chronic deposition of uric acid crystals. Its occurrence is associated with increased uric acid production or decreased excretion in the body. Substantial focus has been put on developing suitable therapeutic measures and palliative drugs to control and prevent the condition. In this study, we hypothesized and verified that plasma-specific miRNA-miR-223-3p and miR-22-3p interact with NLRP3 to regulate the expression of inflammatory factors, thereby inhibiting the development of gout inflammation (Figure 3). The following 4 key observations were made in the study following in vivo and in vitro experiments: (a) the expression of miR-223-3p and miR-22-3p decreased significantly after the onset of gout inflammation; (b) overexpression or silencing of miR-223-3p and miR-22-3p significantly influenced the expressions of NLRP3 inflammasome and IL-1β

FIGURE 2 In vitro experimental analysis of mir-223-3p and mir-22-3p on gout inflammation. A: Screening of optimal THP-1 cell concentration stimulated by monosodium urate (MSU). B: Changes of NLRP3 (nucleotide-binding domain leucine-rich repeat [NLR] and pyrin domain containing receptor 3) messenger RNA (mRNA) expression level over time after MSU stimulation of the differentiated THP-1 cells. C: Transfection of miR-223-3p and miR-22-3p in the differentiated THP-1 cells. D: Expression difference of miR-223-3p and miR-22-3p in cells after MSU stimulation of differentiated THP-1 cells for 6 h. E-F: Expression difference of NLRP3 and interleukin (IL)-1β and the secretion level of IL-1β in extracellular fluid after MSU stimulation of differentiated THP-1 cells for 6 h. G: Protein expression difference of NLRP3 in cells after MSU stimulation of differentiated THP-1 cells for 6 h, and the bar chart of protein expression difference was calculated by Image J software through band gray analysis. H: Mutation site "Seed" of mir-223-3P and mir-22-3p. I: Dual-luciferase report of mir-223-3P and mir-22-3p. Note: *<.05; **<.01; ***<.001


(A)
OD450
MSU(0μg/ml)
MSU(50μg/ml)
MSU(100μg/ml)
MSU(200μg/ml)
MSU(400μg/ml)
NS
(B)
NLRP3 mRNA(fold change)
0h
1h
3h
6h
12h
24h
48h
(C)
miRNA(fold change)
negative inhibitor
miR-223-3p inhibitor
miR-23-3p inhibitor
negative mimic
miR-223-3p mimic
miR-22-3p mimic
(D)
miRNA(fold change)
negative control
mi-RNA-223-3P
mi-RNA-22-3P
(E)
NLRP3 mRNA(fold change)
IL-1β mRNA(fold change)
negative inhibitor
miR-223-3P inhibitor
miR-22-3P inhibitor
negative mimic
miR-223-3P mimic
miR-22-3P mimic
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IL-1β(pg/ml)
negative inhibitor
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miR-22-3p inhibitor
negative mimic
miR-223-3p mimic
miR-22-3p mimic
(G)
NLRP3
Beta actin
negative mimic
miR-223-3p mimic
negative inhibitor
miR-223-3p inhibitor
negative mimic
miR-22-3p mimic
negative inhibitor
miR-22-3p inhibitor
NLRP3 protein(fold change)
negative mimic
miR-223-3p mimic
negative inhibitor
miR-223-3p inhabitor
NLRP3 protein(fold change)
negative mimic
miR-22-3p mimic
negative inhibitor
miR-22-3p inhabitor
(H)
Position 457-464 of NLRP3 3' UTR 5' ...AUGUUGAAAUUUUAUGGCAGCUA...3'
hsa-miR-22-3p 3' UGUCAAGAAGUUGACCGUCGAA 5'
'Seeds'
Position 406-412 of NLRP3 3' UTR 5' ...CGCUAUCUUUCUAUUAACUGACC...3'
hsa-miR-223-3p 3' ACCCCAUAAACUGUUUGACUGU 5'
'Seeds'
(I)
Relative light units
miR-223-3p-wt
negative control-wt
miR-223-3p-mut
negative control-mut
Relative light units
miR-22-3p-wt
negative control-wt
miR-22-3p-mut
negative control-mut

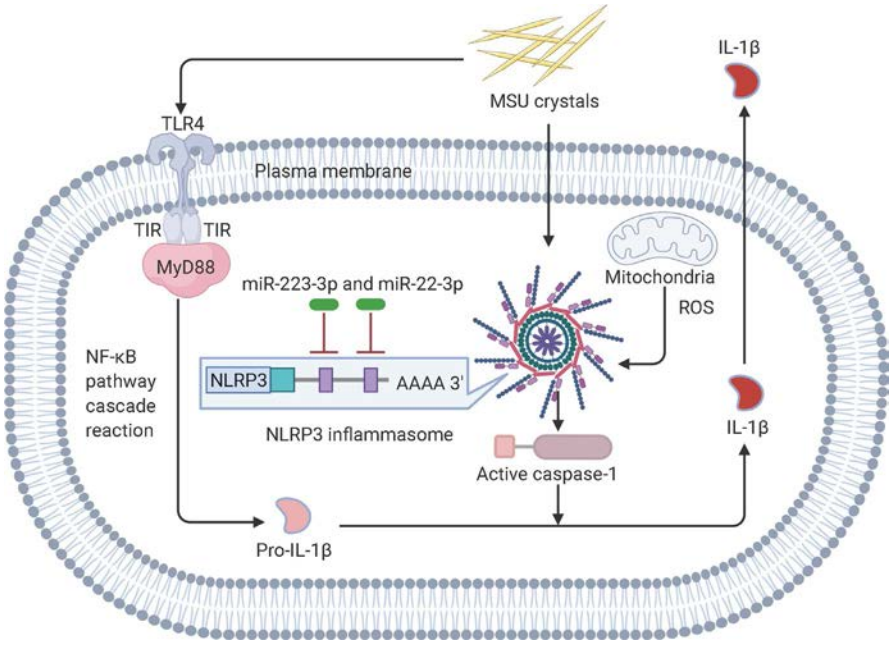

FIGURE 3 The mechanism of mir-223-3p and mir-22-3p regulating NLRP3 (nucleotide-binding domain leucine-rich repeat [NLR] and pyrin domain containing receptor 3) inflammasome activation

in the gout inflammatory models in vivo and in vitro; (c) overexpression of miR-223-3p and miR-22-3p substantially reduced leukocyte chemotaxis and the expression of NLRP3 in the mice air pouch synovium tissues; (d) miR-223-3p and miR-22-3p regulate NLRP3 by binding to its 3'-UTR.

Recent studies have shown that miR-223-3p and miR-22-3p are related to the inflammatory factors in some diseases. MiR-223-3p can serve as a biomarker of disease severity in pelvic inflammatory disease and Alzheimer's disease.[12,13] MiR-223-3p plays a vital role in inflammatory processes and negatively regulates neutrophil chemotaxis and the expression of C-X-C ligand 2, C-C ligand 3, and IL-6 proteins.[14,15] The nuclear factor (NF)-κB signaling pathway (which is the classical inflammatory pathway) was also closely related to miR-223-3p expression. Some studies demonstrated that miR-223 can negatively regulate the activation of NF-κB by inhibiting the phosphorylation and nuclear translocation of p65 and reducing the expression of Cul1a/b, Traf6, and Tab1 proteins.[16,17] Multiple studies have found a strong link between NLRP3 inflammasome and miR-223-3p. Jimenez et al.[18] found that miR-223-3p could improve the infiltration of monocytes, neutrophils, and activated macrophages in acute hepatitis by regulating NLRP3. In an animal study, miR-223-3p inhibited the expression of the NLRP3 inflammasome, promoted the polarization of dendritic cells to the tolerant dendritic cells, and effectively induced the production of regulatory T cells and prevented the mice from developing autoimmune myocarditis.[19]

miR-22-3p also plays a significant role in the inflammatory response. Cui et al.[20] found that the expression of miR-22-3p in plasma could have been related to inflammation caused by tuberculosis infection. Meanwhile, miR-22-3p can induce M2 polarization of macrophages and reduce spinal cord ischemia/reperfusion impairment by inhibiting interferon regulatory factor-5 (IRF5) in macrophages.[21] The expression of miR-22-3p is closely correlated to the NLRP3 inflammasome. Some studies reported that miR-22-3p plays an essential protective role in the inflammatory injury and cell apoptosis by inhibiting the mRNA and protein expression of NLRP3, Caspase-1, and IL-1β through targeting NLRP3.[22-24] However, one study revealed that miR-22-3p could inhibit Sirtuin 1 protein expression and promote the high expression of inflammatory cytokines tumor necrosis factor-α, IL-1β, and IL-8, which are vital for the development of inflammation.[25] The regulatory role of miR-22-3p in inflammation requires further exploration and verification with more inflammatory disease phenotypes. The results of the present experiment further confirmed the relationship between miR-223-3p and miR-22-3p and NLRP3. Meanwhile, it also reported the specific miRNA in plasma of patients with gout and their relationship with gout inflammation, which served as a supplement to gout and miRNA research. The main limitation of this study was that it was restricted to phenotypic observation and gout inflammation models of cells and animals. More studies are still required to validate the potential of miR-223-3p and miR-22-3p in reducing the expression and function of NLRP3 in future clinical trials. Further, it would be crucial to identify the relationship between the specific miRNAs and gout-related risk factors, such as age, gender, and so on.

In conclusion, the findings of this study suggest that miR-223-3p and miR-22-3p can reduce the inflammatory response of gouty arthritis by suppressing the expression of NLRP3 inflammasome. This study might provide a new design direction for the clinical trials of patients with acute gouty arthritis.

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EXPERT COMMENTARY

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WILEY

Recent advances in pediatric rheumatology: October to December 2020

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1. The temporal relationship between juvenile idiopathic arthritis disease activity and uveitis activity

Liebling EJ, Faig W, Chang JC, Mendoza E, Moore N, Ledesma Vicioso N, et al. *Arthritis Care Res (Hoboken)* 2020; Oct 12. https://doi.org/10.1002/acr.24483

While uveitis is an important complication in children with juvenile idiopathic arthritis (JIA), its onset and severity are often unpredictable. There is paucity of literature on the temporal association between arthritis and uveitis. Liebling et al. have carried out a retrospective single center analysis on 98 patients with JIA under ≤21 years. The authors report a close temporal association of active uveitis with active arthritis. The probability of developing uveitis was 65% within 45 days of onset of active arthritis, as compared to 42% in those with no active joints. Further, risk of uveitis was lower in females and patients with rheumatoid factor negative polyarthritis and human leukocyte antigen (HLA)-B27 related arthritis. Uveitis was also less common in patients treated with a combination of biologic and non-biologic disease-modifying anti-rheumatic drugs. Although this is a retrospective study, it has important clinical implications for pediatric rheumatologists. This study highlights the need for urgent ophthalmological evaluation, especially in children with flares of arthritis on follow-up.

2. Autoimmune thyroid diseases, autoimmune hepatitis, celiac disease and type 1 diabetes mellitus in pediatric systemic lupus erythematosus: Results from the CARRA Legacy Registry

AlAhmed O, Sivaraman V, Moore-Clingenpeel M, Ardoin SP, Bout-Tabaku S; CARRA registry investigators. *Lupus* 2020; Dec; 29(14): 1926-1936. https://doi.org/10.1177/0961203320961469.

Systemic lupus erythematosus (SLE) is the prototype autoimmune condition but data on polyautoimmunity (PA) in pediatric SLE (pSLE) are lacking. AlAhmed et al. have analyzed 1285 patients with pSLE within the Childhood Arthritis and Rheumatology Research Alliance (CARRA) Legacy Registry, of which 388 pSLE patients had comorbidities including autoimmune and non-autoimmune manifestations. The authors looked for prevalence of other autoimmune disorders like autoimmune hepatitis, autoimmune thyroid diseases, type 1 diabetes mellitus and celiac disease. It was found that prevalence of PA was 8.8% (34 out of 388 pSLE with comorbidities). It was also noted that patients with PA required hospital admissions more frequently and also needed more aggressive immunosuppression. This study is perhaps the first comprehensive report on the association of PA with pSLE.

3. Differential parameters between activity flare and acute infection in pediatric patients with systemic lupus erythematosus

Luo KL, Yang YH, Lin YT, Hu YC, Yu HH, Wang LC, et al. *Sci Rep* 10, 19913 (2020). https://doi.org/10.1038/s41598-020-76789-6

Infections can cause significant morbidity, and occasional mortality, in patients with pSLE. For the clinician at the bedside, it is often difficult to differentiate SLE flares from infections. This difference is critical for optimal management of patients. In this single center study from Taiwan, Luo et al. took up 50 pSLE patients. These were divided into 4 groups depending on disease activity and presence or absence of infections. It was found that simultaneous analysis of 4 different domains (namely, hematological parameters - lymphocyte percentage, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio; inflammatory parameters – C-reactive protein, erythrocyte sedimentation rate, procalcitonin; complement levels - C3, C4; and clinical disease activity and damage scores - Systemic Lupus Erythematosus Disease Activity Index, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index) is helpful in differentiating infections from SLE flares in pSLE patients. Although the sample size is small, this study has an important message for pediatric rheumatologists.

4. Study of serology and genetics of celiac disease in patients with juvenile systemic lupus erythematosus 'celiac in juvenile systemic lupus'

Shamseya AM, Elsayed EH, Donia HM. *Eur J Gastroenterol Hepatol* 2020; Oct; 32(10): 1322-1327. https://doi.org/10.1097/MEG.0000000000001865

Juvenile SLE (jSLE) has been associated with several autoimmune conditions. Occurrence of these autoimmune diseases in the context of SLE has important therapeutic implications. While there have been several isolated reports on association of celiac disease (CD) with jSLE, there is paucity of prospective data on this aspect. Shamseyaa et al. studied 100 jSLE patients and performed laboratory screening, endoscopic examination and histopathological examination of intestinal biopsies (in those with positive serology) for features of CD. Serological tests for CD (tissue transglutaminase antibody immunoglobulin A [tTGA]) were positive in 10% of patients. Histopathological examination of 10 patients with positive serology revealed that 6 patients had changes consistent with CD, while 4 had normal histopathology. Serological positivity for celiac was associated with significantly higher disease activity index but did not affect response to immunosuppression for SLE. This study represents an important advance in our knowledge of jSLE.

5. Development and testing of reduced versions of the MMT-8 in juvenile dermatomyositis

Rosina S, Varnier GC, Pistorio A, Pilkington C, Maillard S, Civino A, et al., Pediatric Rheumatology International Trials Organization (PRINTO). *J Rheumatol* 2020; Nov 15: jrheum.200543. https://doi.org/10.3899/jrheum.200543.

Juvenile dermatomyositis (JDM) is the commonest inflammatory myopathy in children and has an unpredictable long-term prognosis. Accurate assessment of muscle strength is essential for clinical assessment of disease activity. Tools like Childhood Myositis Assessment Scale (CMAS) and Manual Muscle Testing 8 (MMT8) are gold standards for this purpose but are time-consuming. Rosina et al. have developed a shortened version of MMT-8 that is derived from individual muscle group scores of MMT-8, analyzed retrospectively in 3 multinational datasets. MMT-4 included neck flexors, hip extensors, hip abductors and shoulder abductors, whereas elbow flexors and hip flexors were also included in MMT-6. The authors report that the 2 shortened tools correlated strongly with MMT-8 and responsiveness to change was superior to that of MMT-8. Hence, these shortened tools can be used in routine patient care, particularly in children who may not cooperate for the entire assessment. This is yet another clinical contribution from the PRINTO, and has management implications for these patients.

6. Safety and immunogenicity of the quadrivalent human papillomavirus vaccine in patients with juvenile dermatomyositis: a real-world multicentre study

Grein IHR, Pinto NBF, Groot N, Martins CB, Lobo A, Aikawa NE, et al. *Pediatr Rheumatol Online J* 2020; Nov 11; 18(1): 87. https://doi.org/10.1186/s12969-020-00479-w.

Vaccine coverage is low in patients with autoimmune diseases because of concerns of safety. But this population is also highly vulnerable to infections both due to the disease and also due to the therapy that these patients receive. Grein et al. assessed safety and immunogenicity of quadrivalent human papillomavirus (qHPV) vaccine in a cohort of 47 patients with JDM patients and 41 healthy controls from 10 Brazilian centers. Possible adverse events following vaccination were monitored for 14 days following each dose of vaccination. At each visit, serum samples to test antibody concentrations against HPV 16 and 18 were collected from all participants. The schedule of the visits was as follows: before the first dose; 1 month after the second and third doses; and 6 months after the third dose. Adverse events were mild and there was no difference between JDM and control groups. Seroconversion rates were 100% for HPV 16 and 97% for HPV 18 1 month after the third dose of vaccine. There was no significant difference in seroconversion between study and controls groups. Seroconversion rates were 94% for both HPV types at 6 months of administration of the third dose. The results of this study show that HPV vaccination in JDM patients was safe and immunogenic. The results are reassuring.

7. Association between Kawasaki disease and prenatal exposure to ambient and industrial air pollution: A population-based cohort study

Buteau S, Belkaibech S, Bilodeau-Bertrand M, Hatzopoulou M, Smargiassi A, Auger N. *Environ Health Perspect*. 2020; Oct; 128(10): 107006. https://doi.org/10.1289/EHP6920. Epub 2020 Oct 19.

Kawasaki disease (KD) is an important etiology of acquired heart disease in children the world over. Etiology of KD is still an enigma. Possible etiological factors that have been implicated are infectious agents and environmental triggers in a genetically predisposed individual. Buteau et al. have carried out a longitudinal study on a cohort of 505 336 children, born in Quebec, Canada between 2006 and 2012 and followed until 31 March 2018. The authors have tried to correlate prenatal exposure to outdoor air pollution with incidence of KD in the cohort. Five hundred and thirty-nine children were diagnosed as having KD during the study period. Prenatal air pollutant exposure (annual average concentration of ambient fine particulate matter) was assessed according to the residential postal code at the time of birth. It was found that prenatal exposure to ambient and industrial air pollution was associated with higher incidence of KD. This study provides new insights into the etiology of KD and may have implications for public health authorities.

8. Functional benefits of corticosteroid and IVIG combination therapy in a coronary artery endothelial cell model of Kawasaki disease

Inoue T, Murakami S, Matsumoto K, Matsuda A. *Pediatr Rheumatol* 18; 76 (2020). https://doi.org/10.1186/s12969-020-00461-6

Treatment of KD was hitherto based on use of intravenous immunoglobulin (IVIg) during the acute stage. However, over the last decade, several new agents have been added to the therapeutic armamentarium for this condition. It is now increasingly evident that corticosteroids have an important, and additive, role in management of these patients, especially when there is IVIg resistance. Inoue

et al. have studied an experimental model to demonstrate benefits of combination therapy of IgG and corticosteroids in KD. The investigators stimulated cultured human coronary artery endothelial cells (HCAECs) with tumor necrosis factor (TNF)-α, interleukin (IL)-1α or IL-1β and measured messenger RNA, protein concentrations for high-mobility group box-1 (HMGB1), ILs like IL-1α, IL-6 and granulocyte-colony stimulating factor (G-CSF) and Caspase 3/7 activity assay in culture supernatants. These assays were carried out in presence and absence of dexamethasone and IgG. It was found that apoptotic effects of inflammatory stimuli were blunted more effectively by dexamethasone than by IgG. While dexamethasone and IgG could both inhibit production of TNF-α induced inflammatory cytokines by HCAECs, IL induced cytokines were suppressed to a greater extent by dexamethasone as compared to high-dose IgG alone. The authors have proposed a role for TNF-α in the onset of coronary artery abnormalities (CAAs),and for ILs in the development of CAAs. These cytokine pathways are more effectively inhibited by dexamethasone in combination with IgG, than IgG alone. This is an important study in as much as it provides some experimental proof for clinical observations.

9. Onset age is a risk factor for refractory pediatric IgA vasculitis: a retrospective cohort study

Liao CH, Tsai M, Yang YH, Chiang BL, Wang LC. *Pediatr Rheumatol Online J* 2020; Nov 10; 18(1): 86. https://doi.org/10.1186/s12969-020-00480-3

It is known that prognosis and outcomes of IgA vasculitis are age-dependent. Children >12 years of age have more severe forms of the disease. Liao et al. have reported their cohort of 484 patients with IgA vasculitis seen over a period of 19 years in Taiwan. Of these, 8.3% were aged >12 years. While renal involvement was noted in 26.9% of the cohort, it was significantly higher in children >12 years (55%). Joint involvement was more common in children <6 years (82%), but there was no difference in gastrointestinal involvement with respect to age stratification. It is no surprise that disease-modifying agents (prednisolone and azathioprine) were needed more frequently in older age groups. This study provides added evidence to the fact that IgA vasculitis is significantly more severe in adolescents and young adults.

10. Anti-IL1 treatment in colchicine-resistant paediatric FMF patients: real life data from the HELIOS registry

Sag E, Akal F, Atalay E, Akca UK, Demir S, Demirel D et al. *Rheumatology (Oxford)* 2020; Nov 1; 59(11): 3324-3329. https://doi.org/10.1093/rheumatology/keaa121.

Familial Mediterranean fever (FMF) is a common hereditary autoinflammatory disease which is characterized by recurring febrile episodes, abdominal pain, chest pain, arthritis and elevation of acute phase reactants. Colchicine is the drug of choice for FMF. However, 5-10% patients are either intolerant, or non-responsive, to colchicine. Anti-interleukin-1 (anti-IL-1) therapy is the preferred treatment modality for such patients. Sag et al report a series of 40 patients with colchicine-resistant or intolerant pediatric FMF, enrolled from a single center in Turkey who were treated with anti-IL1 agents (anakinra and canakinumab). The majority (34/40) needed continuous treatment, and 6 were put on intermittent use of anti-IL1 agents. There was a significant clinical improvement with this modality. The authors also demonstrated utility of anti-IL-1 agents on an on-demand basis over short duration. Larger clinical studies are needed to confirm these findings.

11. Serum KL-6 level as a biomarker of interstitial lung disease in childhood connective tissue diseases: a pilot study

Kilinc AA, Arslan A, Yildiz M, Kucur M, Adrovic A, Barut K, et al. *Rheumatol Int* 2020; Oct; 40(10): 1701-1706. https://doi.org/10.1007/s00296-019-04485-4

Interstitial lung disease (ILD) is an important pulmonary manifestation of pediatric connective tissue diseases (CTDs) like juvenile systemic sclerosis (jSSc), JDM, jSLE and systemic juvenile idiopathic arthritis (sJIA). Diagnosis requires clinical examination, pulmonary function tests and radiological assessment (especially high-resolution computed tomography). However, these modalities have low sensitivity, especially during early stages of the disease. Several biomarkers have been used in adults with ILD for the purpose of screening and prognostication. Krebs von den Lungen-6 (KL-6) is one such biomarker that has been frequently used in previous studies. Kilinc et al. enrolled 88 patients with CTD from a single center in Turkey, out of whom 11 (12.5%) had ILD (8 patients with jSSc, 2 with jSLE, and 1 had sJIA). The authors also enrolled 68 healthy controls. KL-6 was measured using enzyme-linked immunosorbent assay. Patients with CTD and ILD had significantly higher levels of KL-6 as compared to patients with CTD without ILD and healthy controls. Receiver operating characteristic curves were used to identify a cut-off value for KL-6. It was found that a cut-off of 712.5 U/mL had 81% sensitivity and specificity of 72% for CTD with ILD. KL-6 is an emergent biomarker in the context of ILD in childhood CTDs, but needs validation among other populations.

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COCHRANE CORNER

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Are non-steroidal anti-inflammatory drugs effective for acute low back pain? A Cochrane Review summary with commentary

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The aim of this commentary is to discuss the published Cochrane Review "Non-steroidal anti-inflammatory drugs for acute low back pain"[a] by Wendelien H van der Gaag et al.,[1] under the direct supervision of Cochrane Back and Neck Group. This Cochrane Corner is produced in agreement with *International Journal of Rheumatic Diseases* by Cochrane Rehabilitation.

1 | BACKGROUND

Low back pain (LBP) is defined as pain, muscle tension, or stiffness localized below the costal margin and above the inferior gluteal folds and restricting the activities of daily living.[2] Acute back pain may last up to 12 weeks.[3] The prevalence rate of LBP is higher in the developed and industrial countries ranging 60%-70%.[4] It has a significant socio-economic burden on society.[5] LBP in some cases may improve without taking any specific pharmacological treatment.[6] But due to the current active life styles and the risk of absence from work, most patients with LBP prefer to take medicines for a quick pain relief. Non-steroidal anti-inflammatory drugs (NSAIDs) and muscle relaxants are considered to be among the first choices of treatment for non-specific LBP.[7]

2 | NON-STEROIDAL ANTI-INFLAMMATORY DRUGS FOR ACUTE LOW BACK PAIN

van der Gaag, Roelofs PD, Enthoven WT et al. (2020).

2.1 | What is the aim of this Cochrane Review?

The aim of this Cochrane Review was to determine the effects of NSAIDs compared to placebo and other comparison treatments for acute LBP.

2.2 | What was studied in the Cochrane Review?

The population addressed in this review was adults aged 18 years and above with acute non-specific LBP. The interventions studied were one or more types of NSAIDs. The comparator was placebo or other type of treatment. The primary outcomes studied were pain intensity (eg, visual analog scale or numeric rating scale [NRS]), back pain-specific functional status (eg, Roland Morris Disability Questionnaire [RMDQ], Oswestry Disability Index [ODI]), global measure (eg, overall improvement, proportion of participants recovered), adverse events (proportion of participants experiencing adverse events) and return to work. No secondary outcomes were studied.

[a]This summary is based on a Cochrane Review previously published in the Cochrane Database of Systematic Reviews 2020, Issue 04, Art. No.: CD013581, https://doi.org/10.1002/14651858.CD013581 (see www.cochranelibrary.com for information). Cochrane Reviews are regularly updated as new evidence emerges and in response to feedback, and Cochrane Database of Systematic Reviews should be consulted for the most recent version of the review.

The views expressed in the summary with commentary are those of the Cochrane Corner authors and do not represent the Cochrane Library or Wiley.

2.3 | What was the search methodology and search date of the Cochrane Review?

This is an update of a 2008 Cochrane Review on the same topic, focusing on acute LBP. The authors searched for studies that had been published up to 7 January 2020 from the Cochrane Central Register of Controlled Trials, MEDLINE, Embase, PubMed, ClinicalTrials.gov and WHO International Clinical Trial Registry Platform. Authors also screened the reference lists of review articles and included trials.

2.4 | What are the main results of the Cochrane Review?

The review included 32 randomized controlled trials (RCTs) with a total of 5356 participants, age ranging from 16 to 78 years. The follow-up period in different studies ranged from 1 day to 6 months. Most of the studies included in the review were conducted in Europe and North America.

The review studied the following comparisons:

NSAIDs compared to placebo: 9 studies compared NSAIDs with placebo. The mean pain intensity in the placebo group ranged from 7.9 to 33.9 and was 7.29 points lower (95% CI 10.98 lower to 3.61 lower) in the NSAID group. There was a clinically unimportant reduction in pain intensity (moderate-quality evidence) reported by 4 RCTs with 815 participants. Two RCTs with 471 participants reported a clinically unimportant reduction in disability. The mean disability in the placebo group ranged from 6.0 to 7.3 and was 2.02 lower (95% CI 2.89 lower to 1.15 lower) in the NSAID group (high-quality evidence). Five RCTs with 1201 participants reported global improvement (risk ratio [RR] 1.40, 95% CI 1.12 lower to 1.75 lower) (low-quality evidence) but the effect size was small and not clinically relevant. Adverse events and return to work were reported by 6 RCTs with 1394 participants and 1 RCT with 266 participants respectively. Very low-quality evidence showed there was no difference between NSAIDs and placebo in the proportion of people experiencing adverse events (RR 0.86, 95% CI 0.63 to 1.18) or returning to work after 7 days (RR 1.48, 95% CI 0.98 to 2.23).

Selective cyclooxygenase (COX-2) inhibitors compared to non-selective NSAIDs: 17 RCTs compared NSAIDs to other NSAIDs. Only 2 of these compared selective COX-2 inhibitors with non-selective NSAIDs. Two RCTs with 437 participants, reported pain intensity with no clear difference between groups, The mean change in pain intensity from baseline in the non-selective NSAID group ranged from 38 to 41 and the COX-2 inhibitors group was 2.60 lower (95%, 9.23 lower to 4.03 higher) (low-quality evidence). One trial reported a mean difference in disability score of −7.00 (95% CI −13.15 to −0.85) after 10 days, showing a statistically significant and clinically relevant difference in favor of the nimesulide arm. Adverse events were reported in 2 trials (444 participants), but the results were inconclusive (RR 0.97, 95% CI 0.63 to 1.50) with no clear difference between groups (very low-quality evidence). Return to work was not reported.

Non-selective versus non-selective NSAIDs: 15 RCTs compared non-selective NSAIDs to other non-selective NSAIDs. Pain intensity was reported in 13 RCTs (1823 participants), disability was reported in 5 RCTs (1006 participants) and global improvement was reported in 7 trials (987 participants) (moderate-quality evidence). The results were not clinically relevant and there was no clear difference between groups except for 1 trial which reported a clinically relevant difference between groups. Thirty-two percent of those who received dipyrone intramuscular injections completely recovered after 2 days compared to 12% of the participants who received diclofenac intramuscular injections. Fourteen trials with 2337 participants reported adverse events out of which 11 trials showed no clinically relevant difference between groups while 3 trials reported statistically and clinically relevant difference between groups. The first trial (100 participants) reported adverse events of 2% in the aceclofenac arm compared to 12% in the diclofenac arm. The second trial (174 participants) reported 5% adverse events in the dipyrone group compared to 1% in the diclofenac arm. The third trial (133 participants) reported 18% adverse events in the diflunisal arm versus 31% in the indomethacin arm (moderate-quality evidence).

NSAIDs compared to paracetamol: 3 trials compared NSAIDs to paracetamol. Two trials with n = 289 and one trial with n = 219 reported no clear difference for pain intensity reduction with standardized mean difference (MD) of −0.12, 95% CI −0.35 to 0.12 comparable to MD of −0.07 (95% CI −0.25 to 0.11) and disability between groups respectively. Global improvement was not reported in any trial while adverse events were reported in 2 RCTs but due to clinical heterogeneity, the results were not pooled (low-quality evidence). Return to work was reported in a single trial of 45 participants but results showed no clear difference between groups (very low-quality evidence).

NSAIDs compared to other drug treatment: 4 trials with 391 participants compared NSAIDs to other drugs. Pain intensity and adverse events were reported in all 4 trials but due to inadequately reported data; meta-analysis was not done (low-quality evidence). Return to work and disability were not reported in any study. Global improvement was reported in 2 trials with 162 participants and pooled RR was 1.01 (95% CI 0.81 to 1.25; moderate-quality evidence).

NSAIDs compared to non-drug treatment: 7 trials compared NSAIDs to non- drug treatment. Due to clinical diversity and statistical heterogeneity in the trials, meta-analysis was not considered appropriate for any of the primary outcomes.

1. NSAIDs compared to spinal manipulation or physiotherapy: pain intensity was reported in 4 trials (n = 391) (low-quality evidence). Two trials (n = 193) reported disability. One RCT showed a clinically relevant difference while the other trial did not (very low-quality evidence). Two RCTs (n = 180) reported global improvement but one of them showed clinical difference for this outcome between groups (very low-quality evidence).

Two trials (n = 189) reported adverse events (very low-quality evidence) and one trial reported return to work with no clear difference between groups (low-quality evidence).

2. NSAIDs compared to bed rest: 2 trials (n = 130) compared NSAIDs with bed rest. Pain intensity and disability were reported in 1 trial and showed no clear difference between groups. The other trial did not report relevant outcomes except for 2 participants in the NSAID group who withdrew because of adverse events.
3. NSAIDs compared to heat wrap: a single trial of 371 participants showed that heat wrap reduced pain intensity and disability better than NSAIDs, but the difference was not large enough to be considered clinically relevant. Adverse events were equal in both arms.
4. NSAIDs compared to motion style acupuncture treatment (MSAT): 1 study (n = 58) compared MSAT with a single intramuscular injection of NSAIDs. The results were statistically significant and clinically relevant. MSAT reduced mean pain intensity from baseline 4.17 (3.05) versus 5.83 (2.61); and disability mean (*SD*) improvement in functional status from baseline 36.34 (29.1) versus 56.41 (24.86); more than NSAIDs, the MSAT group had better pain relief and disability reduction along with improvement in functional status at 2 weeks, than the NSAID group. No adverse events were reported in either groups.

2.5 | What did the authors conclude?

The authors concluded there is moderate-quality evidence that NSAIDs seem slightly more effective than placebo for short-term pain reduction duration. There is also high-quality evidence that NSAIDs are slightly more effective than placebo for reducing disability in acute LBP. In addition, reduction in pain intensity, disability and global improvement were observed in the NSAIDs group compared to placebo but effect size was small so clinical relevance of this finding might not be significant. There was no clear difference for short-term pain relief when comparing selective COX-2 inhibitors with non-selective NSAIDs. Based on the data the authors were unable to present firm conclusions about adverse events and the safety of long-term use of NSAIDs for LBP management. It is also important to note that although the included studies took place in many different countries, more than half of the studies were conducted in Europe and North America and the developing countries were not adequately represented.

3 | WHAT ARE THE IMPLICATIONS OF THE COCHRANE EVIDENCE FOR PRACTICE IN RHEUMATOLOGY?

Acute back pain is one of the most common musculoskeletal issues seen in rheumatology practice. Rheumatologists and physiatrists frequently attend patients with acute non-specific LBP. Although they have a wide range of options to choose for the optimal management, NSAIDs are usually one of the most prescribed medicines. In addition, in many countries across the globe, NSAIDs are available as over-the-counter medicines and can even be procured without a formal prescription by a physician.[8,9] This systematic review suggests that there is low-to-moderate-quality evidence on pain reduction and global improvement, and high-quality evidence on disability reduction; however, these findings are not clinically relevant. It is also not clear if the non-selective COX-2 inhibitors are better than the selective COX-2 inhibitors. The rheumatologists and physiatrists prescribing NSAIDs for LBP must counsel the patient regarding the shortest possible duration of the medicines and to avoid using them on a long-term basis. With so many non-pharmacological treatment strategies available for the management of LBP, the focus should be gradually shifted from medicines to active management wherever feasible.[10]

A lot of work is being done around the globe to generate high-quality evidence on the management strategies of back pain. An international steering committee has recently developed a preliminary core outcome measurement set that specifies outcomes to be included in every clinical trial involving people with non-specific LBP.[11] This will be useful for studies reporting on acute LBP in future and will allow a better comparability of results from different parts of the world.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Your help is needed in the fight against COVID-19: Please contribute to the COVID-19 Global rheumatology alliance registry

The COVID-19 Global Rheumatology Alliance is a global collaboration of rheumatologists, scientists, patients and organisations all committed to addressing the issues in rheumatology created by the COVID-19 global pandemic. To date the alliance has published important data on the effect of COVID-19 infection on outcomes and the effect of rheumatic medications on COVID-19 outcomes.

We currently have 3520 cases from all over the world but we still need to collect many more cases and we need cases from all around the world including the Asia-Pacific region. We are hoping for more cases from the Asia-Pacific region because this is currently under-represented in the registry.

To contribute we ask that you provide details of the case, rheumatic diagnosis details, treatments, and the outcome of the case.

You can join the mailing list for the COVID-19 Global Rheumatology Alliance by signing up on our webpage (top right hand corner)

For more information please visit our website at www.rheum-covid.org, if you have questions or issues and would like to know more information please email rheum.covid@gmail.com.