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EDITORIAL

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A vaccination update for rheumatologists—SARS-CoV-2, influenza and herpes zoster

1 | INTRODUCTION

Vaccination is currently at the forefront of everyone's mind. The SARS-CoV-2/COVID-19 pandemic has resulted in enormous changes to the way we practice rheumatology.[1] The number of worldwide cases, deaths and long-term health and economic impact from SARS-CoV-2 infection has been sobering. With many countries in lockdown or other forms of societal restrictions, widespread vaccination is critical at protecting people and combating the pandemic.

This paper will summarize the currently available evidence regarding SARS-CoV-2 vaccination in patients with autoimmune inflammatory rheumatic diseases (AIIRD) with a focus on vaccine options in the Asia-Pacific region. (We have included only peer-reviewed publications and excluded conference abstracts or preprint manuscripts). The onset of influenza season in Australia with the widespread availability of adjuvant or high-dose influenza vaccines makes this an opportune time to discuss influenza vaccination and its timing with SARS-CoV-2 vaccination. The recent availability of a recombinant herpes zoster vaccine (RZV, or Shingrix®) in Australia with the concern provoked by administration of the live zoster vaccine (ZVL, or Zostavax®) to immunosuppressed AIIRD patients also means this is a fruitful topic for discussion. As there has been dialog regarding a "travel bubble" between Australia and Singapore, we have included a Singaporean perspective on these issues.

2 | SARS-CoV-2/COVID-19 IN RHEUMATOLOGY PATIENTS

2.1 | Risk of hospitalization and death

Recent data from the COVID-19 Global Rheumatology Alliance physician-reported registry (3729 patients, 390 deaths) suggested that higher likelihood of COVID-19-related death was associated with increased age (66-75 years: odds ratio [OR] 3.00, 95% confidence interval [CI] 2.13 to 4.22; >75 years: 6.18, 4.47-8.53; both compared to those ≤65 years of age), male gender (1.46, 1.11-1.91), hypertension with cardiovascular disease (1.89, 1.31-2.73), chronic lung disease (1.68, 1.26-2.25) and prednisolone-equivalent dosage >10 mg/d (1.69, 1.18-2.41; compared to no glucocorticoid).[2]

Moderate/high disease activity compared to remission/low disease activity was also associated with increased odds of death (1.87, 1.27-2.77). Rituximab (4.04, 2.32-7.03), sulfasalazine (3.60, 1.66-7.78), immunosuppressants (azathioprine, cyclophosphamide, cyclosporin, mycophenolate or tacrolimus; 2.22, 1.43-3.46) and not receiving any disease-modifying anti-rheumatic drug (DMARD; 2.11, 1.48-3.01) were associated with higher odds of death, compared with methotrexate (MTX) monotherapy.[2]

Prednisone dose ≥10 mg/d was associated with higher odds of hospitalization (OR 2.05, 95% CI 1.06-3.96). Reassuringly, conventional synthetic DMARD (csDMARD) alone, or in combination with biologic DMARDs (bDMARDs) or Janus kinase inhibitors (JAKi) was not associated with hospitalization (OR 1.23, 95% CI 0.70-2.17 and OR 0.74, 95% CI 0.37-1.46, respectively). Interestingly, tumor necrosis factor inhibition (TNFi) was associated with a lower odds of hospitalization (OR 0.40, 95% CI 0.19-0.81).[3] Despite initial enthusiasm about the benefits of hydroxychloroquine, this association was not observed (OR 0.94, 95% CI 0.57-1.57).[3]

A North American study used a large multicenter electronic database to identify patients with AIIRD infected with SARS-CoV-2 and compared outcomes to matched patients with SARS-CoV-2, but without AIIRD. Reassuringly, once correction for comorbidities was undertaken, there was no difference in outcomes, except for the higher risk of venous thromboembolism in those with AIIRD (relative risk [RR] 1.60; 95% CI 1.14-2.25).[4]

2.2 | Vaccine hesitancy

Despite SARS-CoV-2 vaccination being critical for dealing with the pandemic and protecting immunosuppressed AIIRD patients, there was more hesitation regarding vaccination among Italian patients compared to healthy controls.[5] However, AIIRD patients were willing to undergo vaccination if informed about risks and benefits by their trusted specialist.[5] While a French web-based survey involving participants from 56 countries found the proportion of patients with AIIRD willing to undergo SARS-CoV-2 vaccination was 54.2% (686/1266), uncertainty was reported in 32.2% (n = 408) and unwillingness to undergo vaccination in 13.6% (n = 172).[6] The most trusted healthcare professional for vaccination advice was their medical specialist.[6] The commonest patient concerns were limited

experience/information regarding the new SARS-CoV-2 vaccines, use of relatively "new" messenger RNA (mRNA) vaccine technology, possible flare of their AIIRD and risk of a local reaction or side effects.[6] There is marked national variation in attitudes to vaccination with differences in acceptance rates ranging from almost 90% in China to less than 55% in Russia.[7]

2.3 | Vaccine safety, immunogenicity and efficacy

As immunocompromised patients were excluded from studies of SARS-CoV-2 mRNA vaccines, there is limited evidence regarding vaccine efficacy in immunosuppressed rheumatology and musculoskeletal (RMD) patients. A non-randomized study of 123 RMD patients found that at a median (interquartile range [IQR]) of 22 (18-26) days after the first vaccine dose (52% BNT162b2, Pfizer-BioNTech; 48% mRNA-1273, Moderna), 74% (95% CI 65%-81%) had a detectable antibody response against the receptor-binding domain of the SARS-CoV-2 spike protein.[8] Those treated with mycophenolate or rituximab were less likely to develop an antibody response. Another study from the same group found that at a median time of 20 days (IQR 17-24 days) after the first vaccine dose, antibody to the anti-S1 or anti–receptor-binding domain, was detectable in only 76 participants (17%; 95% CI 14%-21%).[9] Those treated with anti-metabolite immunosuppressants, such as mycophenolate or azathioprine, were less likely to develop an antibody response than those not on these agents (37% compared to 63%, respectively; adjusted incidence rate ratio [IRR] 0.22; 95% CI 0.15-0.34).[9] However, despite their importance in protection against SARS-CoV-2 infection, post-vaccination cellular immune responses were not assessed in these studies.

A small German study of 42 healthy controls and 26 patients with chronic inflammatory disease (mean age 37.5 and 50.5 years, respectively) treated with a range of bDMARDs found that anti-SARS-CoV-2 antibodies and neutralizing activity using a separate enzyme-linked immunosorbent assay was detectable in all participants following the second dose of mRNA vaccine.[10] Immunoglobulin G (IgG) titers were significantly lower in patients compared with controls (2053 binding antibody units/mL ± 1218 vs 2685 ± 1102). However, post-vaccination cellular immune responses were again not assessed in this study. While vaccination was not associated with disease flares, the short follow-up of 7 days may have been insufficient to study this.[10]

Despite assay differences between studies, there is a strong relationship between levels of neutralizing antibodies and vaccine protective efficacy across different SARS-CoV-2 vaccines.[11] Furthermore, the estimated neutralization level for protection from severe infection is approximately 6-fold lower than that required for protection from a symptomatic infection.[11]

A recent small study from New York found that only 18/25 (72%) patients on MTX for treatment of AIIRD had an adequate humoral response (IgG antibodies against the spike protein) following BNT162b2 (Pfizer-BioNTech) compared to 24/26 (92%) of AIIRD patients not on MTX and 96% (25/26) of normal controls.[12] In the same study, post-vaccination cellular immunity was also impaired in patients on MTX, with a lack of CD8+ T-lymphocyte activation.

A British study compared antibody responses and seroconversion rates in patients with inflammatory bowel disease (n = 865) treated with the TNFi, infliximab compared to those treated with vedolizumab (n = 428), a gut-selective anti-integrin α4β7 monoclonal antibody.[13] Geometric mean (SD) anti-SARS-CoV-2 antibody concentrations were lower in patients treated with infliximab compared to vedolizumab following 1 dose of the BNT162b2 vaccine (Pfizer-BioNTech; 6.0 U/mL, SD 5.9 vs 28.8 U/mL, SD 5.4; $P < .0001$) and 1 dose of the ChAdOx1 nCoV-19 (Oxford/AstraZeneca; 4.7 U/mL, SD 4.9 vs 13.8 U/mL, SD 5.9; $P < .0001$) preparation. Despite this blunting of post-vaccination serologic response, following the second vaccine doses, 85% (17/20) of infliximab-treated patients and 86% (6/7) of vedolizumab-treated patients seroconverted.[13] Unfortunately, vaccine supply delay in the United Kingdom at the time limited the number of study participants who received the second dose of both vaccines.

2.4 | Recommendations from various national rheumatology organizations

While the American College of Rheumatology COVID-19 Vaccine Clinical Guidance Task Force suggested with-holding MTX for 1 week after each dose of the mRNA COVID vaccine and for 2 weeks after single dose COVID vaccination in those with well-controlled disease, this was based on expert consensus opinion rather than firm evidence.[14] While a South Korean study found cessation of MTX 2 weeks before and 2 weeks after seasonal trivalent influenza vaccination in patients with rheumatoid arthritis (RA) resulted in better serologic responses,[15] similar data are not currently available for SARS-CoV-2 vaccines. Nevertheless, the Korean College of Rheumatology advised temporary discontinuation of MTX for 1-2 weeks after each vaccine dose can be considered, but that DMARDs should be continued during vaccination since their with-holding can increase disease activity, which is associated with worse SARS-CoV-2 infection severity and outcomes.[16]

The Singapore Chapter of Rheumatologists recommended that immunomodulatory drugs, other than rituximab, can be continued alongside SARS-CoV-2 vaccination, that is without the need for cessation. For those on rituximab, vaccination should be administered a minimum of 6 months after the last dose, and/or 4 weeks prior to the next dose of rituximab. If possible, vaccination should ideally be performed prior to commencing rituximab.[17]

2.5 | Summary

As csDMARDs, bDMARDs and targeted synthetic DMARDs (tsDMARDs) may reduce serologic responses following SARS-CoV-2 vaccination,[8-10,12,13] it is reasonable to minimize immunosuppression around the time of SARS-CoV-2 vaccination by with-holding these

agents – if possible, so long as joint inflammation is quiescent and risk of underlying disease flare is low. Vaccination urgency will obviously be dependent on the prevailing national healthcare burden of SARS-CoV-2 morbidity and mortality. However, the logistics of with-holding DMARDs can be challenging and given the ambivalence toward vaccination already expressed by many rheumatology patients,[5] every effort should be made to remove vaccination hurdles. Reassuringly, despite the lower post-vaccination serologic titers observed following a TNFi, most patients still seroconverted.[13]

There is currently insufficient data to warrant routine measurement of post-vaccination serologic responses.[14]

Medication adherence in rheumatology patients is a major issue compromising optimal care,[18] so clinicians advising with-holding of DMARDs around the time of vaccination should ensure patients recommence immunosuppression as soon as possible afterwards to avoid disease flares. As population vaccination is a critical strategy to fight this pandemic, and their opinion matters to patients,[5] rheumatologists should encourage all patients to undergo SARS-CoV-2 vaccination as per national guidelines. It would also be prudent to encourage vaccination of household and other close contacts to reduce likelihood of SARS-CoV-2 exposure. In Australia, the Pfizer vaccine has now been approved for use in pregnant women.[19]

2.6 | Useful links for patients

- https://rheumatology.org.au/downloads/20210422%20COVID-19%20Vaccination%20for%20Rheum%20Patients%2022Apr21.pdf
- https://rheum-covid.org/covaripad-summary/
- https://creakyjoints.org.au/covid_19/
- https://www.health.gov.au/resources/publications/atagi-covid-19-vaccination-shared-decision-making-guide-for-people-with-immunocompromise

3 | INFLUENZA

Influenza vaccination in the setting of AIIRD has been recently discussed.[20-22] With onset of influenza season in Australia, quadrivalent seasonal influenza vaccines are recommended for those aged 10-65 years, for example FluQuadri®, Afluria Quad®.[23] However, due to waning age-associated immunity, the adjuvant influenza vaccine, Fluad Quad®, is recommended for those aged ≥65 years.[23]

In Singapore, either the trivalent or quadrivalent influenza vaccines are recommended annually for immunosuppressed individuals.[24]

Reduced serologic responses occur in AIIRD patients following vaccination against seasonal influenza – probably due to therapeutic immunosuppression.[20-22] A Canadian study randomized 279 patients with RA to standard-dose quadrivalent influenza vaccine (SD-QIV) and 139 to a high-dose trivalent preparation (HD-TIV). Those who received HD-TIV were more likely to seroconvert than those who received SD-QIV with OR of 2.99 (95% CI 1.46-6.11) for seroconversion to strain A/H3N2, 1.95 (1.19-3.22) for strain B/Bris, 3.21 (1.57-6.56) for strain A/H1N1 (in 2016-2017), and 2.44 (1.18-5.06) for seroconversion to strain A/H1N1 (in 2017-2018).[25] No flare in RA activity was observed following either vaccine.

A British study using a large community-based database (Clinical Practice Research Datalink) found seasonal influenza vaccination reduced the risk of influenza-like illness (adjusted hazard ratio [aHR] 0.70), hospitalization for pneumonia (aHR 0.61) and chronic obstructive pulmonary disease exacerbations (aHR 0.67), and death due to pneumonia (aHR 0.56) in AIIRD patients.[26] No association was seen in the AIIRD population between influenza vaccination and disease flares or vaccine-related adverse events.[27]

The Australian Technical Advisory Group on Immunisation (ATAGI) does not routinely recommend co-administration of a SARS-CoV-2 vaccine with other vaccines, including influenza. Instead, ATAGI suggests a minimum 7-day interval between administration of a SARS-CoV-2 vaccine and other vaccines, including the influenza vaccine.[28] However, this interval can be shortened in certain circumstances, for example during a significant SARS-CoV-2 or influenza outbreak. The Singapore Ministry of Health recommends a 14-day interval between administration of a SARS-CoV-2 vaccine and other vaccines.[29]

3.1 | Summary

Rheumatologists should encourage all patients to undergo seasonable influenza vaccination as per national guidelines. Influenza vaccination should occur as far as possible from a dose of rituximab - the CD-20 depleting antibody.[30] All immunosuppressed AIIRD patients who receive the seasonal influenza vaccine for the first time should receive 2 vaccine doses, at least 4 weeks apart, and 1 dose annually thereafter on an ongoing basis.[31,32] Consideration should be given to vaccinating immunosuppressed AIIRD patients with an adjuvant or high-dose seasonal influenza vaccine – if available.[25]

4 | HERPES ZOSTER

The increased risk of herpes zoster (HZ) reactivation in immunosuppressed AIIRD patients, especially in the setting of JAKi use is well-recognized.[33] Since ZVL was included in the Australian National Immunisation Program (NIP) in November 2016 for those 70 years and older, there has been a marked fall in HZ antiviral prescription rates in this age group, by an average of 13.6% per year (95% CI 1.5-24.2).[34] (As ZVL is not included in the Singapore National Vaccination Program, the high cost and concern for vaccine-related disseminated VZV in immunosuppressed AIIRD patients has led to relatively low uptake). While these results indicate incorporation of ZVL onto the Australian NIP was successful in protecting many 70-79-year-olds against HZ, there have been 3 post-vaccination deaths due to disseminated infection from the vaccine strain used in ZVL. Only 1 of

these has occurred in an AIIRD patient on prednisone and hydroxychloroquine, the other 2 were in oncology patients.[35,36] Current recommendations are that live virus vaccines should be avoided in the setting of immunosuppression.[32,37] However, low-to-moderate doses of corticosteroid (dose equivalent to prednisone ≤10 mg/d), leflunomide, salazopyrine and MTX (≤0.4 mg/kg per week) and AZA (≤3 mg/kg per day) are not listed as a contraindication to ZVL.[32]

The recent availability of Shingrix® in Australia, a RZV with greater sustained efficacy than ZVL[38] should simplify vaccination against this troublesome pathogen. Waning age-related immunity following ZVL was not observed with RZV.[39] While local injection site reactions are common, presumably due to the potent adjuvant, most adverse events were of short duration, with no difference in vaccine-related autoimmune disturbances between the vaccine and placebo groups at 3 years of follow-up.[38] While the above is reassuring and use of a recombinant preparation means no risk of disseminated VZV infection post-vaccination, the use of RZV has not been studied in immunosuppressed AIIRD patients. However, studies in AIIRD patients [40] and those with inflammatory bowel disease[41] are ongoing. Currently, cost considerations for RZV in both Australia and Singapore will probably limit uptake in the foreseeable future.

4.1 | Summary

Availability of the recombinant RZV vaccine will simplify zoster vaccination in AIIRD patients as it can be given concurrently with bDMARDS and tsDMARDS. While it will eliminate the risk of disseminated post-vaccination HZ, cost may limit widespread use.

5 | CONCLUSION

The current pandemic has highlighted the importance of vaccination in preventing disease, especially in immunosuppressed AIIRD patients. There is increasing availability and range of vaccines against SARS-CoV-2, influenza and HZ. Their knowledge of immunosuppression and trust placed in them by patients means rheumatologists should take the lead role in vaccination advice for their patients.

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REVIEW

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Microbiome and osteoarthritis: New insights from animal and human studies

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Abstract

Osteoarthritis (OA) is a common cause of disability, especially among the elderly. With an ageing and increasingly obese population, OA will become more prevalent. Obesity and metabolic syndrome are risk factors for OA and have been implicated in its pathogenesis. The gut microbiome may shed light on this possible common pathogenesis. Recent animal and human studies have gained important insights into the relationship between OA, obesity, and the gut microbiome. Animal studies have demonstrated links between obesity and increased severity of OA and altered gut microbial DNA profile. Use of prebiotics and probiotics in animal trials provides proof-of-concept that interventional options to the gut microbiome can modulate the progression of OA favorably. Current evidence in human studies is limited. Shifts in gut microbial profile and reduced gut microbial diversity have been associated with people with OA, as well as blood and synovial fluid lipopolysaccharide endotoxemia. Linkages between microbiome dysbiosis and host responses may help in the understanding of OA pathogenesis and the discovery of therapeutic targets. This narrative review provides a summary of up-to-date animal and human studies on the gut microbiome and its link with OA.

KEYWORDS

gut, microbiome, microbiota, osteoarthritis

1 | INTRODUCTION

Osteoarthritis (OA) is the most common joint disorder worldwide.[1] The knee is the most common site of OA, followed by the hand and hip.[1,2] Symptomatic knee OA affects more than 10% of the normal population, and up to 30% of elderly[3] and is one of the top ten causes of disability; the burden has risen in the past two decades worldwide.[1,3] The etiology of OA is multifactorial, including systemic and local biomechanical factors.[3] Although OA is considered a prototypical degenerative disease of the joints, an inflammatory component is now well recognized.[4] The hallmark pathological changes of OA are degradation of cartilage and bone, bone formation (osteophytes), and inflammation of the synovial membrane.[5] OA is now recognized as a complex disease with inflammatory mediators released by cartilage, bone, and synovium.[6] The inflammation seen in OA is characteristically chronic and low grade, and is mediated primarily by the innate immune system,[7-9] but may be driven by macrophages.[10-12] As OA pathogenesis remains unclear, a specific disease-modifying treatment target has not yet been defined apart from pain management or replacement of affected joints for end-stage OA.

The gut microbiome comprises multiple microorganisms, including bacteria, yeast, and viruses.[13] Emerging evidence suggests that the composition and diversity of the bacterial microbiome have a major impact on health. Disruption of the composition of the gut microbiome, or dysbiosis may allow external factors or even pathogenic members of the microbiome to invade the host and this has been reported to be associated with various illnesses. Taxonomically, the bacterial microbiome is classified according to phyla, classes, orders, families, genera, and species. The dominant gut microbial phyla are Firmicutes and Bacteroidetes, representing 90% of the gut microbiome; other phyla—Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia—represent lower proportions. The Firmicutes phylum includes over 200 different genera such *as Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus*, and *Ruminococcus*, and the *Clostridium* genera represent 95% of the Firmicutes phyla. Bacteroidetes consists of predominant genera such as *Bacteroides* and *Prevotella*. The Actinobacteria phylum is proportionally less abundant and mainly represented by the genus *Bifidobacterium*.

The gut microbiome and its composition may represent a possible explanation for OA pathogenesis. The composition of the gut microbiome and its dysbiosis have been shown to be involved in many chronic diseases,[14] including obesity and metabolic syndrome, which are associated with OA. The gut microbiome may also have a direct pathological link with OA.[15,16] The gut microbiome is involved in many physiological functions, including mucosal barrier function, immune system regulation, food digestion, energy metabolism, production of bioactive agents such as short-chain fatty acids, estrogens, and serotonin.[17] The dynamic crosstalk between the host and its gut microbiome is important for creating and maintaining homeostasis. Diet, age, and disease may alter the abundance and diversity of bacterial species in the gut. Dysbiosis occurs when there is either a loss of specific health-promoting bacteria or an increase in pathogens in the microbiome, resulting in an imbalance.[18] An imbalance of the gut microbiome leads to increased translocation of microbial metabolites or microbe-associated molecular patterns across the gut endothelium into the systemic circulation.[19] Examples of microbe-associated molecular patterns include lipopolysaccharide (LPS), peptidoglycan, and bacterial DNA. These factors stimulate immune receptors in resident immune cells in bone, cartilage, and synovium, which in turn trigger inflammatory pathways.[20,21] LPS is an endotoxin that is a key molecular component of the outer membrane of Gram-negative bacteria. It has been recognized as a pro-inflammatory product of the gut microbiome that migrates from the gut into the blood, which invokes an inflammatory response.[22]

Currently, the pathogenesis pathways linking gut microbiome dysbiosis to OA manifestations are unclear. Possible linkages between microbiome dysbiosis and host responses may shed light on the understanding of OA pathogenesis and discovery of therapeutic targets. In this narrative review, we aim to summarize the current state-of-the-art evidence of gut microbiome in relation to OA, in both animal and human studies.

2 | MATERIALS AND METHODS

We conducted literature searches in PubMed, MEDLINE®, Web of Science, Scopus, and Google Scholar. The searches were conducted from inception to December 2020. We included human and animal observational studies and clinical trials focusing on gut microbiome and OA of any joints. Search terms included "gut", "microbiome", "microbiota", and "osteoarthritis". Two researchers read the titles and abstracts; relevant articles were retrieved and reviewed. Two researchers read and decided on the final inclusion of articles by consensus.

3 | ANIMAL STUDIES

For in vivo preclinical animal studies, the exact time of disease onset, control of causes, and environmental influences are tightly regulated. Animal studies allow detailed examination of outcomes in various tissues including joints, cartilage, and other organs.[23] This tightly controlled environment is very helpful to elucidate the pathogenesis of diseases.

3.1 | Role of obesity and gut microbiome in osteoarthritis

The effect of diet and obesity on the gut microbiome has been evaluated in numerous animal studies. The initial hypothesis of a possible link between the gut microbiome and obesity was made in homozygous genetically obese mice.[24,25] The homozygous obese mice had 50% reduction of Bacteroidetes and a proportional increase in Firmicutes when compared with lean wild-type mice or heterozygous mice, characterizing the obese host microbiome.[25] Colonization of germ-free mice with gut microbiome from homozygous obese mice resulted in significantly greater increase in total body fat than colonization with gut microbiome from lean mice.[26] This observation suggests that the gut microbiome plays a role in the efficiency of caloric extraction from food.[27] Exposure of animals to a high fat and high sucrose diet induces obesity and a metabolic phenotype, whereas the gut microbiome and their hormonal and anti-microbial networks were altered simultaneously.[28,29] In a study comparing genetically altered obese mice and wild-type mice given a high-fat diet (HFD), diet changes explained more of the total structural variation (57%) in gut microbiome than genetic mutation (12%), suggesting the dominant role of diet in shaping the gut microbiome.[30]

In people with obesity, an increase in the proportion of Firmicutes to Bacteroidetes ratio has been observed.[31] This in turn may stimulate the production of biologically active metabolites such as short-chain fatty acids.[26] Increased levels of short-chain fatty acids may cause an imbalance in energy regulation, leading to the onset of obesity.[31]

Obesity is a major risk factor of OA in both animals and humans.[32-34] Obesity and metabolic syndromes are closely related and

TABLE 1 Animal trials on alteration of gut microbiome in relation to osteoarthritis

Authors/ years	Animal model	Altered group	Control group
Collins et al, 2015[42]	Rats	Diet-induced obese (DIO) rats (n = 21)	Chow-fed rats (n = 11)
Guss et al, 2019[44]	Adult mice subjected to joint loading	1. TLR5KO mice (metabolic syndrome prone) fed with chow (n = 10) 2. TLR5KO mice treated with antibiotics and fed with chow (n = 10)WT mice fed with HFD (n = 10)	WT mice fed with chow (n = 10)
Ulici et al, 2018[50]	DMM mice model; DMM performed at 2 ages: (13.5 wk and 43 wk)	GF mice (n = 20)	SPF mice (n = 23)
Dunn et al, 2020[51]	Mice	OA-susceptible mice (n = 8)	OA-resistant mice (n = 8)
Mendez et al, 2020[52]	Mice subjected to joint loading	1. Antibiotic-treated mice (n = 7) 2. LPS-treated mice (n = 7) 3. Antibiotic & LPS-treated mice (n = 7)	Untreated mice (n = 7)

Outcome measures	Result
- Gut microbiome DNA profiling - Knee cartilage damage by Modified Mankin scores - Blood and synovial fluid pro-inflammatory cytokines - Blood LPS level	Compared with control group, DIO rats had: - Increased ratio of Firmicutes to Bacteroidetes - Increased cartilage damage - Increased levels of blood and synovial fluid inflammatory markers - Increased blood LPS levels The abundance of *Lactobacillus* spp. and *Methanobrevibacter* spp. was negatively associated with cartilage damage ($P < 0.001$). Cartilage damage was associated with body fat ($P < 0.001$), but not body mass.
- Gut microbiome DNA profiling - Knee cartilage damage by OARSI score and modified Mankin score - Blood pro-inflammatory cytokines - Blood LPS level	Each group has distinct gut microbiome composition: - Reduced gut microbiome diversity in antibiotic treated TLR5KO mice - Highest abundance of Bacteroidetes in WT and TLR5KO mice compared with antibiotic-treated mice - Highest abundance of Proteobacteria in antibiotic-treated mice - Highest abundance of Firmicutes in HFD obese mice Metabolic syndrome was not sufficient to increase load-induced cartilage damage: - HFD mice had increased cartilage damage, TLR5KO mice had cartilage damage comparable to WT mice LPS had a significant correlation with cartilage damage in all groups except for antibiotic-treated TLR5KO mice.
- Gut microbiome DNA profiling - Knee cartilage damage by articular cartilage structure (ACS) score and loss of proteoglycan - Osteophyte size - Synovial hyperplasia - Blood pro-inflammatory cytokines - Blood LPS and LBP levels	GF mice did not have any measurable gut microbiome. Among the SPF mice, compared with mice with less cartilage damage, mice with more knee cartilage damage had: - Increase in gut diversity within SPF mice ($P = 0.041$) and overall phylogenetic diversity ($P = 0.049$) - 28 OTUs were significantly different between mice with higher vs lower cartilage damage scores ($P < 0.05$) Compared with SPF mice, GF mice had: - 28% lesser cartilage damage ($P = 0.036$) - 31% lesser proteoglycan loss ($P = 0.009$) Among the mice with DMM performed at younger age, compared with SPF mice, GF mice had: - 36% lesser osteophyte size ($P = 0.0119$) - 34% lesser synovial thickness ($P = 0.006$) - 27% lower blood level of LBP ($P = 0.007$) Among the mice with DMM performed at younger age, cartilage damage correlated with LBP in both SPF and GF mice ($P = 0.0061$).
- Knee cartilage microbial DNA profiling	There is existence of microbial DNA in mouse cartilage. Compared with OA-resistant mice, OA-susceptible mice had: - No differences in microbial diversity - Enrichment of Gram-negative organisms ($P = 0.028$) - Increased family *Lactobacilliaceae* ($P = 0.003$), family *Turicibacteraceae* ($P = 0.01$), genus *Actinomyces* ($P = 0.01$) and members of class Verrucomicrobiae ($P = 0.03$) OA-susceptible mice cartilage contained DNA signature from class Betaproteobacteria, order Burkholderiales, family *Comamonadaceae* and order Clostriadiales, family *Tissierellaceae*. 11 clades were different between OA-resistant vs OA-susceptible mice cartilage: - class Bacteroidia, class Betaproteobacteria, order Lactobacillales, order Turicibacterales, family *Lachnospiraceae*, and genus *Lactobacillus*
- Knee cartilage damage by OARSI score and articular cartilage structure histology - Joint macrophage population and total RNA measurement	Compared with control, antibiotic-treated mice had: - Reduced OARSI cartilage score and articular cartilage structure damage - Greater level of anti-inflammatory macrophages within joint - Reduced expression of inflammatory genes *Tlr5*, *Ccl8*, *Cxcl13*, and *Foxo6* Compared with control, LPS-treated mice had: - Greater OARSI score and articular cartilage structure damage - Greater level of pro-inflammatory macrophage Compared with control, antibiotic- and LPS-treated mice had: - Similar OARSI score as controls

(Continues)

TABLE 1 (Continued)

Authors/ years	Animal model	Altered group	Control group
Luna et al, 2020[46]	Mice subjected to joint loading	1. Diet-induced severely obese mice (HFD) (n = 11) 2. TLR5KO mildly obese mice (TLR5KO) (n = 11) 3. TLR5KO mice treated with antibiotics (n = 11)	WT mice fed with chow (n = 11)
Guan et al, 2020[54]	DMM mice model	1. OA-female (n = 9) 2. Antibiotic-OA-female (n = 9) 3. OA-male (n = 9) 4. Antibiotic-OA-male (n = 9)	WT female and male mice (n = 9 respectively)

Abbreviation: ACS, articular cartilage structure; Ccl8, chemokine ligand 8; Cxcl13, chemokine ligand 13; DIO, diet induced obesity; DMM, destabilized medial meniscus; Foxo6, Forkhead box O6; GF, germ free; HFD, high fat diet; IL-6, interleukin-6; LBP, LPS binding protein; LPS, lipopolysaccharide; MMP-13, matrix metalloproteinase-13; n, sample size; OA, osteoarthritis; OARSI, osteoarthritis Research Society International; OTUs, operational Taxonomic Units; SPF, specific pathogen free; Tlr5, toll-like receptor 5; TLR5KO, toll-like receptor 5 knock-out mice; TNF-α, tumor necrosis factor-α; WT, wild type.

are associated with OA. However, it is unclear if obesity causes OA through mechanical loading on joints or through the consequences of metabolic syndrome. This link of OA to metabolic syndrome, recognized as the "metabolic phenotype" of OA, possibly accounts for nearly 60% of the OA population.[35] In this "metabolic OA", visceral fat deposits release inflammatory cytokines or adipokines,[36] resulting in low-level systemic inflammation.[37] Obesity is associated with local inflammation of synovial membrane, followed by increased pro-inflammatory M1 macrophage infiltration into the OA synovium before cartilage degradation.[38] Some adipokines produced by adipocytes are involved in OA pathogenesis.[39] Inflammation attributed to metabolic syndrome underlies the link between obesity and OA onset and progression.[40] As the dysbiosis of the gut microbiome leads to obesity, insulin resistance, and systemic inflammation, it is possible that the gut microbiome has an important role in the pathogenesis of OA.[41]

The effect of obesity or metabolic syndrome on the gut microbiome in association with OA has been evaluated in three studies (Table 1). Collins et al[42] compared the gut microbiome of diet-induced obese (DIO) rats to chow-fed rats, and found an increased ratio of Firmicutes to Bacteroidetes in DIO rats. Apart from being obese, the DIO rats had higher joint and cartilage damage, higher serum LPS, and higher levels of blood and synovial fluid pro-inflammatory cytokines compared with chow-fed rats.[42] A significant association was noted between joint damage and body fat, but not with body mass, implying a greater role of bio-metabolic effect from obesity than the bio-mechanical effect of body weight in the DIO mice on the pathogenesis of OA. The DIO rats demonstrated an increased Firmicutes to Bacteroidetes ratio, and lower abundance of *Lactobacillus* spp. in the gut microbiome. This lower abundance of *Lactobacillus* spp. was associated with severity of cartilage damage, and blood and synovial fluid pro-inflammatory cytokines in the DIO rats.[42] Lactobacilli had been reported to be protective against fat accumulation, and improved gut barrier function.[43] The higher serum level of LPS in obese rats could reflect a state of increased gut permeability due to decreased lactobacilli, providing the link between the altered gut microbiome and joint damage. In the second study, Guss et al[44] used four groups of mice to explore the diverse links between metabolic syndrome and obesity: (a) Toll-like receptor 5 knockout (TLR5KO) mice, which are prone to metabolic syndrome without weight gain or obesity due to deficiency of TLR5,[45] the receptor for bacterial flagellin, in the gut mucosa; (b) TLR5KO mice treated with broad-spectrum antibiotics; (c) wild-type mice fed with HFD and (d) chow-fed wild-type control mice. The HFD-fed mice developed obesity and had the most cartilage damage. The non-obese TLR5KO mice developed metabolic syndrome, but had cartilage scores similar to those of other non-obese mice, suggesting that metabolic syndrome alone was not sufficient to induce OA. Each of these four groups of mice had a distinct gut microbiome profile, with the HFD obese mice exhibiting the greatest abundance of Firmicutes; this group had the highest cartilage damage score. Most notable was the lowest diversity of gut microbiome in the antibiotic-treated TLR5KO group, which had the lowest cartilage damage among the four groups, suggesting the role of the microbiome in mediating the cartilage damage. In another study examining the effects of antibiotic treatment of TLR5KO mice, gut microbiome richness was found to be reduced.[46] Severely obese

Outcome measures	Result
- Gut microbiome DNA profiling - Knee cartilage damage by OARSI score - Blood LPS level	Compared with control, HFR, TLR5KO and antibiotic-treated TLR5KO mice each had distinct gut microbiome diversity. - Antibiotic treated-TLR5KO mice had reduced richness - Verrucomicrobiae, Gammaproteobacteria, Erysipelotrichi, Mollicutes, Actinobacteria, and Coriobacteria were differentially abundant across groups Compared with control, HFD and TLR5KO mice had: - Similar OARSI scores as WT mice
- Gut microbiome DNA profiling - Knee cartilage damage by OARSI score - Joint tissue immunohistochemistry staining - Blood pro-inflammatory cytokines - Blood LPS levels	Compared with control, antibiotic-OA-female mice had: - Greater abundance of Firmicutes and α-Proteobacteria - Reduced abundance of γ-proteobacteria - Reduced OARSI score of the joint - Reduced MMP-13 expression in subchondral bone - Reduced blood TNF-α, IL-6 and LPS level Compared with control, antibiotic-OA-male mice had: - Reduced abundance of Bacteroidetes - Reduced OARSI score of the joint - Reduced MMP-13 expression in subchondral bone - Reduced blood TNF-α, IL-6 and LPS level Weight gain was increased in antibiotic-OA-male mice compared with antibiotic-OA-female mice.

wild-type mice fed with HFD, TLR5KO mice, and antibiotic-treated TLR5KO mice had distinct gut microbiome diversity from one another, with notable differences at the class level (Verrucomicrobiae, Gammaproteobacteria, Erysipelotrichi, Mollicutes, Actinobacteria, and Coriobacteria). The way in which the gut microbiome influences the cartilage and bone is not well understood but it is believed to do so through interactions with the immune system at the gut lining, translocation of microbe-associated molecular patterns from the gut to circulation, and regulation of nutrients or vitamin absorption.[47]

Using animals raised in a germ-free (GF) environment is often considered the reference standard approach to study the contribution of the gut microbiome in the development and progression of diseases.[48] In the GF environment, these mice are microbiologically sterile and no living organisms can be cultured from them.[49] Whereas, specific pathogen-free (SPF) animals are those guaranteed to be free from certain strains of pathogens to avoid experimental interference. Ulici et al[50] used a murine destabilized medial meniscus (DMM) OA model with mice raised in GF and SPF environments to study the effect of the microbiome on OA development. It was shown that SPF mice with more knee cartilage damage had a higher diversity of gut microbiome compared with mice with less cartilage damage. On the other hand, GF mice had 28% less cartilage structure damage ($P = 0.037$) and 31% less proteoglycan loss ($P = 8.9 \times 10^{-3}$) compared with SPF mice. These differences were even more prominent in mice with DMM performed at a younger age. The blood LPS-binding protein (LBP) level in GF mice was 27% lower ($P = 7 \times 10^{-3}$) compared with SPF mice. Among the mice with DMM performed at a younger age, a positive correlation with blood LBP level and cartilage damage was found in both GF and SPF mice ($P = 6.1 \times 10^{-03}$). This suggests a role of the gut microbiome contributing to blood LBP levels and cartilage damage.

Cartilage is thought to be sterile. However, Dunn et al[51] proved the contrary in both mouse and human knee samples. The authors analyzed murine knee cartilage and uncovered the presence of microbial DNA patterns using 16S ribosomal RNA gene deep sequencing, whereas cartilage from GF mice did not contain any microbial DNA. The authors used an OA-susceptible and an OA-resistant mouse model but found no differences in the diversity of gut microbiome within, and between the two groups of mice at baseline. Eleven clades were able to differentiate cartilage samples from OA-susceptible vs OA-resistant mice: class Bacteroidia, class Betaproteobacteria, order Lactobacillales, order Turicibacterales, family *Lachnospiraceae*, and genus *Lactobacillus*, among others. Cartilage from OA-susceptible mice also showed an enrichment of Gram-negative organism nucleic acid, compared with that from OA-resistant mice ($P = 0.028$). The authors repeated similar experiments in humans comparing cartilages from patients with OA to cadaveric controls without OA and found similar differences as with the cartilage microbial DNA signature between OA-susceptible vs OA-resistant mice (more details given under the human study below).

Reduction of gut microbiome through antibiotic treatment has also demonstrated reduced articular cartilage structure damage in mice subjected to joint compression loading.[52] There was also reduced expression of inflammatory genes and a greater level of anti-inflammatory macrophages within the joint. The gene *Rspo1* was downregulated in antibiotic-treated mice compared with control

TABLE 2 Animal trials comparing effects of prebiotics and probiotics on gut microbiome of animals in relation to OA

Author/ year	Animal model	Intervention	Comparison	Duration of follow up
Prebiotics trials				
Schott et al, 2018[63]	Lean and obese mice subjected to DMM	Prebiotic (Oligofructose) (n = 8)	Control fiber (cellulose) (n = 8)	26 wk
Rios et al, 2019[65]	Sprague-Dawley rats	1. HFS diet (n = 12) 2. HFS diet +aerobic exercise (n = 12) 3. HFS + prebiotic (n = 12) 4. HFS + aerobic exercise +prebiotic (n = 12)	Chow-fed control rats (n = 8)	12 wk
Probiotics trials				
So et al, 2011[68]	Wistar rats, OA induced by MIA injections	1. GLN only (n = 8) 2. *L casei* only (n = 8) 3. CII +GLN (n = 8) 4. *L. casei* +CII+GLN (n = 8)	Placebo (Phosphate-buffered saline; n = 8)	10 wk

Outcome measures	Result
- Gut microbiome DNA profiling - Colonic tissue RNA sequence - Knee cartilage damage (OARSI score) - Peri-articular soft tissue cell cytometry - Histology of knee joint - Micro-CT of knee joint - Blood pro-inflammatory cytokines	Prebiotic reversed key changes induced by obesity in the gut microbiome: - Reversed the loss and increased abundance of Actinobacteria - Partially reduced Firmicutes to Bacteroidetes ratio induced by obesity - Reversed loss of *Bifidobacteria* - Reduction in pro-inflammatory bacteria (*Peptococcaceae* sp.) - Converted obese gut microbiome to lean diversity profile - Mitigated colonic tissue RNA macrophage lineage upregulation induced by obesity Compared with non-treated obese mice, prebiotic treated obese mice had: - Lesser cartilage damage (OARSI score) - Reduced macrophage levels, chemokine MCP-1 level in peri-articular soft tissue - Reduced synovial expression of cytokines MCP-1 and TNF-α - Reduced articular chondrocytes expression of cartilage degeneration products Runx-2, MMP13, Col10 - Reduced osteophytes and decreased meniscus mineralization on micro-CT - Reduced blood pro-inflammatory cytokines (KC, MIP-1B, M-CSF, TNF, MCP-1, IL-12) and increased anti-inflammatory cytokine IL-10
- Gut microbiome DNA profiling - Knee joint damage: OARSI score and Modified Mankin score - Synovial fluid cytokines, adipokines - Blood lipid profile, endotoxin, cytokines, adipokines	HFS diet (groups 1 and 2) rats had: - Reduced abundance of *Lactobacillus* and *Faecalibacterium prausnitzii* - Decreased *Bacteroides* to *Prevotella* ratio - Increased *Enterobacteriaceae* Compared with HFS diet (groups 1 and 2), prebiotic treated rats (groups 3 and 4) had: - Increased abundance of *Bifidobacterium* and *Roseburia* - Increased *Bacteroides* to *Prevotella* ratio - Reduced abundance of *Clostridium*, *Methanobrevibacter*, *Akkemansia muciniphila*, Enterobacteriaceae Compared with chow-fed controls, HFS-fed rats (group 1): - OA-like damage to knee joints - Increased (11%) body fat percentage - Increased blood and synovial leptin levels - Reduced insulin sensitivity - Increased total cholesterol, triglycerides, HDL-cholesterol - Increased blood endotoxin levels Compared with HFS group 1, treatment with prebiotic or exercise (group 2,3,4) had: - Reduced OA-like damage to knee joints - Reduced body fat percentage - Reduced blood and synovial leptin levels - Increased insulin sensitivity - Improved lipid profile - Reduced blood endotoxin levels
- Knee cartilage damage on histology analysis - Synovial tissue and chondrocyte pro-inflammatory and cartilage degradation cytokines - Blood pro-inflammatory cytokines and anti-inflammatory cytokines - Pain behavior/ paw withdrawal threshold (PWT)	Compared with all other groups, treatment with combination of probiotic + CII + GLN (group 4) had: - The least cartilage damage and cartilage thinning - Reduced synovial tissue pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, Cox-2) and MMP (MMP1, MMP3, MMP13) - Increased synovial tissue TIMP-1 - Reduced chondrocyte pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, Cox-2) and MMP (MMP1, MMP3, MMP13) - Increased chondrocyte Col2a and TIMP1 - Inhibited activation of NF-κB in chondrocytes - Reduced blood pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IL-12, IL-17A, TNF-α, IFN-γ) - Increased blood anti-inflammatory cytokines (IL-4 and IL-10) - Greatest pain reduction measured by PWT

(Continues)

TABLE 2 (Continued)

Author/ year	Animal model	Intervention	Comparison	Duration of follow up
Lee et al, 2018[69]	Wistar rats, OA induced by MIA injections	*L. acidophilus* (n = 3)	Placebo (n = 3)	15 d
Kwon et al, 2018[70]	Wistar rats, OA induced by MIA injections	1. Probiotic complex (*Lactobacillus* sp., *Bifidobacterium* sp., *Streptococcus thermophilus*) + rosavin (100 mg/rat) + zinc (20 mg/rat) (n = 3) 2. Celecoxib (n = 3)	Placebo (n = 3)	8 wk
Henrotin et al, 2019[71]	16-wk-old Dunkin Hartley guinea pigs (spontaneous OA model)	1. *B. longum* only (n = 12) 2. *B. longum* + vitamin C (n = 12)	Placebo (sterile water; n = 12)	12 wk
Sim et al, 2018[72]	Sprague-Dawley rats, OA induced by MIA injections	1. Low dose *C. butyricum* at 10^8 CFU/d (n = 10) 2. High dose *C. butyricum* at 10^{10} CFU/d (n = 10) 3. Indomethacin treatment given at 2mg/kg/d (n = 10)	Distilled water (n = 10)	6 wk

Abbreviations: *B. longum:* lyophilized inactivated culture of *Bifidobacterium longum*; CFU, colony-forming units; *C. butyricum*, *Clostridium butyricum*; CII, type II collagen; Col2a, type II collagen; Coll2-1, type II collagen peptide; Col10, collagen X; COMP, cartilage oligomeric matrix protein; Cox-2, cyclooxygenase-2; CT, computed tomography; CTX-II, cross linked C-telopeptide of type II collagen; GLN, glucosamine; DMM, destabilized medial meniscus; HDL-cholesterol, high-density lipoprotein cholesterol; HFS, high-fat/high-sucrose diet (20% fat, 50% sucrose); GAGs, glycosaminoglycans; KC, keratinocyte chemotactic (mouse homolog of IL-8); IL, interleukin; IFN-γ, interferon-γ; *L. casei*, *Lactobacillus casei*; LTB4, leukotriene B4; M-CSF, macrophage colony-stimulating factor; MCP-1, monocyte chemotactic protein-1; MIA, monosodium iodoacetate; MIP-1B, macrophage inflammatory protein-1B; MMP, matrix metalloproteinase; n, sample size; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International; PWT, paw withdrawal threshold; PIIANP, type IIA procollagen amino terminal propeptide; *Runx2*, Runt-related transcription factor 2; TIMP, tissue inhibitor of metalloproteinase; TNF-α, tumor necrosis factor-α.

mice. Rspo1, a modulator of the Wnt signaling pathway, was previously shown to have a role during OA progression.[53] Other inflammatory genes were downregulated in the compression-loaded joint of antibiotic-treated mice. These molecular changes could mediate cartilage structure damage or remodeling following joint injury. Reduced joint damage and reduced blood levels of pro-inflammatory cytokines were also demonstrated in a DMM OA mouse model.[54] The gut microbiome profiles were different between antibiotic-treated female mice with OA vs antibiotic-treated male mice with OA.[54] Antibiotic-treated female mice with OA had greater abundance of Firmicutes compared with control mice, whereas antibiotic-treated male mice with OA had similar levels of Firmicutes

Outcome measures	Result
- Knee cartilage damage (OARSI score) - Micro-CT of knee joints - Synovial tissue and chondrocyte pro-inflammatory and cartilage degradation cytokines - Blood CTX-II level - Pain behavior/ PWT	Compared with placebo, probiotic-treated rats had: - Lesser cartilage damage (OARSI score) - Reduced OA damage on micro-CT images - Reduced synovial tissue pro-inflammatory cytokines (TNF-α, IL-6), and cartilage degradation product (MMP3) levels - Increased synovial tissue anti-inflammatory cytokines (IL-10, TIMP3) levels - Increased chondrocyte anti-inflammatory cytokines (IL-10, TIMP1, TIMP3) levels - Reduced blood CTX-II level (biomarker of OA severity) - Greater pain reduction measured by PWT - Suppressed ganglion pain response
- Knee cartilage damage (Mankin score) - Micro-CT of knee joints - Synovial tissue pro-inflammatory and cartilage degradation cytokines - Pain behavior/ PWT	Compared with placebo, probiotic complex treated rats had: - Less cartilage damage by histology - Reduced subchondral bone damage on micro-CT - Reduced synovial tissue pro-inflammatory cytokines (TNF-α, IL-6), and cartilage degradation products (MMP3) - Increased synovial tissue anti-inflammatory cytokines (IL-10, TIMP1, TIMP3) levels - Greater pain reduction
- Knee cartilage damage (OARSI score) - Synovium inflammation - Blood cartilage degradation markers (Coll2-1 and Fib3-2) - Blood collagen synthesis biomarkers (PIIANP) - Blood bone formation biomarker (osteocalcin)	Compared with placebo, treatment groups had: - No significant difference in OARSI global score - *B. longum* + vitamin C group had significant lower cartilage structure subscore ($P < 0.0001$) - Greater increase in blood PIIANP ($P = 0.0004$) - *B. longum* group had greater reduction in Coll2-1 ($P = 0.0004$) - *B. longum* + vitamin C group had greater increase in blood PIIANP ($P = 0.0003$) - Both *B. longum* treatment groups had greater reduction in blood Coll2-1 to PIIANP ratio ($P = 0.0086$) No significant difference in synovium inflammation, Fib3-2 and osteocalcin in either treatment groups.
- Knee cartilage damage (OARSI score) - Micro-CT of knee joints - Cartilage pro-inflammatory and cartilage degradation products - Blood pro-inflammatory markers, bone metabolism markers - Paw weight-bearing distribution	Compared with control, *C. butyricum* treatment (group 1 and 2) had: - Less cartilage damage (lower OARSI score) ($P < 0.05$) - Preserved femur bone architecture and cartilage volume on micro-CT ($P < 0.05$) - Lower cartilage degradation products (MMP-2, MMP-3, MMP-9, MMP-13, TIMP-1, TIMP3) in cartilage ($P < 0.05$) - Lower blood pro-inflammatory cytokines (IL-6, Cox-2, LTB4) ($P < 0.05$) - Higher blood bone metabolism markers (IFN-γ, GAGs) and lower COMP ($P < 0.05$) - Increased (20%) weight-bearing distribution in hind paw Compared with control, indomethacin-treated group 3 also showed similar effects on: - Reduced cartilage damage - Preserved femur bone architecture and cartilage volume - Reduced cartilage pro-inflammatory cytokines - Reduced blood pro-inflammatory cytokines

to control mice. Antibiotic-treated male mice with OA had reduced abundance of Bacteroidetes compared with control mice, but not so for antibiotic-treated female mice with OA. Weight gain was increased in antibiotic-treated male mice with OA compared with antibiotic-treated female mice with OA. These observations suggest that antibiotic treatment, gut microbiome alterations, and weight gain could be influenced by sexual dimorphism. Acute loss of estrogen increases levels of reactive oxygen species and activates nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and pro-inflammatory cytokine production.[55] The complex relationship between gut microbiome profile differences in males and females warrant further studies.

TABLE 3 Human studies studying the relationship between the microbiome and OA

Study	Study Design	OA site	OA definition	Sample size	Control
Huang et al, 2016[20]	CS	Knee	Radiographic knee OA	25	NA
Zhao et al, 2018[79]	CS	Knee	ACR criteria for OA	OA (n = 58)	RA (n = 125)
Lee et al, 2019[80]	CS	Knee	ACR criteria for OA	OA (n = 9)	RA (n = 9)
Dunn et al, 2020 [51]	CC	Knee, Hip	Joint replacement candidates	1. Eroded knee specimens (n = 21) 2. Intact knee specimens (n = 21) 3. Eroded hip specimens (n = 34 4. Intact hip specimens (n = 33)	Cadaveric control cartilage (n = 10)

Outcome measures	Main results
- Synovial fluid LPS, LBP levels - Synovial CD14 levels - Blood LPS, LBP levels - Blood CD14 levels - NHANES pain score - Activated macrophage by radioisotope ^{99m}Tc-EC20 (Etarfolatide) SPECT/CT - Knee radiographic severity - -WOMAC total pain score	Synovial fluid LPS and LBP levels were associated with: - Synovial fluid CD14 levels ($P = 0.004$; $P = 0.036$) - Activated macrophages in knee synovium ($P < 0.05$ for both) Synovial fluid LPS level was associated with: - Radiographic severity ($P = 0.001$) - Total WOMAC score ($P = 0.008$) Synovial fluid LBP level was associated with: - NHANES pain score ($P = 0.039$) Blood LPS and LBP levels were associated with: - Blood CD14 levels ($P = 0.006$; $P < 0.001$) - Activated macrophages in synovium and joint capsule ($P < 0.05$ for both) - Radiographic knee OA severity ($P = 0.03$, $P = 0.017$) Blood LPS level was associated with: - WOMAC pain score ($P = 0.076$)
- Synovial tissue and synovial fluid microbial DNA signature	Compared with RA, OA synovial tissue had: - Greater abundance of *Bacteroides uniformis*, *Bacteroides fragilis*, *Porphyromonas*, *Streptococcus*, *Atopobium*, *Phascolarctobacterium*, *Rhodotorula mucilaginosa*, *Rothia*, *Megasphera*, *Turicibacter*, *Leptotrichia*, and *Haemophilus parainfluenza* in synovial tissue Compared with RA, OA synovial fluid had: - Greater abundance of *Bacteroides caccae*
- Gut microbiome DNA profiling - Blood inflammatory markers: ESR and hsCRP	Compared with RA, OA patients had: - Greater increase in Bacteroidetes and decrease in Firmicutes abundances at phylum level - Greater abundance of Bacteroidia at class level - Greater abundance of Bacteroidales at order level - Greater abundance of *Rikenellaceae*, *Peptostreptococcaceae* and *Bifidobacteriaceae* at family level - Greater abundance of *Alistipes*, *Anaerotruncus*, *Bacteroides*, and *Bifidobacterium* at genus level
Cartilage microbial DNA signature	Compared with control, OA specimens had: - reduced diversity of microbial DNA ($P < 0.0001$) - Increase in Gram-negative constituents ($P = 0.02$) - Increase in abundance of phylum Proteobacteria, class Betaproteobacteria Compared with control, knee OA had: - Increase in abundance of phylum Firmicutes, class Clostridia, order Clostridiales, genus *Lactobacillus* Compared with control, hip OA had: - Increase in abundance of phylum Actinobacteria, class Betaproteobacteria, order Actinomycetales Knee and hip OA have distinct microbial DNA signatures: - Hip OA had reduced diversity compared with knee OA ($P < 0.0001$) - Knee OA is characterized by phylum Actinobacteria, including family *Micrococcaceae*, and phylum Firmicutes, including genus *Exiguobacterium* - Hip OA is characterized by Proteobacteria phylum, including family *Rhodocyclaceae* There were no significant differences between non-eroded and eroded knee OA cartilage. Eroded hip OA cartilage had increased family *Pseudomonadaceae* and genus *Pseudomonas*, whereas non-eroded hip OA cartilage had increased order Vibronales. In functional analysis, OA was associated with: - Increase in lipopolysaccharide production ($P = 9.9 \times 10^{-3}$) - Increase in phosphatidylinositol signaling ($P = 4.2 \times 10^{-4}$) - Increase in nitrogen metabolism ($P = 8 \times 10^{-3}$) - Decrease in sphingolipid metabolism ($P = 7.7 \times 10^{-4}$)

(Continues)

TABLE 3 (Continued)

Study	Study Design	OA site	OA definition	Sample size	Control
Boer et al, 2019[82]	CS nested in a population-based cohort (Rotterdam study) Validation cohort: independent Dutch cohort, LifeLines-DEEP (LLD)	Knee	Radiographic knee OA (n = 124); WOMAC pain >0 (n = 285)	1. Rotterdam study (n = 1427) 2. LLD cohort (n = 867)	NA
Xie et al, 2020[81]	CS	Knee	Radiographic knee OA	56	Healthy control (n = 53)

Abbreviations: ACR, American College of Rheumatology; BMI, body mass index; CC, case-control; CD, cluster of differentiation; CS, cross sectional; CSA, chondroitin sulfate-A; CT, computed tomography; DNA, deoxyribonucleic acid; ESR, erythrocyte sedimentation rate; hsCRP, high-sensitivity C-reactive protein; LBP, LPS binding protein; LPS, lipopolysaccharide; MRI, magnetic resonance imaging; n, sample size; NHANES-pain, pain score from the First National Health and Nutrition Examination Survey; NSAID, non-steroidal anti-inflammatory drug; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International; PPI, proton pump inhibitors; RA, rheumatoid arthritis; SF, synovial fluid; SPECT/CT, single photon emission computed tomography combined with high-resolution computed tomography; WOMAC, Western Ontario and McMaster Osteoarthritis Index.

3.2 | Role of prebiotics and probiotics on the gut microbiome in osteoarthritis

Prebiotics are defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating growth and/or activity of a limited number of bacteria in the colon, and thus improves host health".[56] Prebiotics that currently fulfil these three criteria are fructo-oligosaccharides, galacto-oligosaccharides, and lactulose.[57] Probiotics are live microorganisms that beneficially affect the host animal by improving the balance in its gut when administered in adequate amounts.[58]

Prebiotics and probiotics are interventional options to the gut microbiome, and were shown to exert immune modulatory effects by reducing systemic inflammation,[58,59] cytokine gene expression,[60] blood LPS level, and LPS-dependent interleukin-1β expression.[61] These mechanisms may be working in a concerted effort to restore gut barrier functionality to reduce endotoxemia in blood,[16,62] leading to reduced systemic inflammation.

3.2.1 | Role of prebiotics in osteoarthritis

A high-fat diet has been consistently shown to shift the gut microbiome towards an increase in the Firmicutes-to-Bacteroidetes ratio.[42,63] Schott et al[63] showed that treating mice with prebiotics reversed the effects of HFD and obesity on the gut microbiome by correcting the Firmicutes-to-Bacteroidetes ratio and restoring abundance of Actinobacteria. In the prebiotic-treated mice, a reduction in pro-inflammatory bacteria (*Peptococcaceae* species), reversal of the obese gut microbiome profile to a lean diversity profile, and a reduction of obesity-induced upregulated macrophage expression in colonic tissue RNA were observed. Prebiotic treatment also reduced levels of infiltrating macrophages and cytokines in synovial tissues; reduced expression of the cartilage degeneration factors Runt-related transcription factor 2 (Runx2), matrix metalloproteinase 13 (MMP13), collagen X (Col10) in articular chondrocytes; and reduced cartilage damage histologically (Table 2). Prebiotic treatment had been shown to reduce blood pro-inflammatory cytokines in obese mice,[64] and the gut microbiome changes observed

Outcome measures	Main results
- Gut microbiome DNA profiling - Knee OA severity on radiography - Knee effusion on MRI (n = 373) - WOMAC pain score	Rotterdam study: - Inter-patient diversity of gut microbiome was associated with WOMAC pain score, but not significant after adjustment of BMI - Intra-patient diversity of gut microbiome was not associated with WOMAC pain score - Both inter- and intra-patient diversity of gut microbiome was not associated with radiographic severity - Greater *Streptococcus* spp. abundance was significantly associated with MRI knee effusion ($P = 0.013$) - *Streptococcus spp.* abundance was strongly associated with WOMAC pain score ($P = 1.2 \times 10^{-5}$), independent of smoking, alcohol consumption, BMI, NSAID, and PPI use Validation (LLD) cohort: - Positive association of *Streptococcus* with WOMAC pain score at four taxonomic levels: o Class Bacilli ($P = 3.6 \times 10^{-5}$) o Order Lactobacillales ($P = 2.4 \times 10^{-4}$) o Family *Streptococcaceae* ($P = 2.3 \times 10^{-2}$) o Genus *Streptococcus* ($P = 3.7 \times 10^{-2}$)
Gut microbiome DNA profiling	Compared with control, OA patients had: - Greater abundance of Actinobacteria, *Bifidobacteriaceae*, *Bifidobacterium*, *Alistipes* - Reduced abundance of *Prevotellaceae*, *Faecalibacterium* - In functional analysis of the gut microbiome o Reduced number of genes related to lipid metabolism, glycan biosynthesis, metabolism involved in gut microbiome, lipopolysaccharide biosynthesis, and adipocytokine signaling pathway o Greater number of genes in apoptosis signaling pathways - Abundance of *Prevotellaceae* was positively correlated with BMI - Abundance of Actinobacteria, *Bifidobacterium* was negatively correlated with BMI

in the Schott et al study perhaps provides a link between prebiotic and reduced blood pro-inflammatory cytokines.[63]

Rios et al[65] showed that prebiotic supplementation, aerobic exercise, and a combination of prebiotic and aerobic exercise prevented OA-like damage in obese rats by normalizing the gut microbiome dysbiosis caused by obesity. Prebiotic or aerobic exercise, or a combination of the two, was associated with normalization of insulin resistance, blood and synovial leptin levels, dyslipidaemia, and endotoxemia. Gut microbiome dysbiosis caused by obesity was also reversed in the all three groups compared with obese rats. *Bacteroides*, *Bifidobacterium*, and *Roseburia* have negative associations with knee joint damage, whereas *Clostridium leptum*, *Akkemansia muciniphila*, and *Faecalibacterium prausnitzii* have positive associations with knee joint damage and increased blood endotoxin levels.

3.2.2 | Role of probiotics in osteoarthritis

Probiotics help in the maintenance of gut microbiome homeostasis via maintenance of the gut epithelial barrier, decrease in oxidative stress, and modulatory effects on the production of pro- and anti-inflammatory cytokines.[66] The most commonly used probiotics are lactic-acid-producing bacteria, such as *Lactobacillus* and *Bifidobacterium*. The genera *Bifidobacterium* and *Lactobacillus* have been described to have positive effects on host health.[41] *Lactobacillus casei* was shown to inhibit experimental arthritis by restoring gut microbiome dysbiosis in adjuvant-induced-arthritis rats compared to normal rats.[67] Table 2 summarized the results of animal studies of probiotics in OA. Overall, the five trials[68-72] have shown that probiotics: *L. casei*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Clostridium butyricum* had protective effects against the development or progression of OA.

In rat OA models induced by monosodium iodoacetate, treatment regimens using *L. casei* or *L. acidophilus* reduced pain, cartilage destruction, and pro-inflammatory cytokine infiltration into synovium or synovial fluid, respectively (Table 2,[68,69]). Combination of *L. casei* with type II collagen and glucosamine reduced pain, cartilage destruction, and pro-inflammatory cytokine infiltration into cartilage and synovium compared with treatment regimens with *L. casei* or glucosamine alone, or collagen and glucosamine in combination.[68] Compared with other

treatment regimens, the triple-cocktail combination treatment had reduced synovial pro-inflammatory cytokines and MMP levels whereas the tissue inhibitor of metalloproteinase 1 level was increased in synovial fibroblasts. Reduction of pro-inflammatory cytokines and transcription factor NF-κB activation, together with increased collagen expression, were observed in chondrocytes. The combination treatment also reduced blood pro-inflammatory cytokines while upregulating levels of anti-inflammatory cytokines in blood.[68] Similarly, treatment using *L. acidophilus* reduced pro-inflammatory cytokines in synovial fluid, cross linked C-telopeptide of collagen in blood, and cartilage MMP3 expression; and increased anti-inflammatory cytokines and tissue inhibitor of metalloproteinase 3 (TIMP3) in the synovium and chondrocytes compared with placebo-treated rats.[69]

Kwon et al[70] evaluated the cartilage protective effects of a mixture of probiotic complex (*L. acidophilus*, *L. casei*, *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactobacillus salivarius*, *B. longum*, *Bifidobacterium bifidum*, and *Bifidobacterium breve*) plus rosavin and zinc in a rat model of monosodium iodoacetate-induced OA. Rosavin is a cinnamyl alcohol glycoside from the plant *Rhodiola rosea*, which possesses antioxidant and anti-inflammatory effects.[73] On the other hand, zinc exerts anti-oxidant effects by downregulating pro-inflammatory cytokine expression.[74] The authors hypothesized that the combination of probiotic complex, rosavin, and zinc can suppress the inflammatory response in OA, and compared this combination with treatment with celecoxib and placebo. The combination regimen improved pain response in rats with OA in a similar way to those treated with celecoxib. In addition, rats treated with the combination regimen had reduced cartilage damage, and reduced expression of pro-inflammatory cytokines and catabolic cytokines in the joint tissue compared with those treated with either celecoxib or placebo.

In a spontaneous model of OA in guinea pigs, treatment with *B. longum* and co-administration of *B. longum* with vitamin C demonstrated prevention of development of OA.[71] Co-administration of *B. longum* with vitamin C significantly reduced the cartilage structure sub-score of the Osteoarthritis Research Society International (OARSI; but not the OARSI global score) compared with placebo. Comparing with placebo, the *B. longum* treatment group had greater reduction in Coll2-1 in blood (type 2 collagen peptide; a cartilage degradation marker), whereas the *B. longum* plus vitamin C combination group had a greater increase in PIIANP (type IIA procollagen amino terminal propeptide, a biomarker of collagen synthesis) in blood. The ratio of Coll2-1 to PIIANP was significantly decreased in both *B. longum* treatment groups ($P < 0.001$). There was a significant correlation of global cartilage damage (OARSI score) with variation of blood Coll2-1 to PIIANP ratio ($r = 0.2794$, $P = 0.0494$). This study suggested that the probiotic *B. longum* decreased cartilage degradation, promoted cartilage matrix synthesis, and prevented progression of cartilage structure damage in spontaneous OA.

Apart from lactic-acid-producing probiotics, *C. butyricum* is a butyric-acid-producing probiotic. It germinates and grows in the gut[75] and produces large amounts of short-chain fatty acids, such as butyrate and acetate.[76] Short-chain fatty acids have immunomodulatory effects, and suppress pro-inflammatory cytokine secretion in cultured epithelial cells.[77] In a rat model of monosodium iodoacetate-induced OA, Sim et al[72] demonstrated reduced cartilage damage (lower OARSI scores), preserved femur bone architecture and cartilage volume, reduced cartilage degradation products (MMP-2, MMP-3, MMP-9, MMP-13), reduced blood pro-inflammatory cytokines, increased bone metabolism markers and increased weight-bearing ability of rats treated with *C. butyricum* compared with rats treated with distilled water (all $P < 0.05$). *C.butyricum* was shown to preserve joint structure to similar extent compared with rats treated with indomethacin. This suggests that *C. butyricum* treatment may have anti-inflammatory effects, reduce cartilage destruction, and play an important role in tissue remodeling of OA in the rat model. This study also supports recent in vitro evidence that butyrate reduced expression of pro-inflammatory cytokines, reduced metalloproteinase production, and inhibited inflammatory signaling pathways, including that of NF-κB, in interleukin-1-stimulated chondrocytes.[78]

4 | HUMAN STUDIES

Human studies evaluating the relationship between the gut microbiome and OA are sparse, with five cross-sectional studies, one case-control study, and two controlled trials (Tables 3 and 4). A cross-sectional study in patients with knee OA demonstrated that there are measurable quantities of LPS in blood and synovial fluid. Synovial fluid and blood endotoxins (LPS and LBP) were associated with the presence of activated macrophages in the knee synovium, and knee joint capsule and synovium, respectively ($P < 0.05$ for all). Synovial fluid and blood LPS and LBP were associated with synovial fluid CD14 levels ($P < 0.05$ for all), a soluble biomarker of activated macrophages. Furthermore, synovial fluid and blood endotoxin levels were associated with clinical outcomes, including radiographic severity of OA and joint symptoms by the total Western Ontario and McMaster Osteoarthritis Index (WOMAC) score. These results suggest a role for endotoxins from bacteria in the pathogenesis of OA, which may mediate via activated macrophages in the knee joint. The authors proposed a two-hit model of OA pathogenesis where (a) endotoxin primes joint tissue macrophages via TLR4;[8] and (b) disease-associated molecular damage patterns produced by the primed TLR4 on macrophages mediate further joint damage through inflammasome activation and result in cartilage degradation.[16]

Zhao et al[79] hypothesized that bacterial antigens may break through the first immune resistance of the gut mucosa to mediate the pathogenesis of arthritis. Challenging the concept that the articular cavity is sterile, the authors detected bacterial nucleic acids in aseptically collected synovial tissue and synovial fluid from both patients with OA and those with rheumatoid arthritis (RA) using 16S rRNA sequencing. The authors postulated that bacteria from the gut may enter joint cavities through certain pathways and so play a role in the occurrence and development of arthritis. The mechanisms and pathways of bacterial entry and how they cause arthritis remain to

TABLE 4 Human trials comparing effects of probiotics and related treatment trials in OA

Reference	Population	Study design	Intervention	Comparison	Duration of follow up	Outcome	Result
Lei et al, 2017[87]	ACR clinical criteria for knee OA (n = 461)	Double-blind RCT	*Lactobacillus casei shirota*-skimmed milk (LcS) (n = 230)	Placebo (plain skimmed milk) (n = 231)	24 wk	- OA symptoms (Total WOMAC score and VAS scores: knee pain severity) - Blood hsCRP levels	Compared with placebo, LcS-treated group had: - Significant decrease in WOMAC total, stiffness, pain, and function ($P < 0.001$, $P = 0.040$, $P = 0.008$, $P < 0.001$ respectively) - Decreased VAS score ($P < 0.01$) - Decreased blood hsCRP levels ($P < 0.05$) - Strong correlations between hsCRP levels and WOMAC, VAS scores ($P = 0.021$, $P = 0.037$ respectively)
Coulson et al, 2013[89]	ACR clinical criteria for knee OA (n = 38)	Open-label RCT	Green-lipped mussel (GLM) extract (n = 21)	Glucosamine sulfate (GS) extract (n = 17)	12 wk	- Stool aerobic and anaerobic culture viable count at baseline and 12 wk - OA symptoms (WOMAC score and Lequesne algofunctional index) - GI symptoms - Quality of Life score - Analgesia use	There was no significant difference in total stool bacterial colony counts between GLM- or GS-treated groups at baseline. Compared with baseline, both GLM- and GS-treated groups at 12 wk showed: - Increase in *Lactobacillus*, *Streptococcus*, *Eubacterium* species - Decrease in *Clostridium* and *Staphylococcus* species Compared with baseline, GLM-treated group at 12 wk showed: - Increased *Lactobacillus*, *Bifidobacterium*, *Eubacterium*, *Bacteroides*, *Streptococcus* species - Decreased *Enterococcus*, *Staphylococcus*, *Clostridium* species, yeasts - Improved OA symptoms: o Decreased WOMAC score ($P = 0.001$) o Decreased Lequesne score ($P < 0.001$) - Improved GI symptoms ($P = 0.02$) - Improved quality of life score by SF-12 ($P = 0.004$) - Decreased use of analgesia Compared with baseline, GS-treated group at 12 wk showed: - Increased coliforms, *Streptococcus*, *Eubacterium*, *Lactobacillus* species - Decreased *Staphylococcus*, *Bacteroides*, *Clostridium* species - Improved OA symptoms: o Decreased WOMAC score ($P = 0.001$) o Decreased Lequesne score ($P < 0.001$) - Improved GI symptoms ($P = 0.044$) - Improved quality of life by SF-12 ($P = 0.001$) - No change in mean analgesia use

Abbreviations: ACR, American College of Rheumatology; CTX-II, collagen type II C-telopeptide; GI, gastrointestinal; GLM, green-lipped mussel; GS, glucosamine sulfate; hsCRP, high-sensitivity C-reactive protein; K/L, Kellgren and Lawrence classification; n, sample size; RCT, randomized controlled trial; SF-12,12-item Short Form Survey; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Osteoarthritis Index.

be elucidated. The abundance of bacterial nucleic acid was found to be different between OA and RA patients both in synovial tissue and fluid. Species of *Atopobium*, *Bacteroides uniformis*, *Haemophilus parainfluenzae*, *Bacteroides fragilis*, and *Streptococcus* were concentrated in OA synovial tissue, whereas *Agrobacterium*, *Comamonas*, *Kocuria*, *Meiothermus*, and *Rhodoplanes* were concentrated in RA synovial tissue (Table 3). Using function-module analysis, the correlation between pathways related to metabolism and transcription factors with microbiome function in the OA vs RA articular cavities were different, once again suggesting a difference in the pathogenesis of the two conditions.

The differences in gut microbiome of patients with OA and RA were also shown in another study.[80] The gut microbiome of OA patients showed increased Bacteroidetes and decreased Firmicutes at the phylum level, in contrast to the study by Zhao et al, where species in both Bacteroidetes and Firmicutes were represented.[79] Individuals with OA had a higher abundance of Bacteroidia at the class level; Bacteroidales at the order level (Table 3). When the gut microbiome of OA patients was compared with that of healthy controls, greater abundance of *Bifidobactericeae*, *Bifidobacterium*, and *Alistipes* was seen.[81] These findings are similar to the gut microbiome profile of OA patients described by Lee et al.[80]

In a case-control study comparing the microbiome of cartilage from 21 patients with knee OA and 34 patients with hip OA with 10 cadaveric controls without OA, Dunn et al[51] demonstrated that the phylum Proteobacteria and class Betaproteobacteria were enriched in cartilage specimens from OA patients, whereas control cartilage specimens had enrichment of class Alphaproteobacteria and class Clostridia. Compared with control cartilage, knee OA and hip OA cartilage had reduced microbiome diversity ($P < 1.0 \times 10^{-4}$) and different microbial DNA signatures. Comparing knee OA cartilage with hip OA cartilage, there were differences in microbial composition at phylum, class, and order level; and hip OA cartilage had reduced microbiome diversity ($P < 1.0 \times 10^{-4}$). No significant differences were seen between eroded and non-eroded knee OA cartilage, but differences were observed in eroded compared with non-eroded hip OA cartilage at the order, family, and genus levels. Knee OA cartilage was characterized by Firmicutes, for which an increase in the Firmicutes-to-Bacteroidetes ratio had been observed previously in obese rats and is associated with greater cartilage damage and increased blood and synovial fluid inflammatory markers.[42] Bioinformatics software prediction identified a reduction in sphingolipid metabolism in OA cartilage ($P = 7.7 \times 10^{-4}$), increase in phosphatidylinositol signaling ($P = 4.2 \times 10^{-4}$), increase in nitrogen metabolism ($P = 8 \times 10^{-3}$), and increase in LPS synthesis ($P = 9.9 \times 10^{-3}$) in OA cartilage.[51] The increase in LPS synthesis in OA cartilage seems to mirror the findings of an association of synovial fluid LPS with increased osteophyte severity and pain score in the study by Huang et al.[20]

In a cross-sectional study of 1427 participants, a random sample from a large population-based cohort in Rotterdam, the diversity of gut microbiome, mainly driven by the abundance of *Streptococcus* spp., was associated with WOMAC pain score.[82] The associations between abundance of *Streptococcus* spp. and WOMAC pain score remained significant after adjustment for smoking, alcohol consumption, body mass index, and use of non-steroidal anti-inflammatory drugs. There was no association between gut microbiome and severity of OA on radiography. However, in a random subset of 373 women with magnetic resonance imaging of knees, the abundance of *Streptococcus* spp. was significantly associated with effusion ($P = 0.013$). Validation using data from an independent population-based cohort (n = 867) showed significant associations of WOMAC pain score with *Streptococcus* at four taxonomy levels ($P < 0.05$). *Streptococcus* spp. are commensal organisms in the oral cavity and gut,[83,84] and are known to produce membrane vesicles that may pass through the gut-blood barrier and enter into the bloodstream.[85] These membrane vesicles may contain immunogenic *Streptococcus* spp. epitopes[86] and trigger macrophage activation through TLR pathways typically seen in OA-related inflammation.[86] It was proposed that bacterial epitopes that have passed through the gut-blood barrier may either activate the synovial macrophages to induce inflammation; or induce a low-grade systemic inflammatory state, which invokes joint inflammation and damage.[82]

4.1 | Role of probiotics and related treatment trials in osteoarthritis

In a 24-week double-blind, placebo-controlled randomized controlled trial, Lei et al[87] showed a significant reduction in WOMAC pain score, stiffness, and function by the probiotic *Lactobacillus casei shirota* (LcS) compared with placebo in patients with knee OA. Blood levels of high sensitivity C-reactive protein (hs-CRP) were also lower in the LcS-treated group ($P < 0.05$). Strong correlations were observed between hs-CRP levels with WOMAC pain score and pain visual analog scale (Table 4), thereby suggesting that improved knee OA symptoms could be mediated through reduction in blood hs-CRP levels in the LcS-treated group. However, a gut microbiome DNA profile was not measured in this study.

Besides prebiotic and probiotic treatments, complementary or alternative medicines are interesting options in therapeutic trials for OA. These alternative medicines, or so-called nutraceuticals, include herbal or non-herbal dietary supplements and are speculated to have positive effects in reducing the development of OA. However, current clinical trials were primitive and limited by non-standardized formulations, small sample sizes, and inconsistent results.[88] In an open-label trial, Coulson et al[89] investigated the effects of green lipped mussel extract vs glucosamine sulfate extract in 40 patients with knee OA for 12 weeks. Both supplements are metabolized by the gut microbiome, potentially modifying and influencing its profile. Aerobic and anaerobic cultures of stool were performed at baseline and end of study. During the study period, there were no significant changes to body mass index, weight and hip-to-waist ratios of the patients. Both green lipped mussel extract-treated and glucosamine sulfate-treated groups had increase in *Lactobacillus*, *Streptococcus*, and *Eubacterium* species and

decrease in abundance of *Clostridium* and *Staphylococcus* species at the end of 12 weeks of treatment (Table 4). At the end of the study, patients from both treatment groups had significantly reduced knee OA symptoms, reduced gastrointestinal symptoms, and improved quality of life, but there were no differences between groups. Nutraceutical supplements may alter the gut microbiome, resulting in small numbers of bacterial count shifts that contribute to mediating knee OA symptom relief in this study. However, this trial was limited by lacking a placebo control arm. Therefore, the link between change in gut microbiome and symptom relief requires more studies.

5 | FUTURE PERSPECTIVES

Current studies focus on investigating the influence of the gut microbiome genome on OA. A deeper understanding of the complexity of the interaction between host and gut microbiome genomes will help to determine the factors that lead to disease pathogenesis. Metatranscriptomic analysis and metabolomic approaches may provide greater insight.[19] Metabolomics provides an approach to measure products of metabolism, leading to identification of diagnostic and prognostic biomarkers and possibly also underlying pathophysiological processes associated with OA.[90] Further studies are required to deepen the understanding of interactions between the host and gut microbiome either locally in the intestine or peripherally in the joint tissue.[40] Pre- and probiotics, oral nutritional supplements, and dietary habits may modulate the symptoms of OA and could be potential therapeutic approaches.

6 | SUMMARY

Published literature pertaining to the gut microbiome and OA were reviewed in this article. Obesity and metabolic syndrome are common risk factors for OA and are associated with the loss of diversity of the gut microbiome. Animal studies showed that the associated shift in the composition of the gut microbiome has the potential to reduce systemic inflammation and preserve cartilage damage in various OA models. Animal trials revealed that supplementation with prebiotics or probiotics has immunomodulatory effects, thereby reducing systemic inflammation, cartilage damage, and OA changes.

Human studies available on this topic were limited. Observational studies suggested that blood LPS and LBP levels were associated with macrophage activation in the knee synovium and OA severity. Loss of gut microbiome diversity and abundance of *Streptococcus* were associated with pain symptoms. These suggest that blood toxemia or differences in gut microbiome may play a role in the pathogenesis of OA. Small clinical trials evaluating the supplementation of LcS, green lipped mussel extract and glucosamine sulfate extract showed modest efficacies in relieving OA symptoms. However, these trials were limited in the lack of standardized formulation, had small sample sizes, inconsistent outcomes and the underlying mechanisms mediating the outcomes remain unknown. Larger and well-planned randomized controlled trials are required to better determine the efficacy of such supplementation.

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CONFLICT OF INTERESTS

The authors do not have any conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article and revising the intellectual content, and all authors approved the final version to be published.

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ORIGINAL ARTICLE

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Coronary (peri)-arteritis in patients with IgG4-related disease: A case series from the Central Anatolia Region of Turkey

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Abstract

Objective: Immunoglobulin G4-related disease (IgG4-RD) is a newly recognized fibro-inflammatory disease which affects many systems, as well as the cardiovascular system. Identifying the coronary involvement like periaortitis, coronary periarteritis and pericarditis is important, as they often cause unfavorable outcomes.

Methods: Eighty-one patients with IgG4-RD were retrospectively evaluated for symptomatic coronary artery involvement from Hacettepe University Vasculitis Research Center (HUVAC) database. The demographic, laboratory, radiologic and clinical characteristics of the patients were assessed.

Results: Among 81 patients with IgG4-RD, 6 patients (M/F:5/1) had coronary artery involvement. The patients' median age was 57 and serum IgG4 levels were above normal except for one case. All patients with coronary arteritis revealed an increased coronary vessel wall thickening and stenotic lesions. The coronary aneurysm and pericarditis were observed in half of the patients. Immunosuppressive treatments were given to all the patients and most of them followed in stable condition.

Conclusion: Coronary arteritis is a rare but notable manifestation of IgG4-RD. Although coronary periarteritis can cause significant morbidity and mortality, it seems better results can be achieved with early diagnosis and treatment.

KEYWORDS

coronary artery disease, coronary periarteritis, IgG4-related disease, periaortitis, periarteritis

1 | INTRODUCTION

Immunoglobulin G4-related disease (IgG4-RD) is an emerging immune-mediated problem with the capability of involving any organ. Some previously defined conditions now are acknowledged as falling within the spectrum of IgG4-RD.[1] The disease may affect various systems, including the cardiovascular (CV) system. Typical cardiovascular manifestations of IgG4-RD are periaortitis, coronary arteritis, and pericarditis.[2] The presence of coronary involvement is critical, while this condition can cause myocardial ischemia, infarction, or sudden cardiac death.[3]

A case of idiopathic retroperitoneal fibrosis (RPF) associated with arteritis was published in 1966.[4] Pan-arteritis in a coronary artery branch with mixed cellularity including lymphocytes, eosinophils, and plasma cells in pathology has been described. However, since then, case reports describing coronary artery involvement are relatively rare. Akiyama et al. reported a systematic literature review of IgG4-related peri-aortitis/periarteritis.[5] Based on the radiological

findings of 27 patients with IgG4-related coronary arteritis, vasculitic lesions were classified into 3 types: stenotic, aneurysmal, and diffuse wall thickening. Although corticosteroid treatment is effective, the disease can be life-threatening secondary to myocardial infarction, aortic dissection, and aneurysmal rupture. Pretreatment evaluation of the severity of stenosis or aneurysms is essential to predict the progression after corticosteroid treatment.

Therefore, we aimed to present the experience of our multidisciplinary vasculitis center in terms of treatment choices and clinical, radiological outcomes of patients with IgG4-RD.

2 | PATIENTS AND METHODS

Hacettepe University Vasculitis Research Center (HUVAC) database is a single-center database in which the data of all primary vasculitides patients has been recorded prospectively since October 2014. Before registration, all patients provided written consent to participate in the HUVAC database. To date, 2111 primary vasculitis patients aged >18 years have been registered. An experienced rheumatologist made the diagnosis of IgG4-RD according to the 2011 Comprehensive Diagnostic Criteria for IgG4-RD.[6] Other systemic diseases mimicking the clinical features of IgG4-RD including Takayasu arteritis, giant cell artertis, polyarteritis nodosa, Behçet's disease, tuberculosis, and syphilis were systematically searched and excluded. The study was approved by Hacettepe University Ethical committee (GO21/01-15).

The demographic and clinical characteristics, co-morbidities, organ involvement, laboratory and disease activity parameters, and medical treatments of IgG4-RD patients were collected from the HUVAC database. Patients' treatment outcomes were evaluated by clinical symptoms, physical examinations, laboratory results, and imaging, including ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography/CT (PET/CT). All available vascular radiology (CT angiography [CTA], magnetic resonance angiography [MRA]) data were reviewed and interpreted independently by 2 vascular radiologists (SA, TH). Chronic periaortitis is defined as idiopathic RPF, peri-aortic vessel wall thickness, wall enhancement, and peri-vascular soft tissue thickening and peri-aneurysmal RPF on CTA or MRA.[7] The presence of coronary periarteritis was defined as circumferential wall thickening with or without coronary artery aneurysms on coronary CTA and classified as stenotic, aneurysmal, and diffuse wall thickening type.[5] Coronary artery aneurysm was defined as a localized dilation of a coronary segment over 1.5 times the diameter of a normal adjacent arterial part.[8] Significant coronary stenosis was defined as a more than 50% reduction in lumen diameter during CTA or invasive coronary angiography.[9]

3 | RESULTS

A total of 81 patients were recorded as having IgG4-RD in our HUVAC database. Of these 81 patients, 59 had undergone a CTA or MRA with the suspicion of vasculitis. Among this patient population, 6 patients (M/F:5/1) revealed a coronary periarteritis due to IgG4-RD (10.1%). The median (interquartile range [IQR]) age was 57 (43.5-70.5) years. Median (IQR) C-reactive protein (CRP), and IgG4 levels were 17.6 (range 5-39) mg/L and 319 (range 125-1357) mg/dL, respectively. Serum IgG4 levels were above normal except for 1 case (Case #4). Additional organ involvement was observed in 5 patients (the most common being lymph nodes, lungs, pleura, and orbits). Furthermore, pericarditis accompanied coronary involvement in half of the patients. Cases 1, 3 and 5 fulfilled the 2019 American College of Rheumatology/European League Against Rheumatism Classification Criteria for IgG4-RD[10] (Table S1).

Coronary arteritis presented with myocardial ischemia symptoms in 3 patients, an acute myocardial infarction in 1 patient, and heart failure symptoms like dyspnea, edema, and cough in the remaining 2 patients. The details of each patient are reported below.

3.1 | Case 1

A 53-year-old man was admitted to our vasculitis center with orbital pain and redness in the eyes with swollen lacrimal glands. The purpuric lesions on the lower limbs preceded his eye symptoms and he had intermittent chest pain for 2 months on admission. The patient had no ear-nose-throat symptoms and had not used any medication recently. Inflammatory markers were normal at presentation, and the patient's serum IgG4 level was elevated (148 mg/dL; reference range, 0-125 mg/dL). His antineutrophil cytoplasmic antibodies and antinuclear antibodies were negative and complement levels were in normal range. His skin biopsy revealed perivascular dermatitis with vasculopathic changes, and the ratio of IgG4-positive cells/IgG-positive cells was >40% in his minor salivary gland biopsy. Proptosis and diffuse thickness with inflammation of the extra-ocular muscles was seen on orbital MRI. CTA revealed mediastinal lymphadenopathies, and diffuse wall thickening in the thoracoabdominal aorta. Coronary CTA revealed multiple partially thrombosed aneurysms in the right coronary artery (RCA). Moreover, the circumflex (Cx) artery was occluded and there was wall thickening in the left anterior descending artery (LAD) and RCA causing significant stenosis (Figure 1A) The patient developed angina during hospitalization and underwent invasive coronary angiography which confirmed CTA findings with total occlusion of the Cx artery, 80% stenosis in proximal LAD artery and total occlusion distally, 90% stenosis in RCA. Due to the rupture risk of coronary aneurysms, CABG (coronary artery bypass graft) was not performed. Then, pulse steroid and cyclophosphamide therapy were administered with a diagnosis of IgG4-RD with coronary periarteritis. After 10 g of intravenous cyclophosphamide therapy, vascular findings on CTA were stable (Figure 1B) and lacrimal gland and orbital symptoms were clinically resolved.

3.2 | Case 2

A 61-year-old man presented with exertional dyspnea, chest discomfort, and bilateral leg edema for 6 months. Past medical history

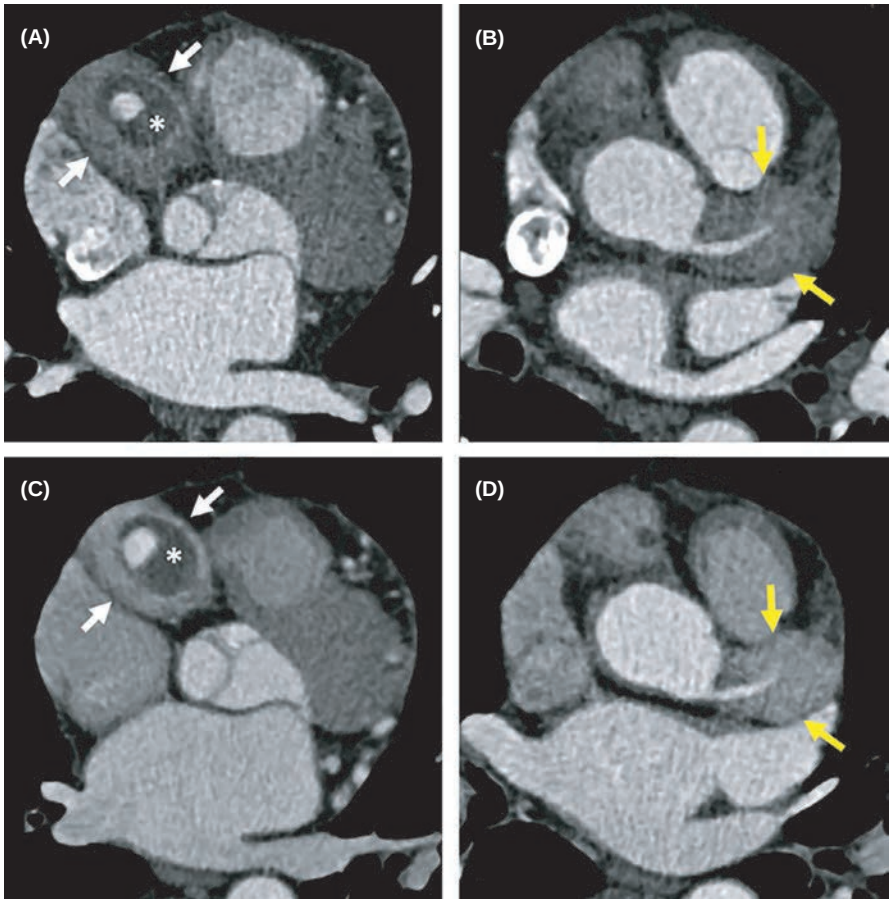

FIGURE 1 Axial coronary computed tomography angiography (CTA) image shows wall thickening and a partially thrombosed (A, asterisk) aneurysm in the right coronary artery (RCA) (A, white arrows). Also, marked wall thickening is observed in the left anterior descending artery (LAD) (B, yellow arrows). In follow-up axial coronary CTA, although the total size of the RCA aneurysm is stable, there was a slight increase in patent lumen size (C, white arrows). Partial thrombus is still observed in the aneurysm (C, asterisk). The wall thickness of LAD is stable (D, yellow arrows). Note that there is wall enhancement in RCA aneurysm (C) and LAD (D)

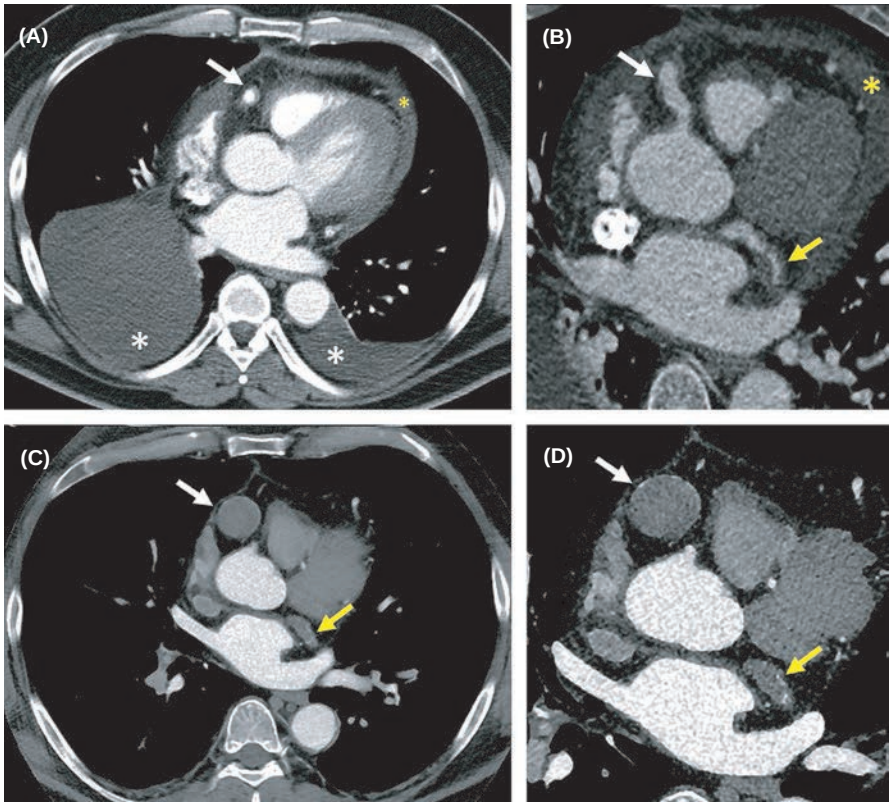

FIGURE 2 In axial post-contrast thorax computed tomography (CT) images, ectasia and diffuse wall thickening are observed in the right coronary artery (RCA) (A, white arrow). Further, there is pericardial effusion (A-B, yellow asterisk) and bilateral pleural effusion (A, white asterisks). Coronary CT angiography (CTA) image shows ectasia and diffuse wall thickening in RCA (B, white arrow) and the circumflex (Cx) artery (B, yellow arrow). Follow-up CTA images show thrombosed RCA aneurysm and occlusion of RCA (C-D, white arrow). Cx artery is also occluded (C-D, yellow arrows). Regression of pleural effusion is seen

included hypertension, coronary artery disease. Massive pericardial effusion and adherent mass to the right atrium wall were detected during transthoracic echocardiography. Plasma cells and active chronic inflammation were observed in the biopsy taken from the mass. He was diagnosed as IgG4-RD with these biopsy findings and high serum IgG4 level (148 mg/dL). CTA showed diffuse periaortitis, contrast-enhancing mass-like wall thickening in RCA (Figure S1 and Figure S2) and pericarditis. Orbital MRI was performed because of the presence of diplopia and revealed an orbital inflammation. The patient was hospitalized, and diuretic, anticoagulant, antiplatelet therapies were initiated after pericardiocentesis. He was discharged with corticosteroid and intravenous cyclophosphamide therapies after clinical stabilization. After 5 g of cyclophosphamide therapy, at the 6-month follow-up visit, he was re-hospitalized due to advanced heart failure and pneumonia. The patient died because of multi-organ failure caused by heart failure and sepsis.

3.3 | Case 3

A 48-year-old man with a previous history of hypertension and coronary artery disease was referred to our vasculitis center with aortic vessel wall thickening and suspicion of vasculitis. He had fatigue and recurrent chest pain for the last 2 years. Laboratory tests were within normal limits except for serum IgG4 level (262 mg/dL). The CTA revealed periaortitis, diffuse ectasia and wall thickening in the main coronary arteries (Figure 2A). Also, bilateral pleural and pericardial effusion with precarinal lymphadenopathies were observed. Pulse steroid and rituximab therapy were initiated. During follow-up, he underwent vascular surgery for a splenic artery aneurysm. After 2 years, he was started on methotrexate therapy. His clinical condition was good for 2 years, but then he was admitted with angina symptoms. Coronary CTA revealed that coronary involvement markedly increased in the interval period (Figure 2B). Coronary angiography was performed because of unstable angina, showing occlusion of RCA and Cx artery and ectasia in proximal LAD artery. Steroid and rituximab treatment was restarted due to the progressive disease and anticoagulant treatment added to his antiplatelet therapy.

3.4 | Case 4

A 30-year-old woman who had bilateral nephrostomy because of ureteral obstruction and RPF performed 1 year ago was admitted to our hospital with an ongoing chest pain. She was diagnosed as having ST-segment elevated myocardial infarction and underwent emergent invasive coronary angiography which revealed critical stenoses in coronary arteries. Coronary CTA was performed because of persistent chest pain and soft tissue masses reaching 7 mm in thickness surrounding all coronary arteries were observed. The concentric luminal narrowing was present in the RCA, Cx artery, and LAD artery (Figure S1 and Figure S2). After the initiation of corticosteroid treatment, she underwent CABG surgery which relieved her myocardial ischemia symptoms. A pericardial biopsy was performed during CABG and revealed fibrosis and rare plasma cell infiltration. Following CABG surgery, rituximab was added to corticosteroid therapy. However, there was no regression in RPF and ureteral obstruction developed after 6 months of treatment. Thus, the treatment was switched to cyclophosphamide. After 3 g of intravenous cyclophosphamide therapy, the drug was stopped according to the patient's decision, and her clinical condition was stable with no treatment for 6 years.

3.5 | Case 5

A 66-year-old man with a history of hypertension, atrial fibrillation and hyperlipidemia was admitted to our emergency department with fever, shortness of breath, cough, and right ankle arthritis. Thoracic CTA revealed ground-glass opacities in bilateral lungs and pleural effusion, and additionally mediastinal lymphadenopathies. The increased wall thicknesses of the RCA and Cx artery, a thrombosed aneurysm in the LAD and an aneurysm in the Cx artery were detected on coronary CTA (Figure 3A). There was a narrow segment that did not create a significant occlusion in the proximal part of the biliary tract on magnetic resonance cholangiopancreatography (MRCP). The detailed questioning of the patient revealed that he underwent a surgical procedure due to an orbital mass 8 years ago. Re-assessment of the pathological material of this surgery was compatible with IgG4-RD. Corticosteroid and intravenous cyclophosphamide treatment (total of 12 g) were initiated, and his clinical condition improved. Furthermore, there was also an improvement in coronary CTA findings with decreased wall thickness and regressed diameter of aneurysmal changes (Figure 3B).

3.6 | Case 6

A 84-year-old man with a history of stroke, coronary artery disease, type 2 diabetes mellitus, hypertension, and hyperlipidemia was found to have cardiomegaly in a routine cardiology visit. During his work-up studies, he was incidentally found to have periaortitis and an infrarenal abdominal aortic aneurysm on CTA. Then, he was referred to our vasculitis center for further evaluation. Furthermore, coronary CTA revealed RCA and Cx artery occlusion with thrombosed aneurysms (Figure 4). There was a soft tissue mass with heterogenous contrast enhancement surrounding LAD. Serum IgG4 level was elevated (910 mg/dL), and corticosteroid therapy was initiated. Because of the reduction in mass size surrounding LAD, the treatment was stopped at 12 months follow-up visit. However, he was re-admitted to our vasculitis center with constitutional symptoms after 1 year. Thrombosed aneurysms in RCA and Cx arteries, the diffuse increment in wall thickness of

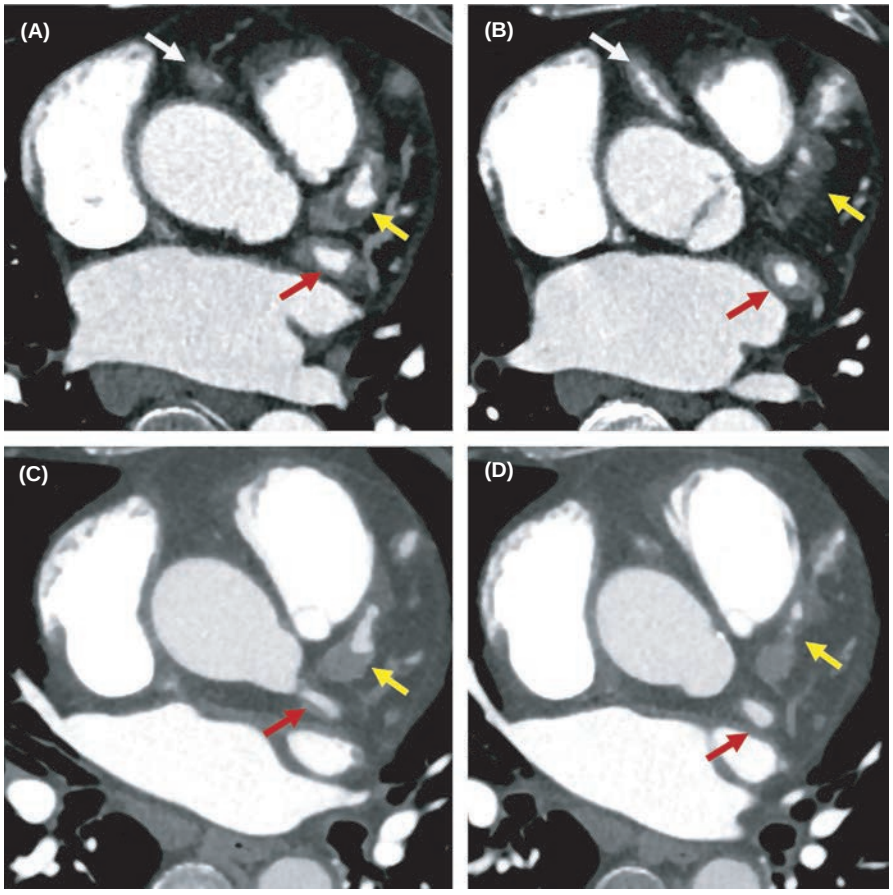

FIGURE 3 Axial coronary computed tomography angiography (CTA) images show fusiform aneurysms in the left anterior descending artery (LAD) (A-B, yellow arrow) and circumflex (Cx) artery (A-B, red arrow) with accompanying vessel wall thickening. There is also wall thickening in the right coronary artery (A-B, white arrow). Follow- up axial coronary CTA images show that the aneurysms and the thickening of the vessel walls in the Cx (C-D, red arrow) and LAD (C-D, yellow arrow) partially regressed

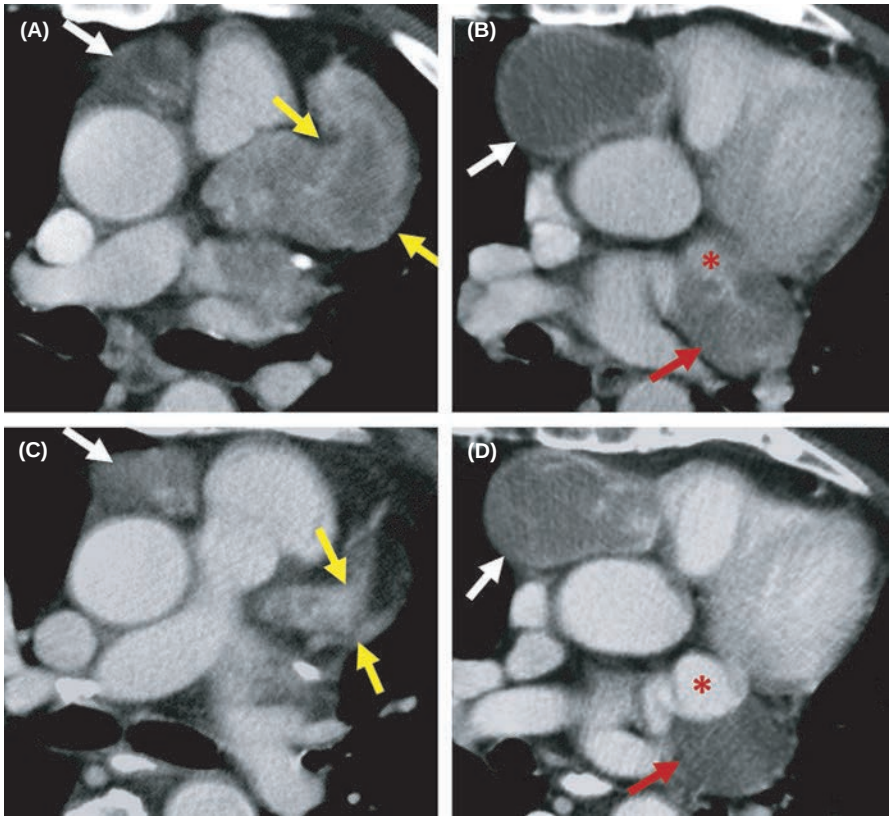

FIGURE 4 Axial post-contrast computed tomography (CT) images show a soft tissue appearance surrounding the left anterior descending artery (LAD) with heterogeneous contrast enhancement (A, yellow arrows). Also, there are aneurysms with luminal thrombus in the right carotid artery (RCA) (A-B, white arrow) and circumflex (Cx) artery (B, red arrow). Cx aneurysm is partially thrombosed with the patent lumen shown as a red asterisk (B). Follow-up axial CT images show that the soft tissue like wall thickening in LAD significantly regressed (C, yellow arrows). RCA (C-D, white arrow) and Cx (D, red arrow) aneurysms are stable in size, but the patent lumen of Cx aneurysm increased in the interval period (D, red asterisk)

TABLE 1 Clinical features, organ involvement, and laboratory data of patients

Cases	Age and gender	Other organ involvement	Serum IgG4 (mg/dL)	CRP (mg/L)	ANCA and APL antibodies	Biopsy site	Histopathology	IgG4+ plasma cell count/high power field	Ratio of IgG4+ plasma cell count over IgG+ plasma cell count	Smoking status	Co-morbidities
#1	53, male	Head and neck gland involvement, retroperitoneum	2700	5	Negative/ negative	Minor salivary gland	Fibrosis	30	>40%	Never smoked	-
#2	61, male	Retroperitoneum	148	15.3	Negative/ negative	Mass adjacent to the heart	Fibro-inflammatory tissue with lymphoplasmocytic infiltration	NA	NA	Current smoker	Hypertension, duodenal ulcer
#3	48, male	Kidney, retroperitoneum	262	5	Negative/ negative	-	-	-	-	Never smoked	Hypertension
#4	30, Female	Kidney, retroperitoneum	56	67.9	Negative/IgG antibodies to beta2-glycoprotein but negative in repeated analysis	Pericarditis	Fibro-inflammatory tissue with rare plasma cell infiltration Fibrosis	NA	NA	Current smoker	-
#5	66, male	Head and neck gland involvement, chest, retroperitoneum	376	39	Negative/ negative	Periorbital mass	Fibro-inflammatory tissue with lymphoplasmocytic infiltration	50	>80%	Previously smoked	Hypertension, atrial fibrillation, hyperlipidemia
#6	84, male	Retroperitoneum	910	20	Negative/NA	-	-	-	-	Previously smoked	Diabetes, hypertension, hyperlipidemia, CVA, osteoarthritis

Abbreviations: ANCA, antineutrophil cytoplasmic antibodies; APL, antiphospholipid; CRP, C-reactive protein; CVA, cerebrovascular accident; IgG4, Immunoglobulin G4; NA, not adjusted.

TABLE 2 Coronary lesions, treatment, and outcomes of patients

Cases	Computed tomography findings				Treatment	Follow-up (mo)	Outcome
	Stenotic	Aneurysmal	Thickening of the wall	Periaortitis			
#1	+	+	+	+	Steroid + cyclophosphamide (IV)	9	Clinical manifestations improved. Radiological findings remained stable, no exacerbation during 1 y of follow-up
#2	+	–	+	+	Steroid + cyclophosphamide (IV)	7	Death due to exacerbation of heart failure, acute renal failure, and sepsis after 6 mo treatment
#3	+	Diffuse ectasia	+	+	Steroid + rituximab	64	Clinically stable after induction treatment. Surgery for a splenic artery aneurysm rupture. Disease progressed while on methotrexate treatment
#4	+	–	+	+	Steroid + IV cyclophosphamide, rituximab Steroid + cyclophosphamide (IV)	72	Coronary bypass surgery was performed – no further improvement with rituximab and corticosteroid treatment after surgery. No relapse without treatment for 6 y
#5	+	+	+	+	Steroid + cyclophosphamide (IV)	13	Clinical manifestations improved. Coronary arteries wall thickness was resolved
#6	+	+	+	+	Steroid + rituximab	25	Regression of aneurysm and no clinical relapse for 2 y

Abbreviation: IV, intravenous.

the LAD artery were found on CTA, and corticosteroid treatment restarted in combination with rituximab. His symptoms resolved, and we observed no relapse in 2 years of follow-up with this combination therapy (Tables 1 and 2).

3.7 | Coronary arteritis, treatment, and outcomes

IgG4-RD patients with coronary arteritis mainly have myocardial ischemic complaints on their admission to the emergency or cardiology department. Invasive coronary angiography was performed in 3 patients (1 patient for acute myocardial infarction [Case #4] and 2 patients for unstable angina pectoris [Cases #1, #3]). The remaining 3 patients were diagnosed with coronary periarteritis by CT angiography. Clinical features, organ involvement, and laboratory data of patients are given in Table 2.

All patients with coronary arteritis revealed an increased coronary vessel wall thickening and stenotic lesions. The coronary aneurysm was observed in 3 of the patients, and only 1 patient had a diffuse coronary ectasia. Three of 4 patients with coronary aneurysms (Cases #3, #5, #6) concomitantly had aortic or splenic artery aneurysms. Periaortitis was present in all patients. Three patients had diffuse periaortitis and with the thoracic aorta being primarily affected. Cyclophosphamide (n = 3) or rituximab (n = 3) therapies with corticosteroids were administered as induction treatment. Coronary lesions, treatment, and outcomes of patients were given in Table 2.

The CABG surgery in 1 patient and a surgery for splenic artery aneurysm rupture in another patient were required during follow-up. Unlike the aortic aneurysms, coronary artery aneurysms did not exacerbate after treatment, and no complications have occurred. Except for Case #2, who died 6 months after diagnosis, all patients were clinically inactive during the median (IQR) 19 months (8.5-66 months) follow-up.

4 | DISCUSSION

IgG4-RD is a systemic fibro-inflammatory disorder characterized by lymphoplasmacytic infiltration of numerous IgG4-positive plasma cells, leading to fibrous thickening in the affected tissues.[1,11] IgG4-RD can affect various organs such as lacrimal and salivary glands, orbits, lungs, pancreas and hepatobiliary tract, kidneys, lymph nodes and the cardiovascular system, including the coronary arteries and pericardium, and especially the walls of medium- and large-sized vessels.

Coronary periarteritis in IgG4-RD was described as soft tissue mass around the coronary arteries, thickening of the vessel walls, and luminal stenosis.[12-15] Moreover, coronary arteritis may be complicated with coronary artery aneurysms (CAA).[14]

Matsumato et al. first pronounced IgG4-RD with coronary periarteritis in an abdominal aortic aneurysm patient in 2008.[16] Since then, several case reports have been published. Six of our IgG4-RD patients had coronary periarteritis, since CTA or MRA was performed in only symptomatic patients, but subclinical cases may have been missed.

Patients with coronary arteritis usually present with symptoms of myocardial ischemia or heart failure. Coronary periarteritis may be the first sign of vasculitis and it can present with sudden cardiac death.[17,18] Less commonly it is detected incidentally during evaluation of a systemic inflammatory disease.[13] IgG4-RD also may present unexpected intraoperative findings. Fibro-inflammatory adherent masses around the vessels, diffuse tissue thickening of the ascending aorta and coronary arteries indicates vasculitis rather than coronary artery atherosclerosis.[19]

IgG4-RD patients with coronary periarteritis mostly present with myocardial ischemia symptoms. When these patients present with acute coronary syndrome, invasive coronary angiography should not be delayed. But after that, when angiography findings suggest vasculitis (diffuse ectasia or aneurysm) or in the presence of systemic symptoms especially in patients without cardiovascular risk factors, further diagnostic work-up should be done considering the differential diagnosis.

Coronary arteritis is a rare but notable manifestation of medium- and large-sized vessel vasculitis. It can cause severe complications in the course of diseases like giant cell arteritis, Takayasu arteritis in adults, and Kawasaki disease in pediatrics.[20] IgG-RD with coronary periarteritis should also be differentiated from other inflammatory vasculitides that may involve coronary arteries and vasculitis mimickers.

Diagnosing of IgG-RD and coronary periarteritis may be challenging. Tissue diagnoses may not be available for all patients. Further, biopsies may not always meet the criteria, especially in fibrotic tissues. Clinical course, typical radiologic involvement of aorta and response to treatment may provide supporting evidence for diagnosis of IgG4-related periaortitis and coronary periarteritis.

Also, it is essential to distinguish between atherosclerosis and coronary arteritis in the management and follow-up of these patients.[20] Clinical history should be taken in detail. A systemic examination should also be performed, and accompanying vasculitis findings should be evaluated, especially in patients without risk factors for coronary artery disease. The presence of an aneurysm, diffuse involvement of a long vascular segment, increased vascular wall thickness, contrast enhancement at the vessel wall on CTA, which is rarely seen in atherosclerosis, are all in favor of periarteritis. When there is diffuse periaortitis, we should also suspect coronary arteritis in these patients. The presence of calcification should imply atherosclerosis, but it should be kept in mind that these patients may have an accelerated atherosclerosis process because of chronic inflammation.

Fatal involvement of the coronary arteries raised concerns about the diagnosis and treatment for coronary arteritis. The optimal treatment method of coronary periarteritis has not yet been clearly established. Corticosteroids with or without other immunosuppressants was effective in most cases.[12,13,15,21,22] Rituximab with corticosteroids also seems useful[18,23] and rational to reduce

long-term corticosteroid exposure as relapses can be seen while corticosteroid tapering.[12] As another option, cyclophosphamide with corticosteroid treatment was reported as effective in a patient with coronary periarteritis and abdominal aortic aneurysm.[24]

On the other side of immunosuppressive drug selection, the timing of immunosuppressive treatment is another matter of thought. In general, when the vasculitis patient needs a percutaneous or surgical procedure, if it is not urgent, is recommended after starting immunosuppressive therapy. However, since several studies show that patients with aortic/luminal dilatation of the affected aortic/arterial lesions have high risk for exacerbations, dilated arterial lesions in IgG4-RD should be carefully monitored to avoid exacerbation of luminal dilatation or rupture after starting glucocorticoid therapy.[25-27]

Although there are patients who remained stable with percutaneous intervention or CABG in the literature, surgical or percutaneous intervention is required in selected cases as it is associated with sudden cardiac death or aneurysms rupture.[16,28]

We treated all of our patients with immunosuppressive treatment, adding on corticosteroids. Although there is a concern about the progression of aneurysms after treatment, 2 patients with CAA remained stable, and 1 patient with CAA had regression after treatment. One patient, treated with corticosteroid and rituximab as an induction therapy, admitted with progressive disease and new thrombosed coronary aneurysms on methotrexate maintenance therapy without corticosteroids. It is difficult to interpret whether this is a long-term consequence of corticosteroid therapy or if it is due to insufficient immunosuppression.

Establishing the diagnosis of IgG4-RD coronary periarteritis is important for several reasons. First of all, IgG4-RD is a systemic disease and affects various other organs, not only the cardiovascular system. Second, treatment of coronary periarteritis is different from atherosclerotic heart disease as it is an inflammatory process and the post-therapeutic clinical course might differ. Future studies should clarify the optimal medical and interventional therapeutic strategy for IgG4-RD patients with coronary periarteritis.

These case series highlight the importance diagnosing IgG4-RD with coronary periarteritis. Although coronary periarteritis can have fatal consequences, it seems better results can be achieved when diagnosed and treated early. Therefore, the cooperation of cardiologist and rheumatologist and a multidisciplinary approach are important in diagnosing these patients and in their follow-up.

CONFLICT OF INTEREST

Professor Karadag: received consultancy fees and/or speaker fees from Abbvie, Amgen, Celltrion, Novartis, Pfizer, Roche, UCB Pharma. Professor Aytemir: proctoring for Abbott, Medtronic, and Biosense Webster. Lecturer for Abbott and Medtronic. Other authors declared nothing to disclose.

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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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Exercise capacity in axial spondyloarthritis and associated factors: A cross-sectional controlled study

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Abstract

Objective: To examine the associations between exercise capacity (EC), cardiovascular (CV) risk factors and disease-related variables in axial spondyloarthritis (AxSpA) patients.

Methods: In this cross-sectional controlled study, CV risk profile data, physical activity, 10-year CV event risk estimated by the Framingham model and Ankylosing Spondylitis Disease Activity Score – C-reactive protein were recorded. A maximal treadmill exercise test by Bruce protocol was administered. Analyses of covariance were performed with adjustments for age, smoking status and physical activity level. Linear regression analysis was performed to study the association between EC and related CV risk factors.

Results: Thirty-eight patients and 38 age-gender matched controls were recruited between May and October 2014. Patients had significantly lower EC than controls (MD 2.2; metabolic equivalents 0.91-3.49; $P = .001$). The difference remained significant after adjustments ($P = .001$). There were significant correlations between EC and age, 10-year CV event risk, body mass index (BMI) and waist circumference for patients and controls ($P < .001$ and $P < .05$, respectively). There was a significant relationship between EC and total cholesterol, triglycerides and heart rate recovery (HRR) in patients ($P = .04$, $P < .001$ and $P = .006$, respectively). High-density lipoprotein - cholesterol was significantly higher, and BMI was significantly lower in nonradiographic AxSpA patients ($P = .026$ and $P = .03$ respectively). Age and triglyceride levels were found as the significant predictors for EC in the AxSpa group (for age $\beta = -.105$, $P = .003$; for triglycerides $\beta = -.016$ $P = .003$).

Conclusion: Exercise capacity was significantly lower and attenuated HRR was significantly associated with low EC and high 10-year CV event risk in AxSpA patients.

The study was registered at clinicalTrials.gov (NCT04706650). Retrospectively registered. Registration date: 12/01/2021

We believe our manuscript provides additional information about the cardiovascular status in axial spondyloarthritis and draws attention to the importance of cardiovascular assessment of these patients in daily routine.

Our manuscript has been published as an abstract in the 4th World Congress on controversies, debates and consensus in *Bone, Muscle, Joint Diseases* (BMJD), 2016.

The study was conducted in Gazi University Hospital but the present adress of Ebru Köseoğlu Tohma is Muğla Sıtkı Koçman University Hospital.

KEYWORDS
ankylosing spondylitis, axial spondyloarthritis, cardiovascular risk factors, exercise capacity, physical activity

1 | INTRODUCTION

Spondyloarthritis (SpA) is a group of inflammatory diseases that affects spine and/or peripheral joints, enthesis. Ankylosing spondylitis (AS) is a subgroup of SpA with a prevalence of 0.1%-1.4%.[1] The typical clinical features of the disease are inflammatory back pain and reduced spinal mobility which reduces quality of life.[1] In 2009, Assessment of Spondyloarthritis International Society (ASAS) defined axial SpA (AxSpA) classification criteria as radiographic or nonradiographic AxSpA. Nonradiographic AxSpA (nrAxSpA) patients have similar clinical presentation and disease activity, albeit there is no radiographically visible sacroiliitis, that is structural damage of the bone.[2]

Exercise capacity (EC), which can be described as peak metabolic equivalents (METs) achieved during an exercise tolerance test, is an established indicator of general body health and is an independent predictor of cardiovascular disease (CVD) risk, cardiovascular (CV) and total mortality.[3,4] An increase of 1 MET in EC is associated with 13% and 15% of reductions in mortality risk of all-cause and CVD, respectively.[4] In healthy populations, it is well known that there is an association between EC and CV risk factors such as waist circumference, blood pressure, level of triglycerides (TG) and high-density lipoprotein - cholesterol (HDL-c), insulin sensitivity and smoking.[5,6] Moreover, lower systemic inflammation is associated with high EC in healthy adults.[6]

Recently, there has been a focus on the increased risk of CV diseases in patients with AS, which requires appropriate CV assessment of these patients.[6-8] The mortality rate in patients with AS is higher than expected, with standardized mortality ratios 1.5-1.9, where the excess mortality seems to result from CV diseases.[6,8] Increased CV risk is considered to be related to the inflammatory process of the disease; inflammation resulting in pain and fatigue leads to physical inactivity.[8] Besides chronic inflammation, an increased prevalence of CV risk factors has been reported in AS patients. There are a few studies investigating EC in AS patients with conflicting results.[6,7,9-11] However, the relationship between CV risk factors and EC has not been elucidated.[6] To our knowledge, there is only 1 study that examines the associations between EC and CV risk factors in AS patients.[6] Considering the inflammatory load of the disease and the fact that inflammation itself exacerbates CV risk factors, the relationship between EC and CV risk factors in AxSpA patients might differ from healthy populations. To date, EC in nrAxSpA patients has not been investigated.

The first objective of this study was to examine the associations between EC and CV risk factors in radiographic and nrAxSpA patients and compare these to population controls. The second objective was to search for possible associations between EC and disease-related variables in AxSpA patients.

Key points

- Exercise capacity was significantly lower and associated with age, BMI, waist circumference, total cholesterol, triglycerides and 10-year CV event risk in AxSpA patients.
- Attenuated HRR was significantly associated with low exercise capacity and high 10-year CV event risk in AxSpA patients but not in controls.
- HDL-c was significantly higher, and BMI was significantly lower in nonradiographic AxSpA patients than patients with radiographic sacroiliitis.

2 | METHODS

2.1 | Design

This cross-sectional, controlled study was conducted between May 2014 and October 2014, at the Department of Physical Medicine and Rehabilitation, Gazi University Medical School in Ankara, Turkey. The study was approved by Gazi University Medical Ethics Committee and was registered at clinicalTrials.gov (NCT04706650). Written informed consents were obtained from all participants. All the procedures were performed according to the World Medical Association Declaration of Helsinki.

2.2 | Participants

Thirty-eight patients (27 men, 11 women) fulfilling the ASAS AxSpA criteria[12] were recruited from the rheumatology outpatient clinic. Thirty-eight control subjects with a similar range of gender and age (24 men, 14 women) without a history of an inflammatory arthritis were recruited among the hospital staff. All participants were between 18 and 70 years old. Participants with an established cardiac disease including structural heart diseases, cardiac rhythm disorder, unstable angina pectoris, uncontrolled hypertension, chronic renal and/or hepatic disease, malignancy, neuromuscular disease, severe musculoskeletal deformity or inability to walk were excluded.

Demographic data (including age, gender and occupation), medical history and medications were noted.

2.3 | Assessment of CV risk profile

Smoking status, body mass index (BMI), waist circumference (measured in a standing position with a measuring tape at the level of umbilicus) was recorded. Brachial blood pressure (BP) was measured after a 5-minute rest in a supine position. Blood samples were drawn after 8 hours of fasting and analyzed immediately. Total cholesterol (TC), HDL-c and low-density lipoprotein cholesterol (LDL-c), TG, fasting plasma glucose, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were analyzed.

Comprehensive systemic and musculoskeletal examinations were carried out in both groups. The International Physical Activity Questionnaire short form (IPAQ) which is reported to be reliable and valid was administered to assess the level of physical activity.[13] The construct validity of the IPAQ (long form) was found to be modest compared with accelerometer activity counts and showed good test-retest reliability in AS patients as well.[14] IPAQ short form provides information on the time spent walking, in vigorous- and moderate-intensity activity and in sedentary activity (sitting) for the last 7 days. Reported minutes within each activity are used for the estimation of total weekly physical activity. There are standardized MET energy expenditure values assigned to each category of activity; which are 8 METs for vigorous activity, 4 METs for moderate activity, 3.3 METs for walking and 1 MET for sitting.[13] Weekly physical activity level in total MET-minutes per week is calculated as duration × frequency per week × MET intensity (standardized MET energy expenditure values). The levels are categorized as low (<600 MET-min/wk), moderate (600-3000 MET-min/wk) or high active (>3000 MET-min/wk).[13]

Framingham risk score (FRS),[15] a validated and commonly used CVD risk prediction tool, was administered to estimate 10-year absolute CVD risk of all participants. Risk factors including age, gender, TC, HDL-c, LDL-c levels, systolic and diastolic BP, smoking status and presence of diabetes were recorded and risk scores were calculated by the calculator on www.framinghamheartstudy.org website. Risk categories were defined according to FRS values as low (<10%), moderate (10%-20%) or high (>20%) risk.

2.4 | Assessment of disease-related variables in the patients

The Ankylosing Spondylitis Disease Activity Score - CRP (ASDAS-CRP) was used to assess disease activity.[16] ASDAS is a well-validated and highly discriminatory instrument for evaluating disease activity in AxSpA.[17] It is based on CRP level and patient-reported outcomes incorporating back pain, duration of morning stiffness, patient global assessment and peripheral joint pain/swelling. Higher values indicate higher disease activity. The online ASDAS calculator is used on http://www.asas-group.org/clinical-instruments/asdas_calculator/asdas.html website. Disease activity states were categorized as inactive disease (<1.3 units), low (1.3 - <2.1 units), high (2.1-3.5 units) and very high (>3.5 units) disease activity.[18]

2.5 | Assessment of exercise capacity

All participants underwent a maximal exercise tolerance test with a 12-lead electrocardiogram (ECG) monitorization on a treadmill (Custo er 2100, Netherlands). Bruce protocol was used, which requires a rise in the inclination and speed of the treadmill in every 3 minutes to increase work output.[19] The tests were performed under the supervision of a physician and an experienced nurse. Brachial BP was measured in a sitting position before and 5 minutes after the test. BP measurements were repeated in each stage of the test and the 1st and 3rd minutes of the active recovery phase. The participants were asked to define their rate of perceived exertion (RPE) on the Borg Scale which ranges from 6 (very, very light) to 20 (maximal exertion) at the end of each workload.[20] The test was terminated when the participants reached at least 85% of the predicted peak heart rate (calculated as 220-age beats/min) or reported a RPE of 17-20 on the Borg Scale or if the participant requested to stop. Peak exercise capacity, expressed in METs where 1 MET is the amount of oxygen consumption in resting state and equals 3.5 mL of oxygen per kilogram of body weight per minute for an average adult, maximal heart rate, maximal systolic and diastolic BP, test duration, the heart rate and BP at the 1st minute after the cessation of exercise test and abnormal ECG changes were recorded.[21] Heart rate recovery (HRR) was calculated as peak heart rate achieved at maximal exercise minus heart rate at the 1st minute after cessation of the exercise test.

A sample size of 38 AxSpA patients was calculated in order to find a correlation coefficient of at least 0.5 between exercise capacity and CV risk factors with 90% power and 0.05 type-I error.

2.6 | Statistical analyses

Data were analyzed using SPSS for Windows statistical package, version 16.0. Continuous variables were presented as mean ± SD, median (minimum-maximum) with categorical variables as numbers and percentage. Normality of the continuous variables was checked by Shapiro-Wilk test while homogeneity of variance was checked by Levene test. Differences for continuous variables between the groups were examined by *t* test if they were normally distributed, by Mann-Whitney *U* test if they were not normally distributed. Differences for categorical variables between AxSpA and control groups were examined by Chi-square test. Analyses of covariance were performed to compare the groups with adjustments for age, smoking status and physical activity level. The relationship between numeric variables was presented as Pearson correlation coefficients. Linear regression analysis was performed to study the association between EC (dependent variable) and related CV risk factors (independent variables). Subgroup analyses were administered according to disease activity (inactive-low; ASDAS <2.1 or high-very high; ASDAS ≥2.1) and the presence of radiographic sacroiliitis (radiographic AxSpA or nrAxSpA) in AxSpA patients. Values of $P < .05$ were considered statistically significant.

TABLE 1 Demographic characteristics and cardiovascular risk factors of AxSpA patients and control subjects

	AxSpA (N = 38)	Controls (N = 38)	Mean difference (95% CI)	*P*[a]
Gender, male, n (%)	27 (71)	24 (63)		.464
Age, y	39.6 ± 11.0	35.2 ± 10.2	4.5 (−0.4-9.3)	.071
Education >12 y, n (%)	14 (37)	18 (47)		.353
BMI, kg/m²	26.4 ± 4.5	25.7 ± 4.8	0.7 (−1.4-2.9)	.489
Disease characteristics				
Disease duration, y	8.0 ± 9.6			
HLA-B27+, n (%)	27 (71)			
Radiographic sacroiliitis, n (%)	28 (74)			
ASDAS	2.6 ± 0.9			
ASDAS ≥2.1, n (%)	26 (68)			
Medication, n (%)				
NSAIDs	18 (47)			
Anti-TNFα	14 (37)			
No medication	6 (16)			
Comorbidity, n (%)	7 (18)	8 (21)		.773
DM	3 (8)	2 (5)		.644
HT	5 (13)	6 (16)		.744
CV risk factors				
Current smoker, n (%)	24 (63)	11 (29)		**.003**
Smoking, pack-y	11.7 ± 13.1	3.3 ± 6.6	**8.4 (3.3-13.1)**	**.001**
Waist circumference, cm	91.5 ± 14.3	88.7 ± 12.7	2.9 (−3.3-9)	.359
Systolic BP, mm Hg	120 ± 16	121 ± 15	−0.9 (−8-6.3)	.804
Diastolic BP, mm Hg	80 ± 11	84 ± 9	−4.1 (−8.8-0.6)	.084
Total cholesterol, mg/dL	186.2 ± 44.0	188.8 ± 35.7	−2.6 (−21-15.7)	.778
LDL cholesterol, mg/dL	115 ± 36	114 ± 27	1.2 (−13.3-15.7)	.869
HDL cholesterol, mg/dL	44.2 ± 10.6	49.8 ± 10.6	**−5.6 (−10.4-−0.8)**	**.024**
Atherogenic index, TC/HDL	4.3 ± 1.0	3.9 ± 0.9	0.4 (−0.01-0.9)	.057
Triglycerides, mg/dL	133 ± 71	115 ± 61	17.6 (−12.7-48)	.250
Glucose, mg/dL	92 ± 13	89 ± 12	2.6 (−3-8.2)	.351
ESR, mm/h	17 ± 13	9 ± 9	**8.2 (3-13.4)**	**.002**
CRP, mg/dL	10 ± 11	3 ± 2	**6.9 (3.4-10.4)**	**<.001**
Framingham risk score	8.7 ± 11.0	5.2 ± 8.0	3.5 (−0.9-7.8)	.115
Framingham risk level, n (%)				
Low, <10%	30 (79)	33 (87)		.361
Moderate, 10%-20%, or high, >20%	8 (21)	5 (13)		
Physical activity level, IPAQ, MET.min/wk	2525 ± 3136	1851 ± 2167	674 (558-1906)	.279
Physical activity level, n (%)				
Low-moderate, <600 MET.min/wk or 600-3000 MET.min/wk	28 (74)	31 (82)		.409
High, >3000 MET.min/wk	10 (26)	7 (18)		

Note: Values are the mean ± SD unless indicated otherwise.

Abbreviations: Anti-TNFα, anti-tumor necrosis factor alpha; ASDAS, Ankylosing Spondylitis Disease Activity Score; AxSpA, axial spondyloarthritis; BMI, body mass index; BP, blood pressure; CI, confidence interval; CRP, C-reactive protein; HDL, high-density lipoprotein; IPAQ, International Physical Activity Questionnaire; LDL, low-density lipoprotein; MET, metabolic equivalent; NSAIDs, nonsteroidal anti-inflammatory drugs; TC, total cholesterol.

[a]Independent-samples *t* test or $\chi 2$ test as appropriate AxSpA.

Significant differences are indicated in bold.

3 | RESULTS

3.1 | Demographic, clinical characteristics and CV risk factors

There were no differences between groups regarding age, gender, educational level or BMI. Smoking rate and tobacco exposure (pack years) were significantly higher in AxSpA patients than that of control subjects. AxSpA patients had lower HDL-c levels and higher ESR and CRP levels. There were no differences in other CV risk factors, Framingham risk scores and the levels of physical activity between AxSpA and control groups (Table 1). None of the participants was on lipid-lowering medication. There was no statistical difference between the 2 groups regarding anti-hypertensive treatment ($P = .744$). Disease characteristics and medications in AxSpA patients are presented in Table 1.

3.2 | Exercise test parameters and their associations

Peak heart rate achieved in maximal exercise was significantly lower in the AxSpA group than that of control group. Exercise capacity, that is peak METs achieved in maximal exercise and test duration were significantly lower in the AxSpA group, which remained significant after adjustment for age, smoking and physical activity level. None of the ECG changes required termination of the test. No cardiac symptoms occurred during the tests. Perceived exertion on the Borg Scale was significantly lower in the AxSpA group (Table 2).

Correlations between peak METs and other clinical, laboratory and cardiovascular parameters are presented in Table 3. Peak METs were significantly correlated with TG and TC levels and HRR in the AxSpA group, but these correlations were not observed in control subjects. Peak METs were significantly correlated with IPAQ score in the control group; however, no significant correlation was observed in the AxSpA group. HRR was inversely and significantly associated with 10-year CV event risk and waist circumference in the AxSpA group ($r = -.326$, $P = .046$ and $r = -.423$, $P = .008$, respectively). HRR was significantly correlated with peak METs and test duration in the AxSpA group ($r = .437$, $P = .006$ and $r = .483$, $P = .002$, respectively). By multiple regression analysis, the model was conducted with EC as a dependent variable, and age, BMI, TC, triglycerides level and HRR as independent variables for the AxSpA group. Based on the model, only age and triglycerides level were found as the significant predictors for EC (constant = 18.182, for age $\beta = -.105$, std-$\beta = -.420$

TABLE 2 Comparison of exercise test parameters between AxSpA patients and healthy controls

	AxSpA	Controls	Mean difference (95% CI)	P^a
At rest				
HR, beats/min	91 ± 13	85 ± 16	5.9 (−0.9-12.7)	.090
SBP, mm Hg	120 ± 16	121 ± 15	−0.9 (−8-6.3)	.804
DBP, mm Hg	80 ± 11	84 ± 9	−4.1 (−8.8-0.6)	.084
At peak exercise				
HR, beats/min	169 ± 16	178 ± 19	**−8.1 (−16-−0.1)**	**.048**
Peak HR/target HR %	94 ± 8	97 ± 7	−3.4 (−6.9-0.1)	.054
SBP, mm Hg	188 ± 26	192 ± 26	−3.1 (−15-8.8)	.602
DBP, mm Hg	97 ± 13	98 ± 17	−1.5 (−8.4-5.3)	.654
Peak METs	11.9 ± 2.8	14.1 ± 2.9	**−2.2 (−3.5-−0.9)**	**.001**
Test duration, min	9.6 ± 2.4	11.3 ± 2.1	**−1.7 (−2.7-−0.6)**	**.002**
Peak METs[b]	12	14		**.001**
Test duration[b], min	9.7	11.1		**.004**
ECG findings, n (%)	10 (26)	5 (13)		.150
RPE	14.7 ± 2.7	16.0 ± 2.0	**−1.3 (−2.4-−0.2)**	**.019**
At 1st min recovery				
SBP, mm Hg	160 ± 26	155 ± 21	4.7 (−6.2-15.6)	.394
DBP, mm Hg	81 ± 11	79 ± 12	2 (−3-7.1)	.429
HRR, beats	25.0 ± 8.6	27.0 ± 7.1	−2 (−5.6-1.6)	.278

Note: Values are the mean ± SD unless indicated otherwise.

Abbreviations: AxSpA, axial spondyloarthritis; CI, confidence interval; DBP, diastolic blood pressure; ECG, electrocardiography; HR, heart rate; HRR, heart rate recovery; METs, metabolic equivalents; RPE, rate of perceived exertion in Borg Scale; SBP, systolic blood pressure.

[a]Independent-samples *t* test or χ^2 test as appropriate.

[b]By analysis of covariance (ANCOVA) with adjustments for age, physical activity and smoking.

Significant differences are indicated in bold.

TABLE 3 Correlations of peak metabolic equivalents with cardiovascular risk factors in patients with AxSpA and controls

	AxSpA		Controls	
	r	*P*	*r*	*P*
Age	**−.585**	**<.001**	**−.639**	**<.001**
IPAQ score	.248	.133	**.435**	**.006**
Framingham score, %	**−.316**	**.045**	**−.395**	**.014**
Smoking, pack-y	−.116	.489	−.184	.268
BMI, kg/m^2	**−.422**	**.008**	**−.425**	**.008**
Waist circumference, cm	**−.411**	**.010**	**−.415**	**.010**
Systolic BP	.177	.287	−.266	.107
Total cholesterol, mg/dL	**−.335**	**.040**	−.041	.807
HDL, mg/dL	.006	.973	.156	.349
Triglycerides, mg/dL	**−.583**	**<.001**	−.189	.255
ESR, mm/h	−.230	.165	−.238	.150
CRP, mg/dL	.016	.924	−.271	.100
ASDAS	−.211	.203		
HRR	**.437**	**.006**	.046	.784

Abbreviations: ASDAS, Ankylosing Spondylitis Disease Activity Score; AxSpA, axial spondyloarthritis; BP, blood pressure; BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HDL, high-density lipoprotein cholesterol; HRR, heart rate recovery; IPAQ, International Physical Activity Questionnaire; *r*, correlation coefficient.

Significant differences are indicated in bold.

[95% CI −0.172 to −0.038], $P = .003$; for triglycerides $\beta = -.016$ std-$\beta = -.417$ [95% CI −0.027 to −0.006], $P = .003$, $R^2 = 0.489$).

When patients with ASDAS ≥ 2.1 (68.4%) were compared to patients with ASDAS <2.1, ESR and CRP were significantly higher in the former group ($P = .012$ and $P = .034$, respectively). There were no significant differences in age, disease duration, smoking status, TG, TC, HDL-c, LDL-c, atherogenic index (TC/ HDL), BMI, FRS and physical activity level between the 2 groups. There was no significant difference between the 2 groups in peak METs or HRR ($P = .323$ and .615, respectively).

When nrAxSpA patients were compared to rAxSpA patients, HDL-c was significantly higher, and BMI was significantly lower in nrAxSpA patients. There was no significant difference between the 2 groups regarding age, disease activity, laboratory or exercise test parameters including EC (peak METs) (Table 4).

4 | DISCUSSION

The results of our study showed that EC was significantly lower in AxSpA patients than controls; however, 10-year CV event risk evaluated with FRS was similar. EC was significantly related to age, 10-year CV event risk, BMI and waist circumference both in patients and controls. However, the significant relationship between EC and physical activity level observed in healthy controls was not confirmed in AxSpA patients. On the other hand, there was a significant relationship between EC and TC, TG and HRR in AxSpA patients, but not in controls. Age and TG level were found as the significant predictors for EC in AxSpA patients.

Lower EC observed in the AxSpA group in our study is in line with previous studies with AS patients, while this is the first study including nrAxSpA patients. In the literature, the mean difference of peak oxygen uptake (VO_{2peak}) varies across studies from 1.3 to 8.4 mL/kg/min (ie, 0.4 to 2.4 METs) between AS patients and control subjects, where most of them were lower than the mean difference of METs in our study.[6,7,9-11,22] This variance may result from different patient characteristics, test protocols and methods of VO_{2peak} estimation. In our study, estimated METs were used since we did not perform ergospirometric test. Some of the previous studies tested EC on a bicycle ergometer which may result in premature termination due to leg fatigue. This may have caused a lower EC difference between groups. In addition, tests on a treadmill provide more accurate data since the stress applied on the CV system is more physiological than that of a bicycle ergometer.[23]

There is only 1 study investigating EC and CV risk in patients with AS in which EC was found to be associated with BMI, waist circumference, and triglyceride levels in AS patients similar to healthy subjects.[6] However, we observed an inverse relationship between EC and TC and triglycerides only in the AxSpA group, but not in control subjects. We also found that EC was inversely associated with BMI and waist circumference in both groups. These results indicate that weight management with prevention of abdominal obesity is crucial for CV health not only for healthy subjects but also for AxSpA patients. In epidemiological studies, TG has been reported to be an independent risk factor for CVD since all CV events are not clarified by elevated LDL-c levels.[24] Atherogenic index of plasma (AIP), the logarithmic transformation of the plasma TG level to the HDL-c level ratio, has been suggested to be a novel biomarker of subclinical CV risk. In AS patients, AIP was reported to provide more accurate information on CV risk than TC/HDL-c ratio or LDL-c levels.[25] Exercise, a recommended treatment of hyperlipidemia, probably alters lipid metabolism via modification of enzymes responsible for synthesis, transport and catabolism of lipoproteins, resulting in adipose tissue and intramuscular TG lipolysis. The major positive effect of exercise training on lipid metabolism was reported to be an increase in HDL-c and decrease in TG levels.[26] These results are in line with our study in which decreased TG levels were found to be a significant predictor of an increased EC in AxSpA patients.

Halvorsen et al.[6] found that EC was not associated with BP or HDL-c in patients with AS, which is in accordance with our findings. However, in our study, HDL-c levels were significantly lower in the AxSpA group than controls, which is in agreement with the literature.[27,28] Low HDL-c is an important cardiovascular risk factor; a 1% increase in HDL-c is accompanied by a 2% decrease in cardiac events. HDL-c particles have anti-atherogenic capacity since they drive reverse cholesterol transport and antagonize pathways of inflammation, thrombosis, and oxidation.[29] There are findings indicating the decrease in HDL-c is correlated with the intensity of the inflammation in AxSpA patients.[6,27] It is an expected finding hence

TABLE 4 Comparison of AxSpA patients in regard to presence of radiographic sacroiliitis

	rAxSpA median (min-max) (N = 28)	nrAxSpA median (min-max) (N = 10)	*P*
Age, y	42 (31-49)	35 (27-49)	.528
Smoking, pack-y	11 (0-20)	2 (0-12)	.152
Disease duration, y	6.0 (3.1-13.0)	0.7 (0.1-3.2)	**<.001**
HLA-B27 + (%)	22 (79)	5 (50)	.087
ASDAS	2.8 (2.0-3.5)	2.6 (1.6-3.3)	.539
ESR, mm/h	12 (7-26)	14 (7-22)	.947
CRP, mg/dL	6.5 (3.1-15.7)	7.2 (2.4-11.7)	.894
TG, mg/dL	114 (82-195)	99 (54-190)	.486
HDL, mg/dL	41 (35-46)	50 (44-56)	**.026**
Atherogenic index	4.7 (3.7-5.2)	4.0 (3.3-5.1)	.233
BMI, kg/m^2	25.9 (24.3-29.6)	23.0 (21.2-27.3)	**.030**
Framingham score, %	4.7 (2.5-15.7)	3.3 (1.8-7.2)	.233
Physical activity, IPAQ	1131 (594-3232)	792 (292-3010)	.445
Peak METs	13.4 (10.0-13.4)	13.4 (9.2-14.3)	.349
Test duration, min	9.4 (7.5-10.8)	11.1 (9.4-12.1)	.104
HRR	21.5 (18.2-28.7)	25.1 (22.2-36.1)	.139

Note: Values are the medians obtained from non-parametric tests (25% and 75% quartiles, respectively).

Abbreviations: ASDAS, Ankylosing Spondylitis Disease Activity Score; AxSpA, axial spondyloarthritis; BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HDL, high-density lipoprotein cholesterol; HLA, human leukocyte antigen; HRR, heart rate recovery; IPAQ, International Physical Activity Questionnaire; METs, metabolic equivalents; nrAxSpA; nonradiographic AxSpA; rAxSpA, radiographic AxSpA; TG, triglycerides.

inflammation contributes to atherosclerosis and plays a key role in CVD exacerbating CV risk factors.

We did not find any relationship between disease activity and HDL-c. The disease activity assessment tools such as ASDAS and inflammatory markers reflect an acute inflammatory status instead of the cumulative inflammation which plays a role on lipid metabolism. However, when we categorize the AxSpA patients in terms of radiographic sacroiliitis, that is rAxSpA and nrAxSpA, HDL-c levels were found to be significantly lower in the rAxSpA group. This is of interest and indicates lipid metabolism is altered by cumulative inflammation which is reflected by radiographic progression and structural damage of sacroiliac joints in rAxSpA. This result recalls a previous study in which radiographic progression in rAxSpA was found to be associated with a high 10-year CV event risk.[28] In our study, there was no difference in 10-year CV event risk or exercise capacity between patients with rAxSpA and nrAxSpA, which may be due to our relatively small sample size. Of interest, the FRS results of our AxSpA group (mean 8.7 ± 11) were similar to the results of the international, multicentric ASAS-COMOrbidities in SPondyloArthritis (COMOSPA) study (mean FRS scores 8.0 and 8.7 respectively, for SpA patients with axial and axial-peripheral involvement).[30]

Halvorsen et al.[6] found that EC was associated with CRP and ESR levels in AS patients. However, after adjustment for physical activity, they found that EC was no longer related to CRP or ESR which can be interpreted as limited physical activity due to active disease might be the reason for low EC.[6] Our results showed that CRP and ESR did not correlate with EC in any of the groups. In addition, there was no significant association between EC and ASDAS, although 68% of the AxSpA group had high or very high disease activity. In line with our study, O'Dwyer et al.[31] reported there was no relationship between physical fitness and disease activity in AS patients. The ASDAS is a composite index, incorporating patient-reported back pain, morning stiffness duration, patient-rated global disease activity, peripheral joint pain-swelling and CRP or ESR as the only objective finding of inflammation.[16] There are studies reporting ASDAS-CRP may evaluate disease activity inappropriately low for the patients with low CRP levels (<2 mg/L).[32] Thirty-four percent of our patients had low CRP levels at the time of assessment, which may have affected the results. Furthermore, disease activity measures reflect an acute inflammatory status more than inflammatory load, which is probably responsible for the increased CVD risk and decreased exercise capacity.

There are several studies confirming that smoking is related to high disease activity, worse clinical, functional and radiographic results in patients with AS.[33] We observed a significantly higher smoking ratio and tobacco exposure in AxSpA patients than controls. The smoking ratio of our study group (63% of AxSpA and 29% of controls) was much higher than the smoking prevalence in the international ASAS-COMOSPA study (38%),[30] while it was similar to a Greek study (61% vs 11% for SpA group and controls, respectively).[34] Smoking is

an established dose-dependent risk factor for atherosclerosis and CVD.[35] In our study, there was no significant relationship between EC and smoking. In addition, the EC difference between AxSpA and control groups remained significant after adjustment for smoking. However, FRS, which incorporates smoking status, was significantly associated with EC in patients and controls. In the literature, there are conflicting results regarding the impact of smoking history on EC where several studies found no impact of smoking on EC changes.[36] These results with ours suggest smoking does not directly affect EC but may have an overall effect with the other traditional risk factors on decreased EC in AxSpA.

The health-related benefits of physical activity are numerous and well documented for the general population. The physical activity profile of AxSpA patients is not clear and there are conflicting results about the benefits associated with physical activity.[37] Most studies reported similar levels of physical activity in AS and control subjects[6,7,38] except for O'Dwyer et al.[11] who reported that AS patients participate in less health-enhancing physical activity than controls. However, AS patients had lower EC than control subjects in several studies[6,7,11] except for Seçkin et al.[38] who found similar EC. Hsieh et al.[9] found no significant difference with regard to exercise tolerance between AS patients with and without exercise habits. In accordance with the literature, we found EC was significantly lower in the AxSpA group than controls while physical activity levels did not differ between the 2 groups. There was no association between physical activity level and EC in AxSpA patients in our study. These findings suggest similar levels of physical activity with healthy subjects are inadequate to maintain EC in AxSpA. On the other hand, although IPAQ is based on a global standard and is the most widely used physical activity questionnaire, it is based on patient-reported data and may cause a bias since an overestimation of moderate-vigorous physical activity was reported in a number of validation studies.[13,39] Further research with criterion measures (eg, accelerometer) would help to clarify the association between EC and physical activity in an AxSpA population.

Abnormal HRR which is an attenuated HR response following exercise cessation, indicates autonomic dysfunction, and is a significant and independent prognostic predictor of CV and all-cause mortality in both men and women.[21,40] There are few studies reporting parasympathetic dysfunction in AS.[41-43] In our study there was no significant difference in HRR between AxSpA and controls. However, we found HRR to be significantly associated with peak METs achieved and 10-year CV event risk in AxSpA patients. These results are of interest and indicate autonomic dysfunction may play a role in increased CV risk and decreased EC in AxSpA. A large prospective cohort of asymptomatic individuals followed up for more than 20 years reported that non-ECG exercise test results (HRR and peak METs) added important and incremental prognostic information to FRS in predicting fatal CVD in low and intermediate risk individuals. Low HRR and low METs were found to be related to increased CV mortality even in individuals with low FRS (<19%).[44] The results of our study suggest the application of these 2 simple exercise test parameters in AxSpA patients in addition to FRS seems feasible in CV risk assessment and may help tailoring management strategies.

This study has some limitations. First, EC was not measured by the criterion measure, that is ergospirometry. Nevertheless, estimating VO_{2peak} from a maximal exercise test is the second most valid test for EC which provides significant information on fatal CVD prediction.[6,44] Because of the cross-sectional design of this study, no causal pathways can be suggested. Since our exercise test protocol required walking on the treadmill, patients unable to walk were not included in the study which may have caused a selection bias. Our sample size was relatively small; however, it included patients with varying degrees of disease activity as well as structural damage. Further, a strength of this study is that it is the first to investigate EC and CV risk factors not only in rAxSpA, but also in nrAxSpA patients, reflecting the whole AxSpA population.

In conclusion, EC was lower in AxSpA patients than controls. EC was associated with age, BMI, waist circumference, TC, triglycerides and FRS in AxSpA patients. Attenuated HRR was significantly associated with low EC and high 10-year CV event risk in AxSpA. Strategies to enhance EC are advised to be implemented in the management of patients with AxSpA.

CONFLICT OF INTEREST

The authors declare they have no competing interest.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ebru Köseoğlu Tohma and Zafer Günendi. The first draft of the manuscript was written by Ebru Köseoğlu Tohma and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL

The study was approved by Gazi University Medical Ethics Committee and was registered at clinicalTrials.gov (NCT04706650). All the procedures were performed according to the World Medical Association Declaration of Helsinki.

CONSENT TO PARTICIPATE

Written informed consents were obtained from all participants.

CONSENT FOR PUBLICATION

All authors have approved the manuscript and agree with submission to *International Journal of Rheumatic Diseases*.

DATA AVAILABILITY STATEMENT

All data and materials support our published claims and comply with field standards.

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ORIGINAL ARTICLE

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Downregulation of miR-98-5p expression induces interleukin-6 expression in rheumatoid fibroblast-like synoviocytes

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Abstract

Aim: The increased level of interleukin-6 (IL-6) plays a significant role in the pathogenesis of rheumatoid arthritis (RA). Specific blockade of IL-6 or its receptor has been used successfully in treating RA. MicroRNAs can regulate gene expression and act as regulators of target genes. Manipulation of specific microRNAs provides a novel therapeutic strategy for treating/preventing diseases. This study explored the role of miR-98-5p in the regulation of IL-6 expression in rheumatoid fibroblast-like synoviocytes (RA-FLSs).

Methods: Real-time PCR was used to detect miR-98-5p expression in RA-FLSs and normal human fibroblast-like synovial cells (HFLSs). Site-directed gene mutagenesis and reporter gene assay were performed to identify the interaction between miR-98-5p and IL-6. Manipulation of miR-98-5p expression in RA-FLS used transfection with miR-98-5p mimic or inhibitor. Stimulation of FLSs with IL-1β induced IL-6 production. Enzyme-linked immunosorbent assay was used to detect the level of IL-6 secreted into the RA-FLS culture supernatant.

Results: Compared with HFLSs, the expression of miR-98-5p in RA-FLSs was significantly downregulated, and was negatively correlated with DAS28 scores and rheumatoid factor. In patients with anti-keratin antibody-positive RA, the expression level of miR-98-5p was lower. miR-98-5p negatively regulated the expression of IL-6 in RA-FLSs. After IL-1β stimulation, the expression of miR-98-5p decreased and the level of IL-6 protein was upregulated during IL-6 secretion.

Conclusion: These data suggest that manipulation of miR-98-5p, which negatively modulates IL-6 expression, may be a potential clinical approach in RA.

KEYWORDS

interleukin-6, microRNA, miR-98-5p, rheumatoid arthritis

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a common systemic autoimmune disease. The clinical manifestations are symmetric swelling and pain in the small peripheral joints, accompanied by a variety of extra-articular symptoms. If you do not provide timely intervention, RA will eventually lead to joint deformity and loss of function, seriously affecting the quality of life of patients and even leading to death.[1,2]

Conventional treatments have focused on disease-modifying anti-rheumatic drugs that can reduce inflammation and progressive

damage, such as methotrexate and leflunomide,[3] but they have the limitations of severe toxicity. During the onset of RA, a large number of pro-inflammatory cytokines are present in the synovial joints. These cytokines lead to the proliferation of synovial cells and the release of matrix metalloproteinases (MMPs), which in turn cause erosion and destruction of cartilage and bone. Biologics that can effectively block and regulate the action of pro-inflammatory cytokines become novel alternative therapies in patients with RA. Compared with conventional treatments, biologics are well tolerated and have higher efficacy and safety in alleviating symptoms, inhibiting bone erosion, and preventing loss of function.[4] Interleukin-6 (IL-6), a pro-inflammatory cytokine, is elevated and participates in the pathogenesis of RA.[5] It has been demonstrated that specific blockade of IL-6 or its receptor can quickly and effectively alleviate the signs and symptoms of RA.[6]

MicroRNAs (miRNAs) are endogenous small non-coding RNAs with a length of about 22 nucleotides. They can regulate gene expression at the post-transcriptional level by base pairing with sequences in the 3′ untranslated region (3′UTR) of target mRNAs.[7] Changes to miRNAs have been confirmed in RA. Several aberrantly expressed miRNAs play a crucial role in RA, and may be therapeutic targets and important biomarkers for its prediction and diagnosis.[8] For example, serum miR-24 and miR-125a are elevated in RA patients. The plasma concentrations of miR-24 and miR-125a-5p are potential diagnostic markers of RA.[9,10] miR-19 downregulates Toll-like receptor 2 expression, and represses the release of IL-6 and MMP-3 in rheumatoid fibroblast-like synoviocytes (RA-FLSs).[11] miR-15a successfully induces cell apoptosis by inhibiting BCL2 expression in the synovium in arthritic mice by the intra-articular injection of double-stranded miRNA into the joint.[12] Targeting miRNA might therefore be a novel therapeutic strategy for the treatment of RA.

Studies have shown that the expression of miR-98-5p in peripheral blood mononuclear cells of female RA patients is significantly lower than in those of male patients. The expression level of let-7f-2, a member of the Let-7/miR-98-5p family, is correlated with the erythrocyte sedimentation rate.[13] Interleukin-6 is a predicted target of the Let-7/miR-98-5p family. Our data suggested that miR-98-5p was significantly downregulated in RA-FLSs and negatively correlated with Disease Activity Scale 28 (DAS28) scores and rheumatoid factor. In patients with anti-keratin antibody (AKA)-positive RA, the expression level of miR-98-5p was lower. miR-98-5p directly regulated IL-6 expression in RA-FLSs; altering miR-98-5p expression affected IL-6 secretion in RA-FLSs. Gain-of-function of miR-98-5p might provide a potential novel strategy for treating RA.

2 | MATERIALS AND METHODS

2.1 | Cell culture and the preparations of rheumatoid fibroblast-like synoviocytes

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen Gibco, Waltham, MA, USA) supplemented with 10% FBS (Invitrogen Gibco, Grand Island, USA). In order to detect the miR-98-5p expression in fibroblast-like synoviocytes of patients with RA and individuals without RA, we collected synovial tissues from 10 cases of RA (seven men and three women, age 61.3 ± 7.9 years) and eight cases of traumatic fracture (five men and three women, age 51.1 ± 14.3 years) during joint replacement. Human fibroblast-like synoviocytes (HFLSs) were isolated by enzymatic digestion of the synovial tissue. Synovial tissues were minced and digested with 0.1% Type-I collagenase (Sigma-Aldrich, St Louis, MO, USA) in DMEM for 4 hours at 37℃. HFLSs were rinsed and re-suspended in high glucose DMEM containing 10% fetal bovine serum.

2.2 | Luciferase reporter constructs

To create 3′UTR luciferase reporter constructs, fragments of 3′UTR of IL-6 containing the predicted miR-98-5p binding site were added to the psiCHECK-2 vector downstream of the firefly luciferase cassette. We constructed a wild-type reporter vector (psiCHECK-2-IL-6-WT) and a mutant type reporter vector (psiCHECK-2-IL-6-MUT). Primers used were as follows: IL-6 wild-type (forward 5′-CACCTC GAGAAAGTATGAGCGTTAGGA-3′, reverse 5′-AAGCGGCCGCAAA CCATTTATACATTAT-3′); IL-6 mutant (forward 5′-CTTGGAAAGTGT AGGCTATAATCCTATAAATGGCTAACT-3′, reverse 5′-AGTTAGCCA TTTATAGGATTATAGCCTACACTTTCCAAG-3′). The DNA sequencing was used to confirm vector construction.

2.3 | Reporter gene assay

Twenty four hours before transfection, Hela cells were plated in 48-well plates at a density of 5×10^4/well. Then, 30 ng of 3′UTR luciferase reporter vector and miR-98-5p mimic or inhibitor, along with miRNA mimic or inhibitor control, were transfected into HeLa cells with lipofectamine 2000, respectively. Twenty-four hours after transfection, luciferase activity was measured using the Dual-Luciferase Reporter assay system. The ratio of Renilla luciferase to firefly luciferase per well was calculated. MicroRNA mimics or inhibitors used were as follows: miR-98-5p mimic (sequence: 5′-UGAGGUAGUAAGUUGUAUUGUU-3′); miR-98-5p inhibitor (sequence: 5′-AACAAUACAACUUACUACCUCA-3′); miRNA mimic control (sequence: 5′-UCACAACCUCCUAGAAAGAGUAGA-3′); miRNA inhibitor control (sequence: 5′-CAGUACUUUUGUGUAGUACAA-3′). MicroRNA mimics and inhibitors were obtained from GenePharma Company Limited (Shanghai, China). MicroRNA mimics are double-stranded RNA oligonucleotides, and miRNA inhibitors are single-stranded oligonucleotides.

2.4 | Re-expression and suppression of miR-98-5p in RA-FLSs

Twenty-four hours before transfection, RA-FLSs were observed in 24-well plates at a density of 5×10^4/well; 100 nM miR-98-5p mimic

or inhibitor was transfected with lipofectamine 2000. RA-FLSs transfected with miRNA mimic or inhibitor control were used as controls. Twenty-four hours after transfection, RA-FLSs were subjected to RNA extraction. The supernatants of RA-FLSs were collected for IL-6 protein analysis.

2.5 | Real-time polymerase chain reaction

Trizol reagent (Invitrogen Gibco, Grand Island, USA, ThermoFisher Scientific Inc., Waltham, MA, USA) was used to extract total RNA from HFLSs following the manufacturer's instructions. SuperScript II reverse transcriptase (Invitrogen; ThermoFisher Scientific Inc.) and oligo-dT primers were used to generate cDNA following the manufacturer's instructions. miR-98-5p expression was detected using a Taqman microRNA assay kit (Applied Biosystems, Foster City, CA, USA, ThermoFisher Scientific Inc., Waltham, MA, USA). RNU66 served as a normalization control. For IL-6 mRNA measurements, the cDNA was amplified by real-time polymerase chain reaction (PCR) with an SYBR Premix Ex Taq™ RT-PCR kit (Takara Biotechnology Co, Ltd, Dalian, China). Ribosomal protein L13A (RPL13a) served as a normalization control. Interleukin-6 primers (forward primer, 5′-GATGAGTACAAAAGTCCTGATC; reverse primer, 5′-CTGCAGCCACTGGTTCTGT) and RPL13A primers (forward primer, 5′-CCTGGAGGAGAAGAGGAAAGAGA; reverse primer, 5′-TTGAGGACCTCTGTGTATTTGTCAA) were used for DNA amplification. Taqman microRNA assay and SYBR assay were performed on a 7900HT real-time PCR system (Applied Biosystems, ThermoFisher Scientific Inc.) in the following conditions: 95°C for 15 seconds followed by 40 cycles at 95°C for 5 seconds and 60°C for 30 seconds, and then at 95°C for 15 seconds, 60°C for 15 seconds and 95°C for 15 seconds, respectively. The relative expression of miR-98-5p and IL-6 were quantified using the $2^{-\Delta\Delta Ct}$ method.

2.6 | Enzyme-linked immunosorbent assay

The contents of IL-6 secreted into the RA-FLSs and normal HFLSs culture supernatants were determined by an enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, Thermo Fisher Scientific, San Diego, CA, USA). We performed measurements following the manufacturer's instructions.

2.7 | Data analysis

Statistical analysis was performed using GRAPHPAD PRISM 5.0 software (GraphPad, San Diego, CA, USA). Comparisons between the two groups and reporter gene activity were performed with the Mann-Whitney test. The correlation between miR-98-5p expression and clinical parameters of RA patients was made using Pearson's correlation test. A P value less than 0.05 was set as a significant difference.

3 | RESULTS

3.1 | miR-98-5p expression was downregulated in RA-FLSs

Rheumatoid fibroblast-like synoviocytes were obtained from 10 individuals with RA and eight individuals with traumatic fracture during joint replacement. Table 1 summarizes the characteristics of RA patients as described above. The expression of miR-98-5p in RA-FLSs and normal HFLSs was detected by RT-PCR, and the results showed that miR-98-5p was significantly downregulated in RA-FLSs compared with normal HFLSs (Figure 1A; $P = 0.020$). Then, we analyzed the correlation between the miR-98-5p level and clinical characteristics. As shown in Figure 1B,C, miR-98-5p was negatively correlated with DAS28 scores and rheumatoid factor ($P = 0.046$;

TABLE 1 Characteristics of the study participants

No.	Gender	Age	RA course (year)	Treatment	ILD	ESR (mm/h)	CRP (mg/L)	RF (IU/mL)	Anti-CCP (U/mL)	AKA	DAS28	miR-98-5p
1	F	62	12	NSAID + LEF + SSZ	Yes	32	0.5	113	200	−	3.06	1.52
2	F	55	8	NSAID + LEF + T-614	Yes	11	0.5	366	200	−	1.68	1.42
3	F	71	12	S + LEF + TNFi	Yes	39	6.17	114	200	−	3.85	1.37
4	M	68	6	MTX + HCQ	No	36	11.3	514	200	+	3.51	1.03
5	F	52	12	NSAID + HCQ	Yes	21	0.96	11.1	200	−	2.99	1.13
6	M	53	15	NSAID + S	No	42	5.62	179	20	−	3.27	1.25
7	F	70	2	NSAID + MTX + LEF	No	45	7.51	1110	200	+	4.31	0.60
8	F	59	5	NSAID + MTX	No	42	27.02	161	200	+	5.01	0.84
9	F	70	20	NSAID + LEF + T-614	No	43	14.18	477	129	−	4.89	1.12
10	M	53	4	NSAID + LEF	No	59	26.06	1100	100	−	3.92	0.93

Abbreviations: −, negative; +, positive; AKA, anti-keratin antibodies; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; DAS, Disease Activity Scale; ESR, erythrocyte sedimentation rate; F, female; HCQ, hydroxychloroquine; ILD, interstitial lung disease; LEF, leflunomide; M, male; MTX, methotrexate; NSAID, nonsteroidal anti-inflammatory drugs; RF, rheumatoid factor; S, corticosteroids; SSZ, sulfasalazine; T-614, iguratimod; TNFi, tumor necrosis factor inhibitors.

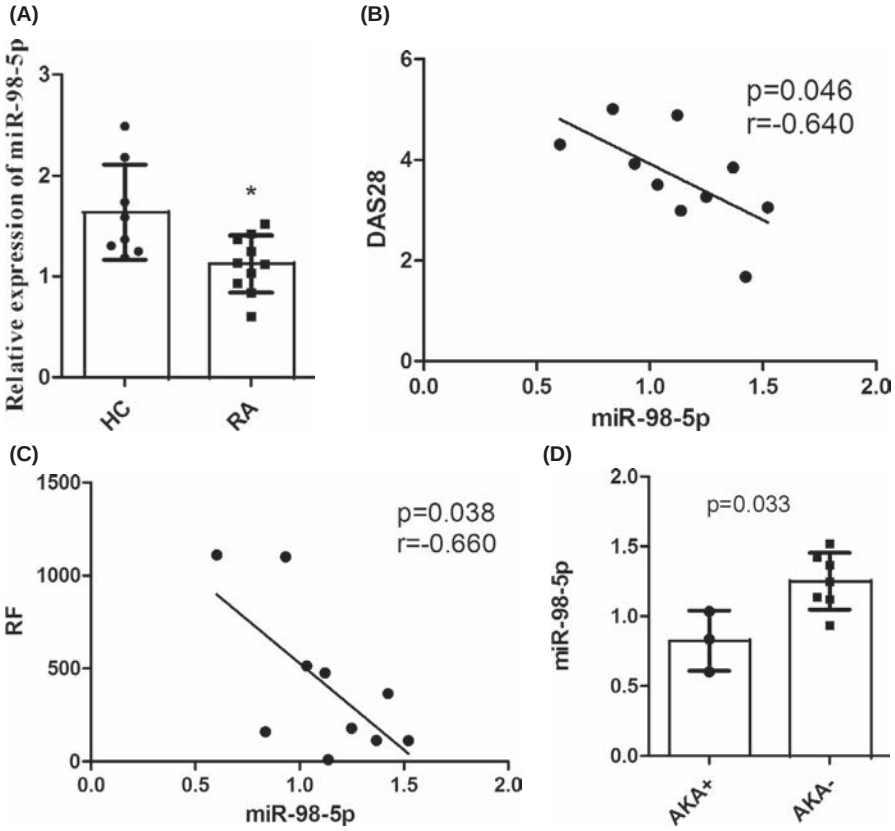

FIGURE 1 (A) Comparison of expression levels of the microRNA miR-98-5p in normal human fibroblast-like synoviocytes (HFLSs) and rheumatoid fibroblast-like synoviocytes (RA-FLSs). (B) Correlation between the expression of miR-98-5p and DAS28 in patients with rheumatoid arthritis. (C) Correlation between the expression of miR-98-5p and rheumatoid factor (RF) in patients with rheumatoid arthritis. (D) Comparison of expression levels of miR-98-5p in anti-keratin antibody (AKA) -positive and negative-patients. Data are presented as the mean ± standard deviation. *$P < .05$, compared with the control

$P = 0.038$, respectively). The expression level of miR-98-5p in AKA-positive patients was lower (Figure 1D; $P = 0.033$).

3.2 | IL-6 was a predicted target of miR-98-5p

To search for the predicted target of miR-98-5p, TargetScan (http://www.targetscan.org/) was used to scan for the potential miR-98-5p target genes sites in IL-6. TargetScan predicted that IL-6 had a putative target site for miR-98-5p in the 3′UTR region.

3.3 | miR-98-5p directly interacted with IL-6

By TargetScan analyses, we found that the putative target site of miR-98-5p was located in the IL-6 3′UTR. To verify whether IL-6 was indeed a target of miR-98-5p, we constructed a wild-type reporter vector (psiCHECK-2-IL-6-WT), which contained the IL-6 3′UTR with the putative target site for miR-98-5p (Figure 2A). We also constructed a mutant-type vector (psiCHECK-2-IL-6-MUT), which contained the mutation of the binding sequences TACCTCAA to ATAATCCT (Figure 2A). Dual-luciferase reporter assays with wild-type or mutant-type reporter vector were performed by gain-of-function or loss-of-function of miR-98-5p, respectively. miR-98-5p mimic, which can overexpress endogenous mir-98-5p, reduced luciferase activity of the psiCHECK-2-IL-6-WT in a dose-dependent manner in HeLa cells (Figure 2B). miR-98-5p inhibitor, which can inhibit endogenous mir-98-5p, increased luciferase activity of the psiCHECK-2-IL-6-WT in Hela cells (Figure 2C). However, miR-98-5p had no effect on the psiCHECK-2-IL-6-MUT (Figure 2D). Collectively, these results supported the idea that IL-6 was the direct target of miR-98-5p.

3.4 | Induction of IL-6 by IL-1β in normal HFLSs and RA-FLSs

miR-98-5p was significantly downregulated in RA-FLSs compared with normal HFLSs. miR-98-5p could directly target IL-6. To determine whether the expression of IL-6 was abnormal in synovial fibroblasts of RA patients compared with normal synovial fibroblasts, we stimulated RA-FLSs and normal HFLSs with IL-1β (1 ng/mL), and then used ELISA to detect the expression of IL-6 in the supernatant. The results showed that the expression of IL-6 in RA-FLSs was greater in response to IL-1β compared with normal HFLSs (Figure 3).

3.5 | Manipulation of IL-6 with altered miR-98-5p expression in RA-FLSs

To evaluate whether miR-98-5p had any influence on endogenous IL-6 expression in RA-FLSs, we transfected RA-FLSs with mir-98-5p mimic or inhibitor for 24 hours. Then we detected IL-6

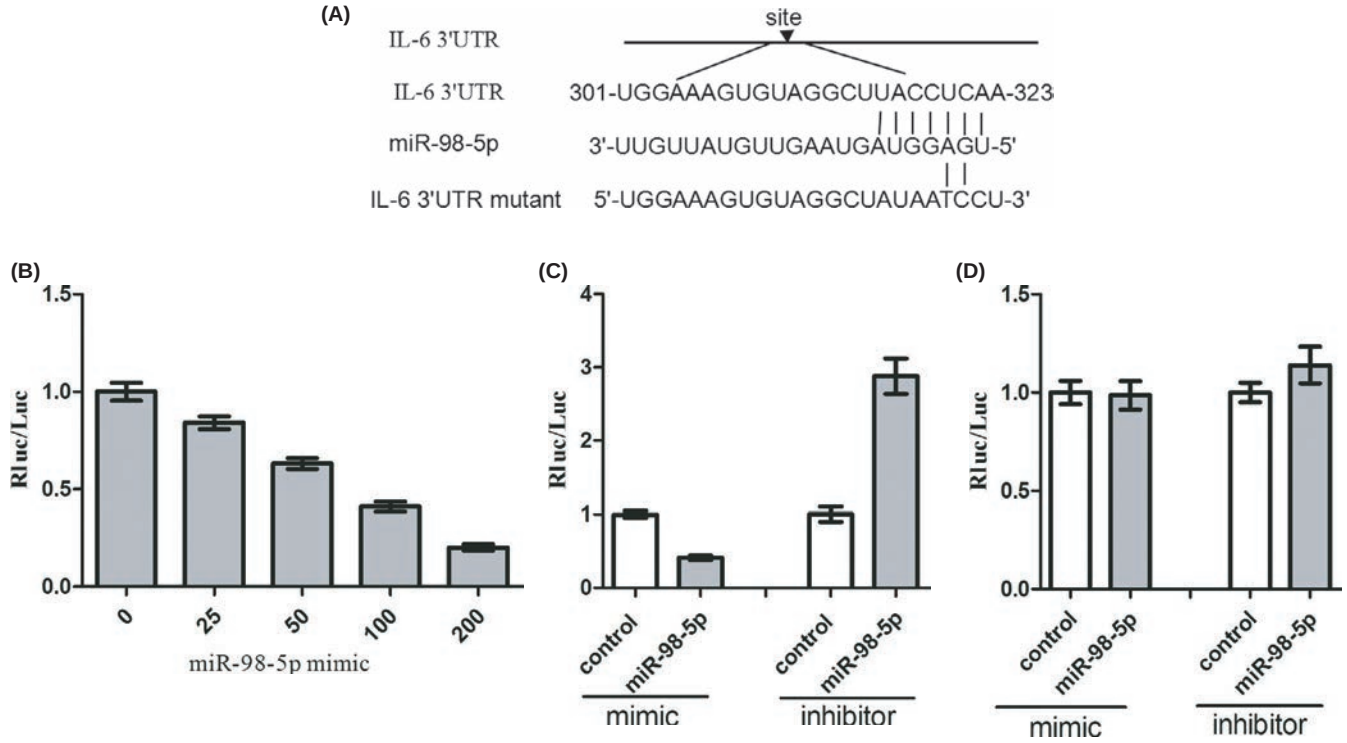

FIGURE 2 (A) The 3′ untranslated region (3′UTR) of interleukin-6 (IL-6) contains an miR-98-5p predicted binding site. Upper panel, the putative miR-98-5p binding sequence in the 3′UTR of IL-6 mRNA; Lower panel, the IL-6 3′UTR mutant. Numbers indicate positions of nucleotides. (B) mir-98-5p mimic decreased luciferase activity of the psiCHECK-2-IL-6-WT in a dose-dependent manner in Hela cells. (C) miR-98-5p mimic decreased, whereas miR-98-5p inhibitor increased luciferase activity of the psiCHECK-2-IL-6-WT in HeLa cells. (D) miR-98-5p did not impact luciferase activity of the psiCHECK-2-IL-6-MUT. Mimic or inhibitor control was labelled as 'control'. Data are presented as the mean ± standard deviation

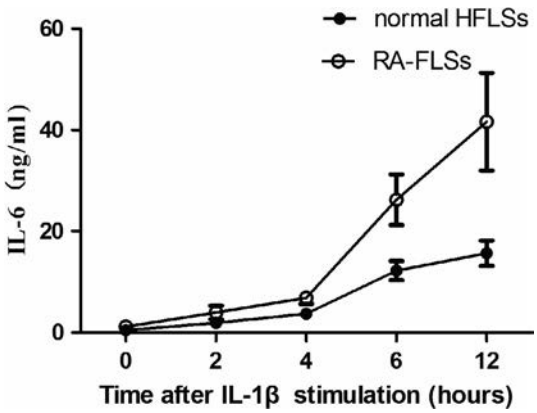

FIGURE 3 Induction of interleukin-6 (IL-6) by IL-1β in normal human fibroblast-like synoviocytes (HFLSs) and rheumatoid fibroblast-like synoviocytes (RA-FLSs)

mRNA and protein levels by quantitative RT-PCR and ELISA, respectively. These assays showed that miR-98-5p mimic suppressed IL-6 mRNA expression and the protein levels of IL-6 secreted into the RA-FLSs culture supernatant. In contrast, miR-98-5p inhibitor increased IL-6 expression (Figure 4). These data suggested that miR-98-5p could regulate IL-6 expression at mRNA and protein levels in RA-FLSs.

3.6 | miR-98-5p expression downregulated in IL-6 secretion process

Previous research found that IL-1β can induce IL-6 production in RA-FLSs. To examine whether miR-98-5p played roles in IL-6 secretion, we investigated the expression of miR-98-5p and IL-6 after the stimulation of IL-1β in RA-FLSs. We stimulated RA-FLSs with IL-1β, and then measured miR-98-5p and IL-6 levels using quantitative RT-PCR and ELISA at different times, respectively. In the process of IL-6 secretion, the expression of miR-98-5p decreased and the level of IL-6 protein increased (Figure 5). The result demonstrated that miR-98-5p was involved in IL-6 secretion and probably serves as regulator of the inflammatory process.

4 | DISCUSSION

Rheumatoid arthritis is a chronic inflammatory disease. There are a large number of inflammatory cytokines in the RA joint cavity, such as tumor necrosis factor-α (TNF-α), IL-6, and IL-1. These inflammatory cytokines lead to the proliferation of synovial cells and the release of MMPs, which in turn cause erosion and destruction of cartilage and bone.

Interleukin-6 is a crucial cytokine among a variety of inflammatory cytokines. Human IL-6 is a four-helix protein containing

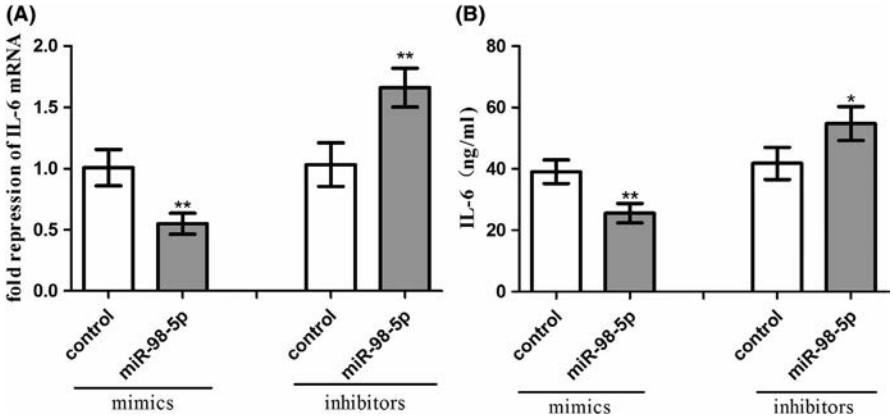

FIGURE 4 A, Interleukin-6 (IL-6) mRNA was regulated by the microRNA miR-98-5p in rheumatoid fibroblast-like synoviocytes (RA-FLSs). B, IL-6 protein was regulated by miR-98-5p in RA-FLSs. Data are presented as the mean ± standard deviation.*$P < 0.05$ and **$P < 0.01$, compared with the control

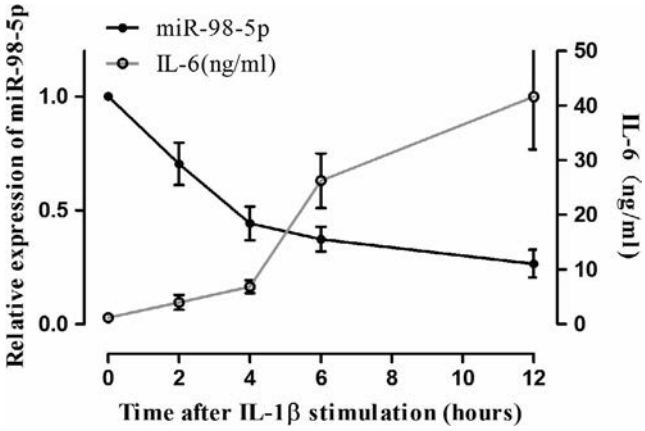

FIGURE 5 Expression levels of the microRNA miR-98-5p and interleukin-6 (IL-6) in rheumatoid fibroblast-like synoviocytes (RA-FLSs) stimulated with IL-1β (1 ng/mL), Data are presented as the mean ± standard deviation

184 amino acid residues.[14] It serves a crucial role in many physiological responses, for instance, acute phase response, T-cell proliferation, B-cell differentiation, and leukocyte chemotaxis.[15] In patients with RA, IL-6 is elevated and triggers various systemic inflammatory processes, resulting in acute reactions, fever, fatigue, and anemia.[16] Interleukin-6 can mediate crosstalk between endothelial cells and fibroblast-like synoviocytes, and promote the recruitment of inflammatory leukocytes (such as neutrophils and monocytes) into the rheumatoid synovium.[17] Meanwhile, IL-6 is an excellent stimulator of osteoclastic bone resorption by activating the expression of nuclear factor-κB ligand.[18] Moreover, IL-6 is involved in the activation of $CD4^+$ T helper (Th) cells. The activated $CD4^+$ Th cells can secrete interferon-γ (Th1) and IL-17, and then break the balance between Th17 cells and protective Treg cells.[19,20] Furthermore, IL-6 promotes the differentiation of B cells to autoantibody-producing plasma cells and triggers degenerative and inflammatory processes.[21]

Precursor experiments revealed that IL-6 is elevated in serum and synovial fluid of RA patients. The elevated IL-6 levels have positive correlation with imaging joint destruction and disease activity.[22,23] Based on the crucial role of IL-6, IL-6 antagonists have been used for the treatment of inflammatory disease, especially RA. Interleukin-6 antagonists have been shown to be effective in treating patients with early-diagnosed RA who did not receive disease-modifying anti-rheumatic drugs[24] and patients with RA that failed to respond to other anti-rheumatic therapies, including TNF inhibitors.[25-29]

MicroRNAs have been identified as regulators of target gene expression and are involved in a variety of biological processes, such as cell differentiation, proliferation, apoptosis and immunity.[30] miR-98 has been found to contribute to the pathogenesis of arthritis. It was reported that miR-98 was upregulated in osteoarthritic tissue. miR-98 reduced the production of IL-1β induced TNF-α and MMP13 in isolated human chondrocytes, indicating that miR-98 has an anti-inflammatory effect.[31] Moreover, miR-98 could regulate Dicer Expression, which was associated with disease activity and balanced the production of TNF-α in RA.[32] Furthermore, overexpression of miR-98 inhibited chondrocyte apoptosis in osteoarthritis.[33]

miR-98-5p was significantly downregulated in peripheral blood mononuclear cells of female vs male RA patients and let-7f-2, which was one member of the Let-7/miR-98-5p family, was correlated with the erythrocyte sedimentation rate.[13] Our data showed that miR-98-5p was significantly downregulated in RA-FLSs compared with normal HFLSs. miR-98-5p was negatively correlated with DAS28 scores. miR-98-5p could be used as a marker of RA disease activity. Rheumatoid factor and AKA were important diagnostic indicators for RA. Our research had shown that miR-98-5p was negatively correlated with the titer of rheumatoid factor. In patients with AKA-positive RA, the expression level of miR-98-5p was lower. Hence, miR-98 may be a potential diagnostic indicator for RA. Due to the small sample size of this study, further verification is required.

Manipulation of specific miRNAs seems to be a new therapeutic strategy in the treatment/prevention of human disorders.[34] Our research demonstrated that miR-98-5p negatively regulated the expression of IL-6 in RA-FLSs. After IL-1β stimulation, the expression of miR-98-5p decreased and the level of IL-6 protein was upregulated during IL-6 secretion. These data suggested that miR-98-5p might serve as a regulator of the inflammatory process and play a positive role in RA-FLSs. We believe that our data provide an important clue

for elucidating the regulation of IL-6 expression, and indicate that manipulation of miR-98-5 may be a potential clinical intervention in IL-6 expression in RA.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Approved by the Ethics Committee of Zibo Central Hospital. Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

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Circulating CD4$^+$ FoxP3$^-$ CXCR5$^-$ CXCR3$^+$ PD-1^{hi} cells are elevated in active rheumatoid arthritis and reflect the severity of the disease

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Abstract

Objective: To examine the expression and clinical significance of circulating CD4$^+$ FoxP3$^-$ CXCR5$^-$ CXCR3$^+$ PD-1^{hi} cells in rheumatoid arthritis (RA).

Methods: CD4$^+$ FoxP3$^-$ CXCR5$^-$ CXCR3$^+$ PD-1^{hi} cells in peripheral blood of 35 patients with active RA, 17 with RA in stable remission, and 24 healthy controls were analyzed by flow cytometry. Serum IgG and circulating plasmablast percentages were measured and correlations with CD4$^+$ FoxP3$^-$ CXCR5$^-$ CXCR3$^+$ PD-1^{hi} cells were systematically analyzed. Disease Activity Scale 28 (DAS28) scores were also calculated and correlation analysis with CD4$^+$ FoxP3$^-$ CXCR5$^-$ CXCR3$^+$ PD-1^{hi} cells was conducted. The levels of CD4$^+$ FoxP3$^-$ CXCR5$^-$ CXCR3$^+$ PD-1^{hi} cells were compared before and after disease-modifying anti-rheumatic drug treatment. Cytokine levels in plasma and cytokine secretion in CD4 cells were measured and their correlations with CD4$^+$ FoxP3$^-$ CXCR5$^-$ CXCR3$^+$ PD-1^{hi} cells were further analyzed.

Results: The levels of CD4$^+$ FoxP3$^-$ CXCR5$^-$ CXCR3$^+$ PD-1^{hi} cells in the peripheral blood of patients with active RA were significantly increased compared with healthy controls. CD4$^+$ FoxP3$^-$ CXCR5$^-$ CXCR3$^+$ PD-1^{hi} cells in patients with active RA were positively correlated with serum IgG and DAS28 scores. CD4$^+$ FoxP3$^-$ CXCR5$^-$ CXCR3$^+$ PD-1^{hi} cells were significantly decreased in patients after treatment. Plasma interleukin-10 concentrations and interleukin-10-positive CD4 cell percentages were significantly positively correlated with CD4$^+$ FoxP3$^-$ CXCR5$^-$ CXCR3$^+$ PD-1^{hi} cell levels.

Conclusion: Circulating CD4$^+$ FoxP3$^-$ CXCR5$^-$ CXCR3$^+$ PD-1^{hi} cells in patients with active RA are increased and could reflect the severity of the disease, which may play a potential role in the pathogenesis of RA.

KEYWORDS
autoantibodies, CXCR3, CXCR5, rheumatoid arthritis, T cells

Lei Zhao and Zhenxue Li contributed equally to this work.

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a common clinical chronic autoimmune disease with symmetrical peripheral polyarthritis as the main manifestation. Its main pathological manifestations include synovial tissue inflammatory cell infiltration, pannus formation, and progressive destruction of articular cartilage and bone, and even loss of function.[1,2]

One characteristic of RA is the production of a variety of autoantibodies.[3] At present, it is believed that the high activation of lymphocytes in vivo and the production of pathogenic autoantibodies caused by the interaction between T and B lymphocytes are important in the pathogenesis of RA.[4] However, the detailed pathogenesis of RA has not been fully elucidated, and the regulatory mechanism is still unclear. Therefore, in-depth research on the pathogenesis of RA is of great significance for promoting the cure of RA.

$CD4^+$ T-cell subsets play very important roles in the pathogenesis of RA. Related studies have shown that T helper type 17 (Th17) cells and regulatory T (Treg) cells with pro-inflammatory effects have opposite effects on autoimmunity and inflammation.[5–7] The occurrence of RA is related to the imbalance of peripheral Th17/Treg cells. The imbalance further promotes the continuous production of Th17 cells. This pro-inflammatory cytokine microenvironment was believed to play an important role in the occurrence of RA.[5–7] In recent years, studies have shown that follicular helper T (Tfh) cells, a specific $CD4^+$ T-cell subset, play a key role in the body's humoral immunity.[8–13] Tfh cells can help B cells in the germinal center perform class switching and somatic mutations, and to produce high-affinity antibodies.[9–11] It has been reported that circulating Tfh cells are increased in RA patients.[4,14,15]

In addition to Tfh cells, there are other T-cell subpopulations that have been reported to help the differentiation of B cells, thereby contributing to the production of antibodies. PD-1^{hi} $CXCR5^-$ $CD4^+$ T cells are phenotypically similar to Tfh cells and studies have found that infiltrating PD-1^{hi} $CXCR5^-$ $CD4^+$ T cells induce B-cell responses in breast cancer and RA,[16,17] which are functionally similar to PD-1^{hi} $CXCR5^+$ $CD4^+$ Tfh cells. Rao et al. defined PD-1^{hi} $CXCR5^-$ $CD4^+$ T cells as peripheral helper T cells and found that this subpopulation mainly expanded in the synovial fluid and inflamed tissues of patients with seropositive RA.[17] Caielli et al. reported a $CXCR5^-$ $CXCR3^+$ PD-1^{hi} $CD4^+$ helper T-cell population distinct from Tfh cells that was expanded in blood from individuals with systemic lupus erythematosus.[18] These $CXCR5^-$ $CXCR3^+$ PD-1^{hi} $CD4^+$ cells could provide B-cell help through interleukin-10 (IL-10).[18] However, the role of $CXCR5^-$ $CXCR3^+$ PD-1^{hi} $CD4^+$ cells in the peripheral blood of RA patients is still unclear. It is of great theoretical significance and potential diagnostic value to explain their expression changes and their clinical significance.

In this study, we collected the peripheral blood of RA patients with different stages of disease to analyze the changes of $CD4^+$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells. In order to remove the influence of Treg-related subgroups, we analyzed $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cell subsets and studied their relationship with the patient's condition, serum immunoglobulin and circulating plasmablast levels, and inquired into the potential role of related cytokines.

Highlights

1. The levels of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells in peripheral blood of patients with active RA were significantly increased.
2. $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells in patients with active RA were positively correlated with serum IgG and DAS28 scores.
3. $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells were significantly decreased in RA patients after treatment.
4. Plasma interleukin-10 concentrations and interleukin-10-positive CD4 cell percentages were significantly positively correlated with $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cell levels.

2 | MATERIALS AND METHODS

2.1 | Patients

A total of 52 RA patients and 24 healthy controls were enrolled from Peking University People's Hospital between October 2019 and April 2020. All RA patients were diagnosed according to the American College of Rheumatology criteria.[19] These 52 patients included 35 with active RA (any joint with active disease or any sign of systemic disease) and 17 patients in stable remission according to the 2014 update of the treat-to-target recommendations (absence of signs and symptoms of significant inflammatory disease activity),[20] and remission lasted for no less than 3 months. Twenty-four healthy controls were recruited from the Physical Examination Center. All included individuals were without any other autoimmune diseases or cancers or infectious diseases. None of the patients had received any glucocorticoid and/or immunosuppressive drug treatments within the previous 3 weeks. Within the active RA group, 11 patients who had undergone one course of standard disease-modifying antirheumatic drug treatment were followed and cell subsets were analyzed before and after the treatment. All the research was performed in accordance with the Declaration of Helsinki and was approved by the Medical Ethical Committee of Peking University People's Hospital. Informed consent was obtained from each individual involved.

2.2 | Flow cytometry

Peripheral blood samples were collected from these individuals for routine blood tests and the remaining blood was used for flow

cytometry analysis. Peripheral mononuclear blood cells (PBMCs) were isolated using Ficoll separation (Ficoll-Paque, Pharmacia, Uppsala, Sweden). PBMCs were washed twice in phosphate-buffered saline and incubated for 30 minutes together with fluorescent antibodies against surface markers. After being washed twice in phosphate-buffered saline, the PBMCs were intracellularly stained using a FoxP3 staining Buffer Kit (eBioscience, San Diego, CA, USA), and then incubated with allophycocyanin-conjugated anti-FoxP3. All the antibodies used in this study were from BioLegend (San Diego, CA, USA). After being washed again, samples were acquired by FACSCanto (BD Bioscience, San Jose, CA, USA).

2.3 | Cytokine measurement

Six cytokines were determined in the plasma using bead-based multiplex flow cytometry with the Aimplex® Human IL-2/IL-4/IL-6/IL-10/TNF-α/IFN-γ 6-plex kit (Quantobio, Beijing, China), according to the manufacturer's instructions.

2.4 | In vitro cell culture

PBMCs were cultured in RPMI-1640 medium containing 10% fetal bovine serum. The cells were stimulated with phorbol 12-myristate 13-acetate (50 ng/mL) and ionomycin (1 μg/mL) for 5 hours in 96-well flat-bottom plates at 37℃, with brefeldin A (3 mg/mL; Sigma-Aldrich, St Louis, MO, USA) in the medium. The cells were then harvested, stained with CD4 and then intracellularly stained with interferon-γ (IFN-γ), IL-4, IL-21, tumor necrosis factor-α (TNF-α), IL-10, IL-17A and FoxP3, and then analyzed by flow cytometry.

2.5 | Clinical parameters measurement

White blood cells and lymphocytes were measured using Sysmex XE-2100 (TOA Medical Electronics, Kobe, Japan). IgG was measured by IMMAGE800 (Beckman Coulter Inc., Brea, CA, USA). Disease activity was assessed by the Disease Activity Score in 28 joints (DAS28), using the C-reactive protein level, as previously reported.[21] C-reactive protein was detected using i-CHROMA (Boditech Med Inc., Chuncheon, Korea).

2.6 | Statistics

All analyses were completed using GraphPad Prism 5.0 software (GraphPad Software Inc., San Diego, CA, USA). The differences between two groups were analyzed by Student's *t* test or paired *t* test. The correlations between parameters were carried out by Spearmen correlation analyses. All statistical tests were two-tailed and *P* values less than 0.05 were considered to be significant.

3 | RESULTS

3.1 | The level of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells was significantly up-regulated in the peripheral blood of patients with active RA

We included 35 patients with active RA, 17 RA patients in stable remission, and 24 healthy controls, and extracted their PBMCs for flow analysis. To exclude the interference of Treg-cell-related subsets, we further analyzed $CXCR5^-$ $CXCR3^+$PD-1^{hi} cells in $CD4^+$ $FoxP3^-$ cells (Figure 1A). The results showed that the percentages of $CXCR3^+$ PD-1^{hi} cells in $CD4^+$ $FoxP3^-$ $CXCR5^-$ cells and the absolute number of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells (per mL) were significantly higher in patients with active RA than in healthy controls ($P < 0.05$), although there was no significant difference between the healthy controls and RA patients in stable remission, or between active RA and stable remission RA (Figure 1B).

3.2 | $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cell level was significantly correlated with serum IgG level and circulating plasmablasts in patients with active RA

We further analyzed the relationship between $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells and related clinical indicators in RA patients. We analyzed the levels of IgG, IgA, and IgM in the serum of patients with active RA, and used correlation analysis to study their relationship with circulating $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells. The results showed that $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells, including both percentages and absolute numbers (per mL), were significantly positively correlated with only the IgG level ($r > 0$, $P < 0.05$), (Figure 2A), but there were no significant correlations with IgA or IgM levels, and the correlation with serum rheumatoid factor was also not significant (results not shown).

We further analyzed the levels of $CD38^{hi}$ $CD24^-$ plasmablast in $CD19^+$ cells by flow cytometry,[22] and then analyzed the correlation between $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells and plasmablast cells (Figure 2B). Results showed that the percentages and absolute numbers (per mL) of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells were significantly positively correlated with plasmablast levels.

3.3 | $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells are related to the severity of the disease in patients with active RA

We further analyzed the relationship between $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells and the severity of RA patients. We calculated the DAS28 scores for patients with active RA, based on C-reactive protein levels and joint tenderness and swelling.[21] The proportions and absolute numbers (per mL) of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells and the DAS28 scores showed significant positive correlations (Figure 3).

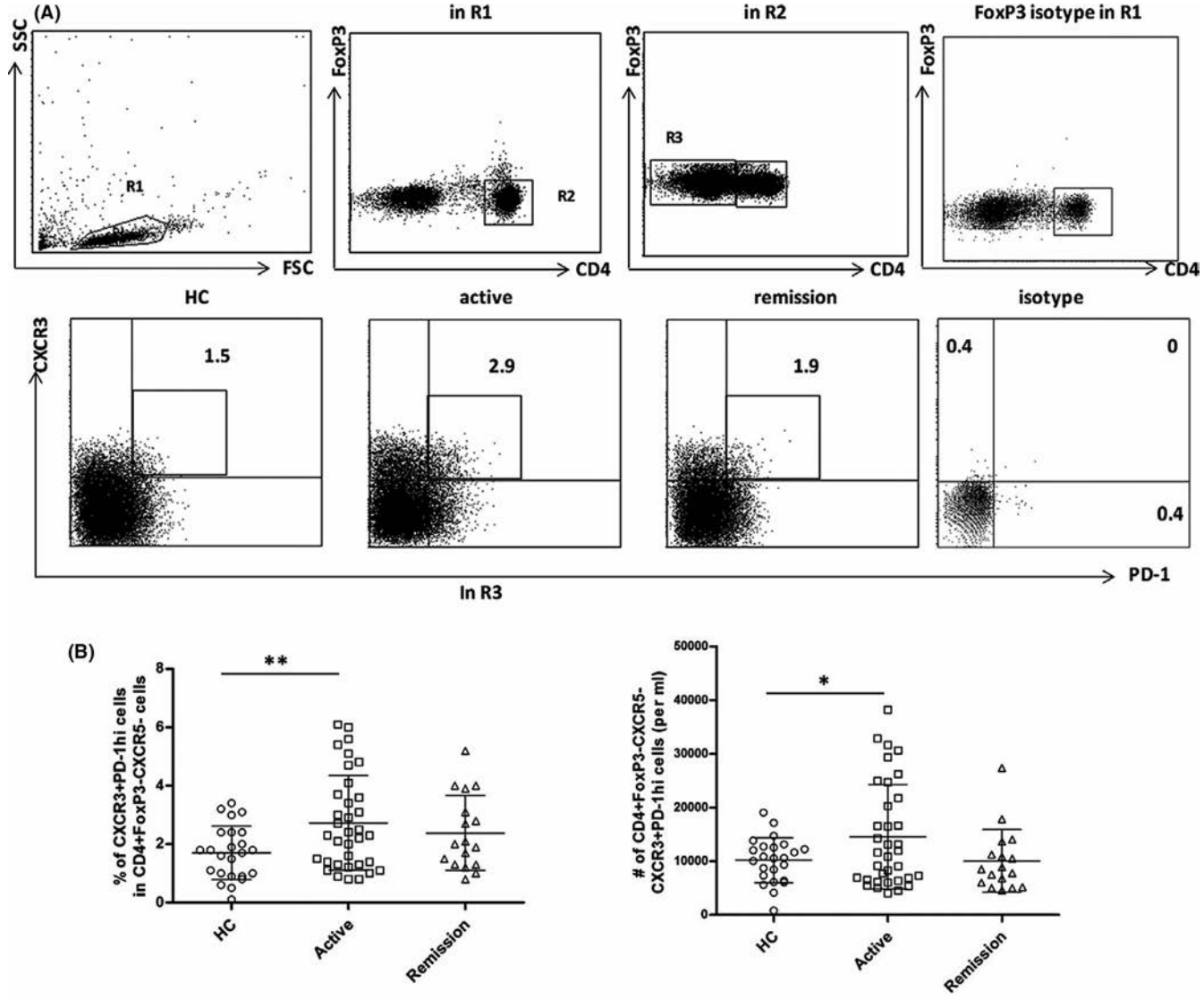

FIGURE 1 Increased $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells in peripheral blood of patients with active rheumatoid arthritis (RA). Peripheral blood mononuclear cells from peripheral blood of patients with active RA ($n = 35$), stable remission RA patients ($n = 17$) and healthy controls (HCs) ($n = 24$) were collected and $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells were measured by FACS through staining of CD4, CXCR5, CXCR3, PD-1 and FoxP3. (A) Representative flow cytometry dot plots used in this study. Numbers indicate $CXCR3^+PD\text{-}1^{hi}$ percentages in $CD4^+$ $FoxP3^-$ $CXCR5^-$ lymphocytes. The isotype comparison chart of FoxP3, CXCR3, and PD-1 is also displayed. (B) The comparisons of the frequencies and absolute numbers (per mL) of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells in active RA, stable remission RA and healthy controls. Data are expressed as mean ± SD and symbols represent individual participants. $^{**}P < 0.01$; $^{*}P < 0.05$

3.4 | The level of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells showed a significant decrease after treatment in patients with active RA

We followed up 11 of the patients with active RA and they achieved remission after one course of standard disease-modifying antirheumatic drug treatment. We acquired their peripheral blood samples after treatment and analyzed the levels of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells and compared them with those before treatment. The results showed that $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cell percentages and absolute numbers (per mL) after treatment were significantly lower than those before treatment (Figure 4).

3.5 | Plasma IL-10 and IL-10/IL-21-secreting CD4 cell levels are significantly positively correlated with circulating $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cell levels

We further analyzed the potential effects of cytokines on $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells. On the one hand, we used bead-based multiplex flow cytometry to detect the level of IL-2/IL-4/IL-6/IL-10/TNF-α/IFN-γ in plasma, and on the other hand we detected the secretion of IFN-γ/IL-4/IL-21/TNF-α/IL-10/IL-17A in CD4 cells by culturing PBMCs in vitro and calculated their positive percentages in CD4 cells. For plasma cytokines, we found that the concentration of IL-10 was significantly

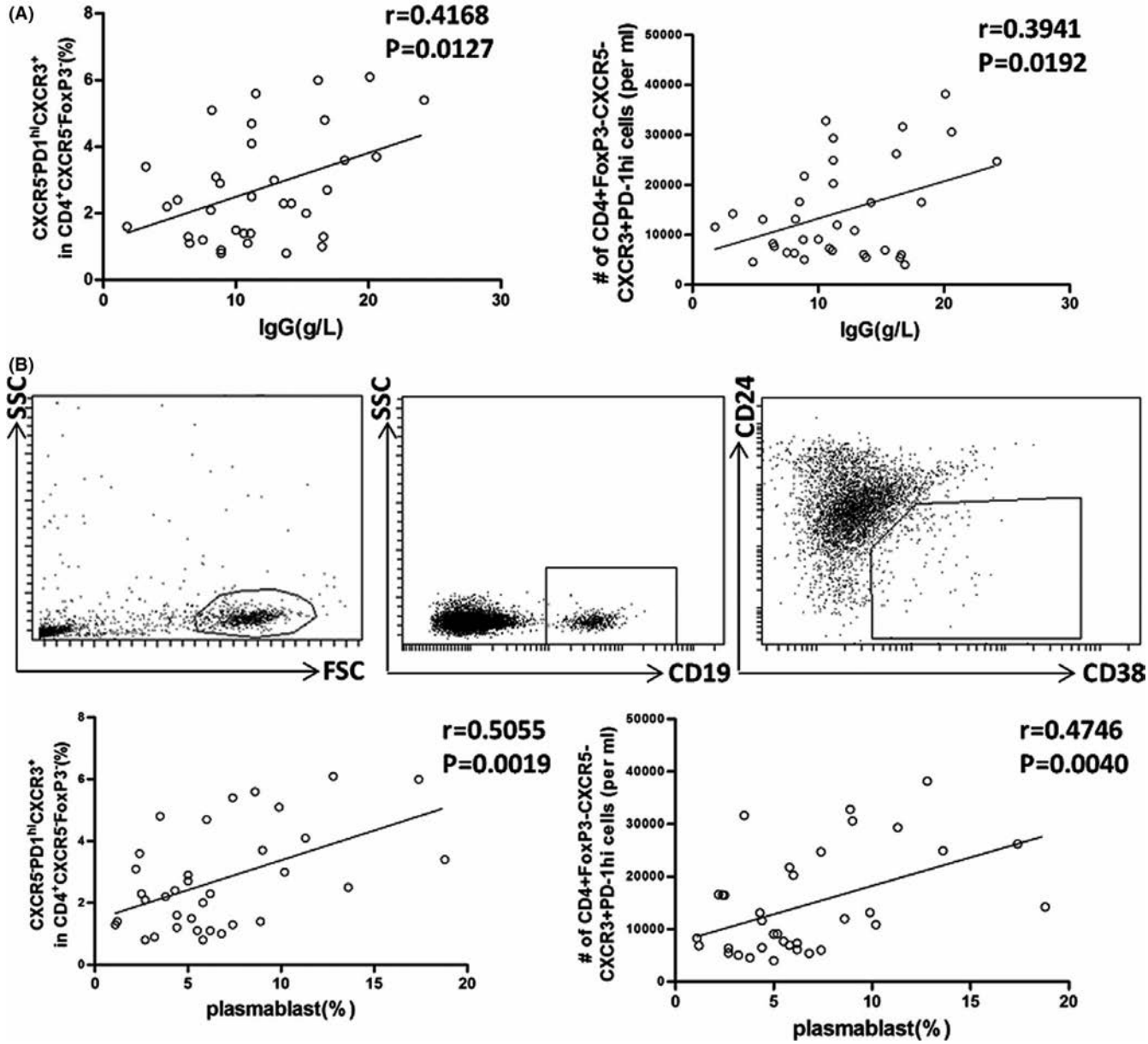

FIGURE 2 Correlation analyses between $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells, serum IgG and plasmablast percentages in peripheral blood B cells. A, Serum IgG levels of patients with active rheumatoid arthritis (RA) ($n = 35$) were analyzed and correlation analyses were performed between serum IgG concentrations and percentages and absolute numbers (per mL) of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells. B, Peripheral blood from patients with active RA ($n = 35$) was collected and plasmablast levels were analyzed by staining for CD19, CD24 and CD38. Representative dot plots for analyzing $CD19^+$ $CD24^-$ $CD38^{hi}$ plasmablasts were shown. Correlation analyses were conducted between plasmablasts and percentages and absolute numbers (per mL) of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells. The *r*-values were the Spearman's correlation coefficients, and $P < 0.05$ was linearly regressed to show relevant trends

positively correlated with the percentages and absolute numbers (per mL) of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells (Figure 5A), whereas other cytokines did not show a significant correlation. For intracellular cytokine staining, we found that $IL\text{-}10^+$ percentages in $CD4^+$ $FoxP3^-$ cells and $IL\text{-}21^+$ percentages in $CD4^+$ $FoxP3^-$ cells were significantly positively correlated with the percentages of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells (Figure 5A). For other cytokines, the proportions of positive cells did not show significant correlations with $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells.

4 | DISCUSSION

In this study, we for the first time inquired into the role of $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ $CD4^+$ cells in RA patients. We found that levels of circulating $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells in patients with active RA were significantly increased compared with healthy controls. In addition, this subset was positively correlated with serum IgG and DAS28 scores in patients with active RA. In terms of treatment, $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells were significantly decreased in patients with active RA after treatment with

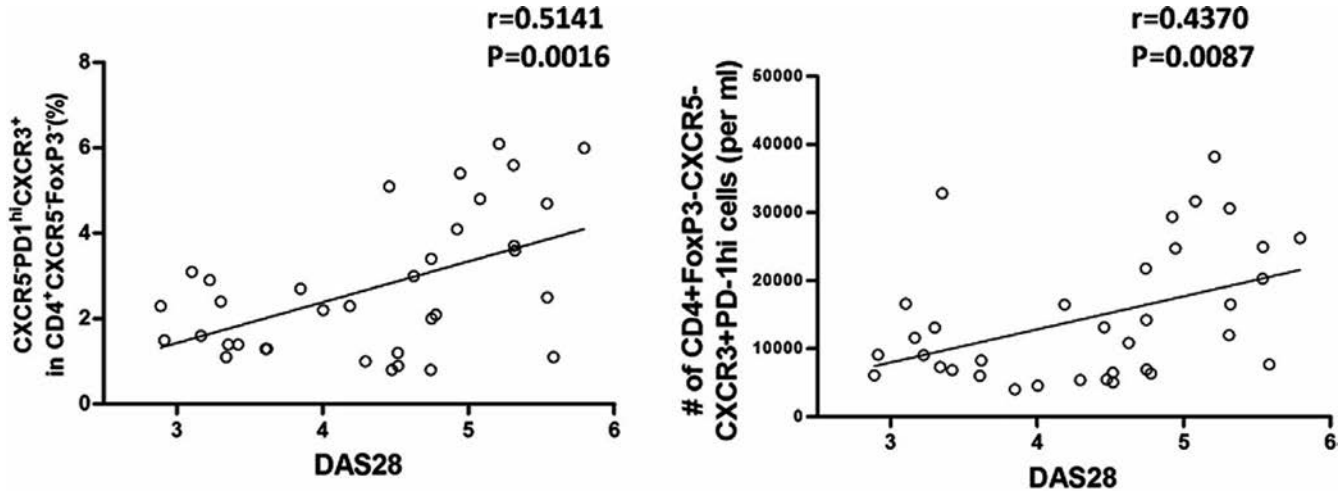

FIGURE 3 The correlation between circulating $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cell levels and DAS28 scores in patients with active rheumatoid arthritis (RA). The disease activity of patients with active RA ($n = 35$) was evaluated by DAS28 scores, and their correlations with $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cell percentages and absolute numbers (per mL) were performed. The *r*-values were the Spearman's correlation coefficients, and $P < 0.05$ was linearly regressed to show relevant trends

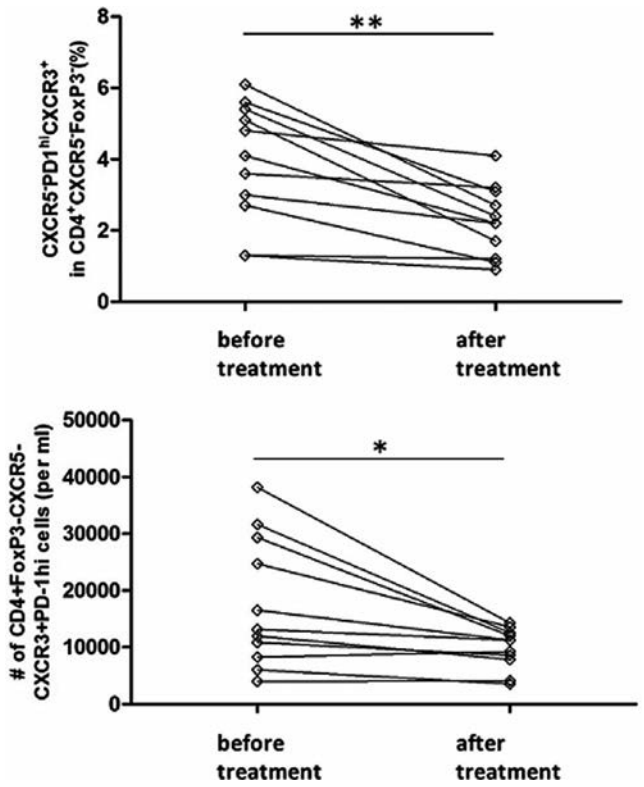

FIGURE 4 Analysis of changes in circulating $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cell levels before and after treatment in patients with active rheumatoid arthritis (RA). Peripheral blood was collected from 11 patients with active RA who were followed and had achieved stable remission after being treated with disease-modifying antirheumatic drugs. $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells were analyzed by flow cytometry and compared with their levels before treatment, by paired *t* test. $^{**}P < 0.01$; $^{*}P < 0.05$

disease-modifying antirheumatic drugs. Furthermore, plasma IL-10 concentrations and $IL\text{-}10^+$ CD4 cell percentages were significantly positively correlated with $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cell levels.

Changes in Tfh cells in RA have been studied, and it has been reported that circulating $CD4^+$ $CXCR5^+$, $CD4^+$ $CXCR5^+$ $PD\text{-}1^+$ and $CD4^+$ $CXCR5^+$ $PD\text{-}1^{hi}$ Tfh cells in RA patients are increased;[4,14,15] it is also suggested that the imbalance of Tfh and follicular Treg cells could reflect the conditions of RA patients.[23] However, the B-cell-related T-cell subsets that play a role in RA are not limited to Tfh cells. $PD\text{-}1^{hi}$ $CXCR5^-$ $CD4^+$ T cells are reported to be phenotypically similar to Tfh cells and induce B-cell responses in breast cancer and RA.[16,17] $PD\text{-}1^{hi}$ $CXCR5^-$ $CD4^+$ T cells are defined as peripheral helper T cells, which mainly expand in the synovial fluid and inflamed tissues of patients with seropositive RA.[17] In addition to peripheral helper T cells and Tfh cells, Caielli et al. reported a $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ $CD4^+$ helper T-cell population that is different from Tfh cells and is expanded in blood from individuals with systemic lupus erythematosus.[13] However, so far, the role of $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ $CD4^+$ cells in the peripheral blood of RA patients remains unclear. In this study, we initially explored the role of this subgroup of cells in RA, which has important theoretical value.

According to our results, only in patients with active RA can a significant increase in $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells be observed, whereas this situation is not obvious in patients whose RA is in remission, which suggests that $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells may affect autoantibody responses by participating in the regulation of humoral immunity. However, according to our results, this subset was only significantly positively correlated with IgG levels, and was not correlated with other immunoglobulins and rheumatoid factor, suggesting that this cell subset has limited regulatory effects. In addition, we proved that $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells were significantly correlated with peripheral blood plasmablast

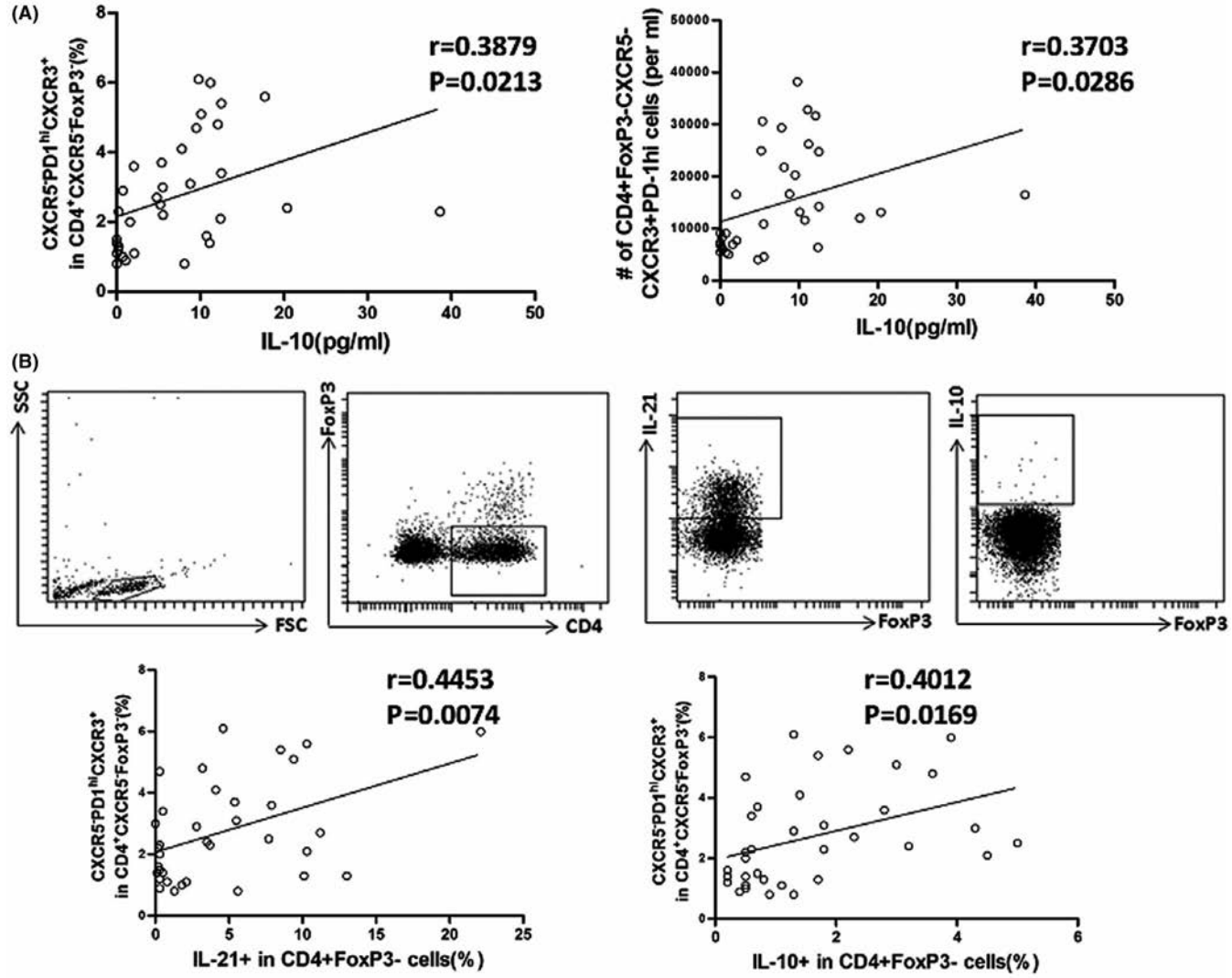

FIGURE 5 Analysis of the relationship between cytokine secretion in patients with active rheumatoid arthritis (RA) and circulating $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cell levels. A, Correlation analyses were performed between plasma interleukin-10 (IL-10) and $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cell percentages and absolute numbers (per mL) of 35 patients with active RA . B, Representative dot plots of IL-10 and IL-21 secretion levels in $CD4^+$ $FoxP3^-$ cells. Correlation analyses between IL-10^+/IL-21^+ percentages and $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cell percentages were carried out in 35 patients with active RA. All symbols represent individuals. The *r*-values were the Spearman's correlation coefficients, and $P < 0.05$ was linearly regressed to show relevant trends

levels, which suggests that $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells have the potential to help B-cell differentiation in active RA.

There are a few limitations to the present study. First, the sample size is not large enough, which will affect the accuracy of the results through sampling errors. But as an exploratory research, this research can provide a very important reference value for further multicenter large-sample research. Our study only used residual blood samples for testing after routine blood tests and did not take any intervention measures. Therefore, as an observational study, it is difficult to collect enough peripheral blood for in vitro culture. We still lack direct evidence to prove that changes in $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells can cause the onset of active RA, and we can only prove that this subset of cells is related to disease severity. Ethical considerations mean that we are not allowed to conduct intervention studies, and the role of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells can only be demonstrated through preliminary changes in the cell level before and after treatment. In the future, we need to conduct follow-up studies to directly demonstrate the role of this cell subset on the basis of animal experiments. We followed up only 11 patients with active RA who were treated, which is too few but the coronavirus disease 2019 pandemic led to a reduction in patient follow-up visits.

According to the reports of Caielli et al. $CXCR5^-$ $CXCR3^+$ PD-1^{hi} $CD4^+$ cells could provide B-cell help through IL-10 and succinate.[18] We conducted a preliminary exploration of the mechanism, and analyzed the relationship between cytokines and $CD4^+$$FoxP3^-$ $CXCR5^-$ $CXCR3^+$PD-1^{hi} cells by detecting the cytokines in plasma and the levels of cytokines secreted by CD4 cells. Among the six plasma cytokines analyzed, only IL-10 was significantly related to $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ PD-1^{hi} cells, suggesting that IL-10 has a potentially important relationship

with $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells. Among the six cytokines stained intracellularly, the levels of $IL\text{-}10^+$ and $IL\text{-}21^+$ cells were significantly positively correlated with the levels of $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells. Considering that IL-21 is critical for Tfh cell function and development,[24,25] this result suggests that $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells have a synchronous effect with Tfh cells, and therefore were positively related to $IL\text{-}21^+$ cells.

To conclude, we demonstrated in this study that $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells were significantly increased in patients with active RA and this up-regulated subset was related to IgG concentrations and plasmablast levels. In addition, $CD4^+$ $FoxP3^-$ $CXCR5^-$ $CXCR3^+$ $PD\text{-}1^{hi}$ cells can reflect the severity of RA, which has a potential role in the pathogenesis of RA.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

CL designed the research studies and revised the manuscript; LZ and ZL conducted most of the experiments and wrote the manuscript; CX and LX acquired and analyzed the data and contributed to the methods; YS did part of the experiments; QX contributed to methods; and XZ modified the manuscript.

DATA AVAILABILITY STATEMENT

Data can be provided by the corresponding author on request.

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ORIGINAL ARTICLE

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Turkish version of modified Hand Mobility in Scleroderma test: A translation and validation study

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Abstract

Objective: Hand Mobility in Scleroderma (HAMIS) is a hand function test used to determine the degree of dysfunction of hand movements. The Modified Hand Mobility in Scleroderma (mHAMIS), on the other hand, was developed later and consists of 4 items. The aim of this study was to determine the reliability and validity of mHAMIS.

Methods: This study included a total of 39 patients with systemic sclerosis (SSc) who were assessed with mHAMIS. The Cronbach's α coefficient, Kappa concordance, and intraclass correlation were respectively used to assess the internal consistency, intra- and inter-observer agreement, and inter-observer reliability of the test. The correlation between the Health Assessment Questionnaire (HAQ), the Duruoz Hand Index (DHI), and the Turkish version of mHAMIS were evaluated.

Results: The internal consistency of the test items was between .912 and .939. The total internal consistency of the test was excellent, with a Cronbach's alpha value of .954. The intra- and inter-observer agreement were good, with Kappa values of 0.954 (95% CI 0.89-1.6) and 0.965 (95% CI 0.82-1.4), respectively. The inter-observer reliability was 0.966 (95% CI 0.936-0.982; $P<.0001$). There was a strong correlation between DHI, HAQ, and mHAMIS (r: .7-.8).

Conclusion: The Turkish version of the mHAMIS test showed good intra- and inter-observer agreement, intra-observer reliability, and internal consistency. This test is a reliable and valid tool to assess hand functions in Turkish SSc patients.

KEYWORDS

disability, Duruoz Hand Index, HAMIS, SSc

1 | INTRODUCTION

Systemic sclerosis (SSc) is a connective tissue disease characterized by autoimmunity, vasculopathy, and fibrosis of the internal organs and skin.[1] Fibrosis causes the skin to thicken, reducing the mobilization of different segments of the body.[1] Although SSc involves multiple organs and large areas of skin, the distal segments of the limbs, accordingly the hands, are more affected. Approximately 90% of SSc patients experience difficulty in holding objects.[2,3] Hands, which are of particular importance for work, intellectual, artistic, and leisure activities, are critical for humans. Therefore, conditions that affect the functional capacity of the hands tend to negatively affect patients' quality of life.[3]

There is a need for specific tests to determine the extent of hand movement dysfunction in patients with SSc. To serve this purpose, the HAMIS (Hand Mobility in Scleroderma) test has been commonly used in countries where it has been validated and translated. This instrument was developed by 2 Swedish authors to assess the specific hand movements required for the instrumental activities of daily living.[2]

Previous studies have demonstrated that HAMIS can adequately distinguish hand functions of patients with SSc from those without SSc, with its high inter-observer and intra-observer reliability.[4,5] Cross-sectional and longitudinal follow-up studies on SSc patients have also shown a correlation between this test and organ involvement, thickening of the hand skin, as well as the effect of this condition on activities of daily living.[2,5-8]

In 2014, the authors of HAMIS developed the Modified Hand Mobility in Scleroderma (mHAMIS) as a shorter version of the test including 4 of the 9 original items of HAMIS.[9] It has been reported to correlate with the original HAMIS and the modified Rodnan skin score (mRSS). Moreover, it has been demonstrated to be able to distinguish between limited and diffuse forms of SSc in the first 3 years of the diagnosis. Accordingly, mHAMIS shows cutaneous disease activity in the early stage, while it can assess the extent of damage caused by vascular and fibrotic involvement in the later stages of the disease.[10]

Although this test has proven to be valuable for clinical follow-up of SSc patients and in studies regarding the subject, there is no Turkish translation or validation study on mHAMIS. Therefore, this study aimed to validate the Turkish version of mHAMIS for Turkish patients diagnosed with SSc.

2 | MATERIALS AND METHODS

2.1 | Patients and Methods

This cross-sectional study included a total of 39 adult patients with SSc who presented to the outpatient clinic of Pamukkale University between 15 January 2021 and 1 March 2021. After obtaining ethics approval for the study from the local institution, all patients who were followed up by our outpatient clinic for SSc were informed about the purpose of the study and invited to participate in the study on a voluntary basis, and consent was obtained from those who accepted to participate in the study.

Age, gender, medical treatments, disease duration, scleroderma type, Health Assessment Questionnaire (HAQ), and Duruoz Hand Index (DHI) scores of patients were recorded. Following a thorough physical examination, patients were divided into 2 groups based on the presence of arthralgia, deformity, digital ulcer, and pitting. Especially, those with comorbidities that may affect the hands, including neurological diseases, sequelae resulting from previous traumatic injuries, and clinically significant osteoarthritis were excluded from the study.

2.2 | Assessment Instruments

2.2.1 | Modified hand mobility in scleroderma

Modified hand mobility in scleroderma is a functional test used to assess 4 specific movements of the hand: extension, flexion and abduction of the fingers, and the dorsal extension of the hands. For this purpose, a variety of objects with a standardized measure like handles of cutlery tools, pencils, coffee packaging, a line spool, milk packaging, and a table are used. Using these objects, each movement of the hand is rated between 0-3 points. A score of 0 indicates normal movement, while a score of 3 indicates total failure to perform the relevant hand movement. The total score is calculated by the sum of the scores obtained from each movement, with the highest score of 12 points.[9,10]

2.2.2 | Health Assessment Questionnaire

The HAQ evaluates the functional ability of patients using 9 general categories (grooming and dressing, walking, arising, eating, hygiene, reach, grip, outdoor activity and sexual activity). For each category, one or more questions specific to the activity are asked of the responder. For example, "Can you shampoo your hair?" is one of the questions for assessing the grooming and dressing category. The score of each question ranges from 0 to 3 points. The total index score is calculated by summing the scores obtained from each question and dividing by the total number of categories.[11]

2.2.3 | Duruoz Hand Index

This includes 18 items to assess the activities of the hand and wrist. Responding to each item, patients rate their activities from 0 to 5 points, with a total score ranging from 0 to 90 points. A higher score indicates increased functional activity.[12]

2.3 | Translation and Face Validity

Permission to use the study was obtained from the authors of the original scale. The guidelines for cross-cultural adaptation were followed in the translation stage.[13] The original English version of mHAMIS was translated into Turkish by 2 independent translators, 1 of the study authors and a professional translator, who were native Turkish speakers fluent in English. The text was independently translated, the translations of the text were then compared. The differences between the 2 independent translations were discussed, and a consensus was reached on the final translation. The final Turkish version of mHAMIS was translated back into English by 2 independent native English speakers who were blinded to the original test. This back-translation and the original text were then compared to identify and review discrepancies. The differences between the translated versions were discussed, and the original scale was satisfactorily adapted to Turkish by the consensus of the translators. The Turkish version of mHAMIS was finalized after the translation and back-translation stages (Appendix 1).

2.4 | Inter-observer and Intra-observer Validation

Two researchers, including a previously trained medical student and an experienced rheumatologist, administered mHAMIS to patients

of a third group consisting of 39 consecutive patients with clinically stable SSc to assess one hand. The test was administered by the first researcher on day 1 and re-administered on day 15 to verify test-retest or intra-observer reproducibility. The patients had no change in their regular treatments during this interval. The second researcher administered the test to the same group of patients only on day 15 to verify inter-observer reproducibility.

2.5 | Statistical Analysis

IBM SPSS Statistics version 20.0 software (Armonk, NY, USA) was used for the analysis of the study data. The results of quantitative variables were presented as means, medians, and amplitudes, while categorical variables were presented as frequencies and percentages. The 2-group comparisons were performed using the non-parametric Mann-Whitney test. A *P* value of <.05 was considered statistically significant.

Spearman's correlation coefficients were estimated to evaluate the correlation between 2 quantitative variables and their significance was assessed. A correlation coefficient (*r*) value of >.91 was considered very strong, .90-.71 strong, .51-.7 moderate, .50-.31 weak, and an *r* value of <.30 was considered as absent.

The means of the Kappa concordance coefficient and a 95% confidence interval (95% CI) were used to verify the intra- and inter-observer agreement for each hand movement evaluated using the instrument, with a Kappa value of 0.81-1.0 indicating perfect agreement, 0.61-0.80 good agreement, 0.41-0.6 moderate agreement, 0.21-0.40 fair agreement, and 0-0.20 slight agreement.[2]

The intraclass correlation coefficient (ICC) and a 95% CI were used to evaluate the concordance (or reliability) of the continuous variables (final score obtained by summing the scores for each movement) for the Turkish version of mHAMIS, with an ICC value of >0.90 indicating excellent reliability, 0.75-0.9 good reliability, 0.5-0.75 moderate reliability, and <0.50 poor reliability.[4]

The Cronbach's alpha coefficient was used to evaluate internal consistency, with a Cronbach's alpha coefficient of >.9 indicating very good internal consistency, .8-.9 good internal consistency, .7-.8 acceptable internal consistency, .6-.7 weak internal consistency, and <.6 unacceptable consistency.[14]

3 | RESULTS

The test was administered to a total of 39 patients with SSc aged 28-74 years, with a median age of 55 years. The majority of participants had an advanced stage disease, with a median disease duration of 10 years. The median mRSS score was 23 (range 0-51) points. Table 1 shows further information on the clinical characteristics of the patients.

Each parameter of mHAMIS showed a very good internal consistency (Cronbach's alpha >.90). The exclusion of question 3 increased Cronbach's alpha value to .788. The Cronbach's alpha value for the entire scale was found to be .958 (Table 2).

TABLE 1 Characteristics of patients with systemic sclerosis (SSc)

	Patients with SSc (N = 39)
Sociodemographic data	
Women, n (%)	35 (90)
Disease duration, median (IQR)	11 (9)
Civil status, n (%)	
Married	35 (90)
Single	4 (10)
Level of education	
College or university	9 (23)
High school	8 (20)
Vocational school	12 (31)
Elementary school	10 (26)
Professional status, n (%)	
Employed	7 (18)
Student or unemployed	29 (74)
Retired	3 (8)
Disease variables	
Disease duration in y, median (IQR)	14 (8)
Limited cutaneous SSc, n (%)	14 (40)
Diffuse cutaneous SSc, n (%)	25 (60)
mRSS 0-51, median (IQR)	23 (11)
Organ involvement n (%)	
Skin	30 (77)
Lung system	28 (72)
Heart system	8 (21)
Kidney system	2 (5)
Comorbidity n (%)	
Cardiovascular disease	5 (13)
Thromboembolism	1 (3)
Diabetes mellitus	3 (8)
Depression or other psychological symptoms	6 (15)
Treatment	
Calcium channel blockers	14 (36)
Immunosuppressive treatment	16 (41)
Biologic agent	9 (23)
Corticosteroids	12 (30)
Antidepressants, anxiolytics	13 (33)

Abbreviation: IQR, interquartile range; mRSS, modified Rodnan Skin Score

The Turkish version of mHAMIS also showed excellent intra-observer and inter-observer reliability for the total score, which was calculated by summing the scores obtained from each item (Table 3). Moreover, an association was found between the Turkish version of the mHAMIS and clinical parameters such as type of disease, arthralgia, pitting, and digital ulcers (*P* <.05) (Table 4).

TABLE 2 Internal consistency of the Turkish version of the modified Hand Mobility in Scleroderma

Test item	Test mean if item excluded	Test variance if item excluded	Item-total correlation	Cronbach's alpha if item excluded
Finger flexion (Q1)	7.8	58.76	0.914	.801
Finger extension (Q2)	8.00	58.5	0.912	.799
Finger abduction (Q3)	8.0	56.9	0.962	.788*
Dorsal extension (Q4)	7.87	56.16	0.939	.794
All	4.46	18.25	0.958	

Note: *The Cronbach's alpha value increased to .788 when question 3 was excluded.

TABLE 3 Intra-observer and inter-observer agreement for each movement evaluated

Movement	Intra-observer agreement (Kappa)	Inter-observer agreement (Kappa)
Finger flexion (Q1)	0.889 (CI 95% 0.86-1)*	1*
Finger extension (Q2)	1*	0.848 (CI 95% 0.70-1)*
Finger abduction (Q3)	0.924 (CI 95% 0.65-1)*	0.849 (CI 95% 0.68-1)*
Dorsal extension (Q4)	0.891 (CI 95% 0.78-1)*	0.814 (CI 95% 0.76-1)*
All	0.954 (CI 95% 0.89-1)*	0.965 (CI 95% 0.82-1)*

Note: *P <.0001 for all movements evaluated.
Abbreviation: CI, confidence interval.

TABLE 4 Correlation between clinical parameters and the Turkish version of the mHAMIS

		Turkish version of mHAMIS			
Clinical parameters		Mean	Median	Range	*P* value
Form of disease	Diffuse SSc	6.3	5.5	0-12	.004*
	Limited SSc	1.8	1	0-8	
Digital ulcers	Presence	8.9	10	0-8	<.01*
	Absence	12.2	1	0-2	
Pitting scars	Presence	7.7	8	0-11	<.01*
	Absence	1.6	1	0-11	
Arthralgia	Presence	6.6	6	0-12	.04*
	Absence	3.0	1	0-2	

*P <.05, statistically significant; mHAMIS; Modified Hand Mobility in Scleroderma; SSc, systemic sclerosis

TABLE 5 Correlation between HAQ, DHI, and the Turkish version of mHAMIS

	mHAMIS	
	r	*P*
HAQ	.882	<.001*
DHI	.769	<.001*

Abbreviations: *r*, Spearman's rho coefficient; DHI, Duruoz Hand Index; HAQ, Health Assessment Questionnaire; mHAMIS, modified Hand Mobility in Scleroderma.
*P <.05, statistically significant

There was a strong correlation between the total scores of DHI and HAQ and mHAMIS (r = .762-.882) (Table 5). Furthermore, the ICC for the test-retest reliability of the Turkish version of mHAMIS was 0.998 for the total score (ICC=0.966; 95% CI 0.936-0.982; P<.0001) (Table 6).

4 | DISCUSSION

This study was conducted for cross-cultural translation and validation of mHAMIS for Turkish patients by evaluating the reliability, intra- and inter-observer agreement, and internal consistency. The results of our study revealed that the Turkish version of mHAMIS showed good psychometric properties in patients with SSc. The test-retest reliability of the Turkish version the mHAMIS seemed to be good. Therefore, the Turkish version of mHAMIS was reliable and valid in Turkish patients with SSc.

TABLE 6 Stability of the Turkish version of mHAMIS

	Initial score Mean ± SD (N = 39)	Retest score Mean ± SD (N = 39)	ICC (95% CI)
Finger flexion (Q1)	1.1 (0.7)	1.06 (0.1)	0.966 (0.936-0.982)
Finger extension (Q2)	1.05 (0)	1.1 (0)	1
Finger abduction(Q3)	1 (0.5)	1.2 (0)	0.981 (0.963-0.990)
Dorsal extension (Q4)	1.1 (0.7)	1.5 (0.5)	0.975 (0.952-0.987)
All	4.5 (0.2)	4.3 (0.8)	0.998 (0.996-0.999)

Abbreviations: ICC,intraclass correlation coefficient; mHAMIS, modified Hand Mobility in Scleroderma; SD, standard deviation.

There are numerous validation studies on HAMIS in the literature. In their study on 45 patients with SSc, Sandqvist et al. reported that the HAMIS test correlated with joint range of motion and mRSS. They also concluded that it could distinguish between healthy controls and scleroderma patients.[4] Another study by Sandqvist et al. also found this test was feasible for longitudinal assessment.[7] Rosso et al. also reported that this test was valid for Italian SSc patients.[5] Moreover, they noticed that patients with flexion contracture and arthritis had higher total HAMIS scores. However, in 2014, the modified version of the HAMIS test consisting of only 4 items of the original test was found to correlate with mRSS and skin involvement of the hand. This test was even evaluated to be useful for distinguishing between limited and diffuse scleroderma.[9] Azevedo et al. also found that mHAMIS and HAMIS were correlated. The same study emphasized that the test had a high Cronbach's alpha value, with good intra- and inter-observer reliability.[14] The high Cronbach's alpha coefficient and high intra- and inter-observer agreements found in our study also showed that this test was valid for Turkish patients. One of the advantages of this test is that it consists of a small number of questions and is therefore practical.

Cutaneous fibrosis of the hands is the most advanced and limiting dysfunction caused by SSc. Studies aiming to assess the effect of SSc on these patients need objective instruments to assess hand movement limitations caused by this condition. In the literature, HAQ and DHI have been validated for the assessment of hand functions in patients with SSc.[15,16] Another study showed that the Italian version of the Cochin hand function scale was valid for scleroderma.[5] In our study, HAQ and DHI showed a good correlation with mHAMIS. This shows the reliability of mHAMIS in Turkish SSc patients.

Factors affecting hand functions in patients with SSc have been described in the literature. A study by Kwakkenbas et al. on 1193 SSc patients found that hand functions were negatively affected by female gender, high body mass index, contracture, rheumatoid arthritis, and diffuse form of SSc.[17] Another study showed that hand functions were affected by Raynaud's phenomenon, digital ulcer, contracture, deformity, and arthritis.[18] However, the effects of these factors on tests that assess hand functions have been the subject of few studies. Azevedo et al. divided SSc patients into 2 groups based on the presence of digital ulcer, arthritis, pitting scar on the hand, and SSc type. However, they found no difference between the mHAMIS scores of the groups.[14] In our study, patients with digital ulcers, deformity, and diffuse form of SSc had significantly higher mHAMIS scores. The different results of the 2 studies can be explained by the different rates of active and painful digits, ulcers, pitting scars, and form of the disease.

4.1 | Limitations

Since the original version of HAMIS has not yet been validated in Turkish, its correlation with mHAMIS could not be evaluated in our study. This is the most important limitation of our study. Another limitation was evaluating the movements of only 1 hand. Furthermore, this study was conducted using the methodology of Sandqvist et al., who preferred to evaluate only the dominant hand of patients in the analysis of data from the original validation study of HAMIS.[4]

5 | CONCLUSION

This study showed that the Turkish version of mHAMIS could be used to assess hand movements in Turkish patients with SSc, with good reliability and internal validity. The correlation of assessment parameters with the mHAMIS score suggests that the mHAMIS score may be an appropriate measure of outcome, assessing the extent of hand dysfunction in SSc patients.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

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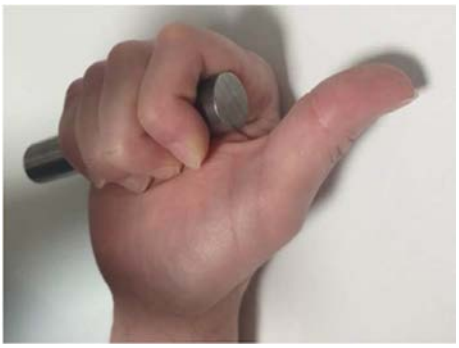
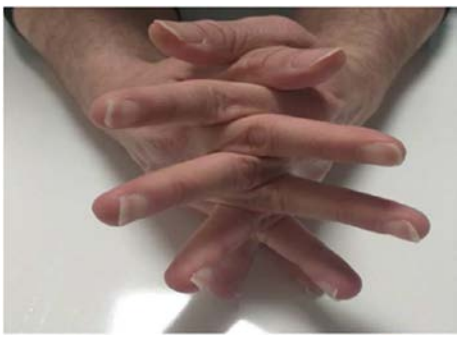
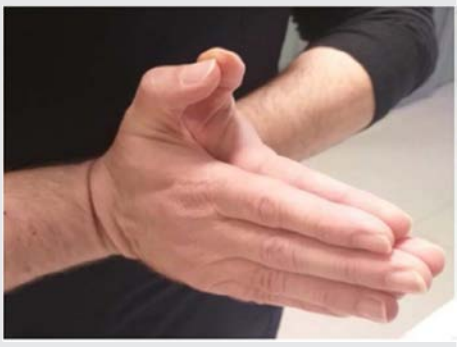
APPENDIX 1

TABLE A1 The Turkish version of modified Hand Mobility in Scleroderma

Modified HAMIS	
Parmak fleksiyonu 0. Bir kalemin etrafında (5 mm çaplı) 2-5. parmakları bükebilir. Tüm parmaklar nesneye sıkı olmalıdır. 1. Bir çatal veya bıçak parçasının etrafında (15 mm çaplı) 2-5. parmakları bükebilir 2. Gidonunun etrafında (30 mm çaplı) 2-5. parmakları bükebilir 3. Önceki maddeleri yapamaz	
Parmak ekstansiyonu 0. 2-5.parmaklarıyla masayı tamamen hissedebilir 1. 2-5. parmaklarıyla 5 mm çapında kalemi hissedebilir 2. 2-5. parmaklarıyla 15 mm çapında çatal bıçak parçasını hissedebilir 3. Önceki maddeleri yapamaz	
Parmak abduksiyonu 0. Parmakları birbirinden ayırıp (uzaklaştırıp) sonrasında elleri parmakların dibinde çaprazlama kavuşturabilir 1. Parmakları birbirinden ayırıp (uzaklaştırıp) sonrasında elleri ilk falanksta çaprazlama kavuşturabilir 2. Parmakları birbirinden ayırıp (uzaklaştırıp) sonrasında elleri ikinci falanksta çaprazlama kavuşturabilir 3. Önceki maddeleri yapamaz	
Dorsal ekstansiyon 0. Avuç içlerini bir arada tutabilir ve bilekleri mideye dayayabilir 1. Avuç içlerini bir arada tutabilir ve baş parmakları boğaza dayayabilir 2. Avuç içlerini bir arada tutabilir ve başparmakları ağzına dayayabilir 3. Önceki maddeleri yapamaz	

(Test ekipmanları; parmak fleksiyonu için (5, 15, 30 mm çaplı), parmak ekstansiyonu (5, 15 mm çap) ve başparmak abduksiyonu için (60, 70 ve 90 mm çaplı) standartlaştırılmış silindirler gerekmektedir. Kısaltmalar: MKF =metakarpofarangealeklemler; PIF =proksimal interfalangeal eklem; DIF =distal interfalangeal eklem)

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ORIGINAL ARTICLE

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Invalidation in fibromyalgia and rheumatoid arthritis and its effect on quality of life in Indian patients

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Abstract

Aim: Fibromyalgia (FM) and rheumatoid arthritis (RA) patients face invalidation in the form of "discounting" and "lack of understanding". Invalidation can have effects on the quality of life (QoL) in these patients. We planned this study to look for invalidation in FM and RA Indian patients and see the correlation between invalidation and QoL.

Methods: Invalidation was measured by the Illness Invalidation Inventory (3*I) to look for "discounting" and "lack of understanding" across sources, that is, spouse, family, medical professionals, work environment. QoL was measured using the World Health Organization QoL-BREF (WHOQoL). It covers mental, physical, psychological, and environmental domains.

Results: Fifty-five FM and 102 RA patients were included in the study. Compared to RA, FM patients had significantly higher discounting by spouse, family and medical professionals ($P < .001$). FM patients suffered more lack of understanding from spouse and medical professionals as compared to RA patients ($P < .001$). In RA patients discounting by spouse had weak to moderate negative correlation with psychological, social and environmental domains of WHOQoL (r −.26 to −.48). Lack of understanding by spouse had moderate negative correlation with all the domains of WHOQoL (r −.30 to −.40) and a weak correlation with disease duration (r .23) in RA. In FM discounting by spouse and medical professionals had weak to moderate negative correlation with the physical health domain of WHOQoL (r −.26 to −.30).

Conclusion: FM patients faced more invalidation as compared to RA patients. Invalidation from spouse leads to poor QoL in RA and FM patients.

KEYWORDS

fibromyalgia, invalidation, life quality, rheumatoid arthritis

1 | INTRODUCTION

Patients with rheumatic diseases like fibromyalgia (FM) and early stages of rheumatoid arthritis (RA) usually do not have any deformities. Other symptoms of these diseases like stiffness, pain, fatigue, mood alterations are not evident to the people around the patients. This leads to family members and society underestimating the impact of these diseases on the patient. The immediate family of the patient, healthcare workers and colleagues end up doubting the severity of the patients' disease. This behavior of people toward the patient has been summed as "invalidation" and encompasses "discounting" and "lack of understanding".[1] Discounting includes

behavior such as criticizing, denying, patronizing, giving unhelpful advise or failure to understand the fluctuation of disease severity. Lack of understanding represents inability of people around the patient to provide emotional support to the patient in the form of a sympathetic hearing and understanding the impact of the disease on patients.[2]

Negative social responses in the form of invalidation can lead to adverse impact on the mental and physical wellbeing of the patient. Social rejection has been shown to increase pain sensitivity and perception.[3,4] Invalidation can have impact on pain and quality of life (QoL) in FM patients.[5] It can also lead to loneliness in patients with rheumatic diseases especially in patients with FM.[6] Other psychological factors such as happiness, agreeableness, and conscientiousness are also affected by invalidation. Invalidation is not unique to a particular rheumatic disease like FM but may be observed in a spectrum of all rheumatic diseases like RA, systemic lupus erythematosus (SLE) or spondyloarthropathy (SpA).[2]

As invalidation is a psychosocial phenomenon which depends on cultural, social and economic factors, it may vary from one culture, society or country to other cultures and societies. Most of the studies on invalidation in RA and FM were conducted in Europe and the Americas. As we do not have studies on invalidation from Asia or India we conducted this study to assess the evidence of invalidation and compare the amount of invalidation between FM and RA in Indian patients and see its effect on QoL.

2 | METHODS

RA and FM patients aged more than 18 years consecutively presenting to the outpatient department of a tertiary care hospital were enrolled in the study. None of the patients refused enrollment in the study. FM patients were classified as per the 1990 American College of Rheumatology (ACR) criteria for the classification of FM and patients of RA were diagnosed as per 2010 RA classification criteria.[7,8] Patients having a diagnosis of both RA and FM were excluded from the study. Patients with other chronic diseases like diabetes mellitus, chronic renal failure, malignancy were also excluded from the study. Data about demographics were collected, such as age, gender, disease duration, marital, work and education status. Invalidation was measured by the Illness Invalidation Inventory (3*I).[1] This questionnaire measures invalidation by looking at 8 items out of which 5 items were in "discounting" which were as follows. (1) Finds it odd that I can do much more on some days than on other days. 2. Thinks I should be tougher. 3. Gives me unhelpful advice. 4. Makes me feel like I am an exaggerator. 5. Thinks I can work more than I do. There were 3items in "lack of understanding" scored in reverse. (1) Takes me seriously. (2) Understands the consequences of my health problems or illness. (3) Gives me the chance to talk about what is on my mind. These were across 5 sources, that is, spouse, family, medical professionals, work environment and social services. The participants indicated on a 5-point Likert scale (1 = never; 2 = seldom; 3 = sometimes; 4 = often; 5 = very often) as to how people in the last year reacted to them in each category. A source category which did not apply was skipped. As social services are not available to patients with chronic diseases in India, none of the patients were evaluated for this source. QoL was measured using the World Health Organization QoL-BREF (WHOQoL).[9] WHOQoL has 26 questions with a possible score of 1-5 for each question. It covers mental, physical, psychological, and environmental domains. Scores for each domain are added separately and the raw scores transformed to a score of 0-100. Higher scores reflect better QoL. A validated Hindi version of WHOQoL was used for Hindi speaking patients.[10] For patients who could not read Hindi or English, the investigators filled the forms after reading the questionnaires to the patients.

Data were analyzed using OpenStat software. Student's *t* test was used to compare the means of continuous variables, categorical variables were compared by Fisher's exact test, and a value of $P < .05$ was considered significant. Pearson's correlation coefficient was calculated between various sources of "discounting" and "lack of understanding" and domains of WHOQoL, disease duration and age. For correlations, $P < .01$ was considered statistically significant. Bonferroni correction was applied and the corrected *P* value was calculated for multiple comparisons. The study was approved by the Institution Ethics Committee. All the patients gave written informed consent.

3 | RESULTS

A total of 102 RA and 55 FM patients were enrolled in the study. RA patients had higher age as compared to FM patients (45.2 vs 36.6 years, $P < .01$) and disease duration was more in RA patients as compared to FM patients(5.7 vs 2.4 years, $P < .01$). There was no significant differences in gender distribution, marital, work and educational status between the 2 groups (Table 1). All the RA patients were receiving disease-modifying antirheumatic drugs with methotrexate and/or hydroxychloroquine as primary therapy; none of the patients were receiving biologicals. Of all 44 FM patients were receiving amitriptyline and 9 patients were receiving pregabalin. None of the FM patients were receiving opiates.

3.1 | Comparison of invalidation between RA and FM patients

FM patients had significantly higher discounting by spouse, family and medical professionals ($P < .01$), while there was no difference in the discounting in the work environment. This could be due to fewer patients in the work environment source. None of the patients were receiving any social service support thus this source was excluded from analysis (Table 2). FM patients suffered more lack of understanding from spouse and medical professionals as compared to RA patients ($P < .01$). There was no significant difference as far as lack of understanding from family and work environment was concerned (Table 2).

TABLE 1 Baseline characteristics of rheumatoid arthritis (RA) and fibromyalgia (FM) patients

	RA (N = 102)	FM (N = 55)	*P* (uncorrected)	*P* (corrected)
Age, y, mean (SD)	45.2 (12.5)	36.6 (10.2)	<.001	<.01
Female, n (%)	80 (78)	50 (90)	<.05	NS
Disease duration, y, mean (SD)	5.7 (6.2)	2.4 (3.5)	<.001	<.01
Marital status (%)				
Married	92 (90)	47 (85)	NS	NS
Single	6 (6)	5 (9)		
Widowed	5 (6)	3 (5)		
Divorced	1 (1)	0 (0)		
Work status (%)				
Housewife	71 (69)	43 (78)	NS	NS
Employed	22 (21)	9 (16)		
Retired	5 (5)	0 (0)		
Student	4 (4)	3 (5)		
Education status (%)				
No education	43 (42)	23 (41)	NS	NS
Primary	6 (6)	7 (13)		
Secondary	45 (44)	22 (40)		
Tertiary	8 (7)	3 (5)		
Quality of life				
Physical health, mean (SD)	51.2 (10.6)	46.3 (11.8)	.009	NS
Psychological, mean (SD)	52.8 (11.8)	45.2 (11.4)	.0001	<.001
Social, mean (SD)	65.6 (14.6)	65.6 (14.6)	NS	NS
Environmental, mean (SD)	52.9 (11.5)	47.5 (11.4)	.005	<.05

Abbreviations: NS, not significant; SD, standard deviation.

3.2 | Correlations between invalidation and WHOQoL domains, disease duration and age in RA patients

Discounting by spouse had weak to moderate negative correlation with psychological, social and environmental domains of WHOQoL (*r* −.26 to −.48) and discounting by family had a weak negative correlation with age (*r* -.25) (Table 3). Lack of understanding by spouse had moderate negative correlation with all the domains of WHOQoL (*r* −.30 to −.40) and a weak correlation with duration of disease (*r* .23). Lack of understanding by the family had weak negative correlation with physical, psychological and environmental domains (*r* −.20 to −.27). Lack of understanding by medical professionals had weak negative correlation with social domain (*r* -.26). Due to fewer RA patients in the work environment source, correlations were not calculated between work environment and WHOQoL domains.

3.3 | Correlations between invalidation and WHOQoL domains, disease duration and age in FM patients

Discounting by spouse and medical professionals had weak to moderate negative correlation with physical health domain of WHOQoL (*r* −.26 to −.30). No correlation of discounting or lack of understanding was found with disease duration and age in FM patients (Table 4). Due to fewer FM patients in the work environment source, correlations were not calculated between work environment and WHOQoL domains.

4 | DISCUSSION

In our study we compared the invalidation between RA and FM patients. We found that both RA and FM patients faced invalidation

TABLE 2 Comparison of invalidation between rheumatoid arthritis (RA) and fibromyalgia (FM) patients

Type and source of invalidation	RA mean (SD)	FM mean (SD)	*P* (uncorrected)	*P* (corrected)
Discounting				
Spouse (n RA = 90, n FM = 47)	1.24 (0.81)	2.6 (1.94)	<.001	<.01
Family (n RA = 102, n FM = 55)	1.29 (0.83)	1.99 (1.68)	<.001	<.01
Medical professional (n RA = 102, n FM = 55)	1.13 (0.39)	3.13 (1.82)	<.001	<.01
Work environment (n RA = 9, n FM = 4)	2.76 (1.85)	2.35 (1.56)	NS	NS
Lack of understanding				
Spouse (n RA = 90, n FM = 47)	2.09 (1.41)	3.05 (1.84)	<.001	<.01
Family (n RA = 102, n FM = 55)	2.06 (1.42)	2.44 (1.73)	NS	NS
Medical professional (n RA = 102, n FM = 55)	1.79 (2.29)	3.40 (1.77)	<.001	<.01
Work environment (n RA = 9, n FM = 4)	3.73 (1.62)	2.97 (1.35)	NS	NS

Abbreviations: NS, not significant; SD, standard deviation.

TABLE 3 Correlations of "discounting" and "lack of understanding" with WHOQoL domains, disease duration and age in rheumatoid arthritis patients

	Physical	Psychological	Social	Environmental	Disease duration	Age
Discounting						
Spouse	-0.20	-0.26*	-0.48***	-0.34**	0.07	-0.11
Family	-0.06	-0.12	-0.02	-0.02	-0.04	-0.25*
Medical professional	-0.13	-0.26**	-0.14	-0.14	-0.02	-0.10
Lack of understanding						
Spouse	-0.30**	-0.36***	-0.35**	-0.40***	0.23*	0.00
Family	-0.20*	-0.27**	0.01	-0.20*	0.18	-0.06
Medical professional	-0.04	-0.15	-0.26**	-0.14	0.05	-0.18

Abbreviation: WHOQoL, World Health Organization Quality of Life.
*P <.05,; **P <.01,; ***P <.001.

from the surrounding environment. FM patients suffered more invalidation in the form of discounting from spouse, family and medical professionals as compared to RA patients. FM patients also faced more lack of understanding from spouse and medical professionals. A study from the Netherlands which compared invalidation between RA and FM patients found higher discounting and lack of understanding in FM patients by family, medical professionals and work environment but no significant discounting or lack of understanding by the spouse.[1] We on the other hand, found there was both discounting and lack of understanding by spouse, family, medical professionals but not by the work environment. The possible reason appears to be cultural or social where the spouse in the Western world may be more aware and appreciative of the disease in the partner, leading to less invalidation by the spouse. We did not find any difference in invalidation in the work environment between RA and FM patients; this could be due to small sample size in the work environment source (9 RA and 4 FM patients). Another study from Portugal compared invalidation between different rheumatological diseases and found that FM patients reported highest invalidation.[2] One of the main reasons for this phenomenon appears to be lack of physical signs, deformities and laboratory abnormalities in FM patients.[11] The people surrounding the patient may doubt the accuracy and severity of patients' symptoms in FM.[12] Invalidation can be such a strong phenomenon that the presence of invalidation can discriminate between FM patients and other non-FM patients with chronic pain. This is especially true if the source of invalidation is from spouse and family.[13] Invalidation in patients of FM can lead to increase in the use of healthcare resources and poor health-related outcomes, especially related to psychological morbidity in the form of emotional dysregulation and emotional distress.[14,15]

TABLE 4 Correlations of "discounting" and "lack of understanding" with WHOQoL domains, disease duration and age in fibromyalgia patients

	Physical	Psychological	Social	Environmental	Disease duration	Age
Discounting						
Spouse	-0.30*	-0.07	-0.09	-0.07	0.03	-0.01
Family	-0.02	-0.13	-0.01	-0.15	0.00	-0.10
Medical professional	-0.26*	-0.06	0.16	-0.16	0.00	0.25
Lack of understanding						
Spouse	-0.19	0.01	0.05	-0.04	0.01	-0.06
Family	0.09	0.00	-0.10	-0.03	-0.01	-0.18
Medical professional	-0.20	0.01	0.06	-0.10	-0.04	0.21

Abbreviation: WHOQoL: World Health Organization Quality of Life.
*$P < .05$.

Invalidation from the spouse and family was associated with poor QoL in physical, psychological, social and environmental domains, while invalidation from healthcare professionals was associated with poor QoL in psychological and social domains in RA patients. This suggests that RA patients who suffered invalidation from their immediate surroundings, that is from spouse and family had greater compromising in their QoL. This is more important in Asian societies as there is a culture of joint families with 2 or 3 generations living together and all the members of the family are expected to contribute to household work. The effect of disease on one member of the household may be reflected in the disruption in the smooth working of joint family dynamics. All the members of the family may not be aware of the physical or psychological impact of the disease on the patient, leading to invalidation. The disease duration and age of the patient had only weak correlation with invalidation by spouse and family. In FM patients only physical domain of the WHOQoL had significant correlation with invalidation by spouse and medical professionals. This suggests that although FM patients face more invalidation as compared to RA patients, this invalidation may not affect the QoL of FM patients as much as compared to RA patients. This might be due to better coping by FM patients to invalidation.

Both RA and FM patients face invalidation but FM patients face significantly more invalidation as compared to RA patients. This has been seen in other studies where the amount of invalidation was compared between FM and other rheumatic diseases such as RA, SLE and SpA.[12] This could be due to the fact that FM patients may have lots of symptoms but have little or no signs of the disease. We suggest that all RA and FM patients need to be screened for invalidation as invalidation is seen to impact the QoL in these patients. Education of the patients, spouses and family members about the disease may help to mitigate the invalidation. Healthcare providers also need to be sensitized about the need to avoid discounting patients' disease due to lack of physical manifestations.

We did not include a healthy control group in our study as the term "invalidation" is used to describe the patients' perception that their medical condition is not recognized by the other people in the surrounding environment. As such this term / questionnaire cannot be applied / administered to healthy controls to quantify their invalidation. We used the ACR 1990 criteria for the classification of fibromyalgia instead of 2010 ACR preliminary diagnostic criteria for fibromyalgia, as the 2010 ACR criteria are proposed as "diagnostic criteria" as opposed to the 1990 ACR criteria which are "classification criteria". The authors of the 2010 diagnostic criteria have themselves mentioned in the paper that the diagnostic criteria suggested are not meant to replace the ACR classification criteria.[16] We used WHOQoL to assess QoL, as this questionnaire was developed by the WHOQoL Group with 15 international field centers, simultaneously, in an attempt to develop a QoL assessment that would be applicable cross-culturally. India was one of the centers.[17] It is more likely to capture the QoL status in Indian settings as compared to other questionnaires developed in Western settings. We excluded RA patients who also had FM so as to study the magnitude of invalidation independently for each disease. It has been seen that around 15% of RA patients may have overlying FM and these patients have higher disease activity and functional disability.[18] By excluding RA patients with overlying FM we might have excluded RA patients with more functional disability and more invalidation, thus skewing the results more toward FM patients. Most of our FM patients were receiving amitriptyline which also has antidepressant and mood elevating activity. This effect of the amitriptyline might have mitigated some of the impact of invalidation which FM patients in our study would have faced. In spite of such an effect the FM patients in our study were found to have more invalidation as compared to RA patients.

Our study is limited by the fact that it was done in a single center in a tertiary care setting. The data may not be reflective of the patients seen at the community level of care, as patients with more severe and difficult to control disease are referred to our center. As this is a cross-sectional study, we were not able to capture the progression of invalidation over a period of time. A significant number of our patients were not educated and needed help of investigators to fill the questionnaires. As these questionnaires are designed to be filled by the patients themselves, any help by the investigators might have altered the desired meaning of the questions, leading to changes in the results.

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AUTHOR CONTRIBUTIONS

Gurmeet Singh: contribution in concept and design of study, analysis of data, writing manuscript. Abdul Hamid: contribution in concept and design of study, collection of data, review of manuscript.

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ORIGINAL ARTICLE

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Discriminative ability of trabecular bone score over bone mineral density for vertebral and fragility fracture in patients treated with long-term and low-dose glucocorticoid

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Abstract

Aim: To evaluate the ability of the trabecular bone score (TBS) to discriminate vertebral fracture (VF) and fragility fracture (FF) in patients with chronic inflammatory rheumatic diseases on long-term and low-dose glucocorticoid (GC) treatment and those without exposure to GC.

Methods: This study assessed TBS and bone mineral density (BMD) in chronic GC users, defined as ≥2.5 mg/d of prednisone for >3 months (n = 89, mean age: 62.5 ± 11 years), and in controls (n = 59, mean age: 60.3 ± 9.6 years). Osteoporosis risk factors, radiographs of the thoracolumbar spine, non-VF history, osteoporosis drugs, and current/cumulative GC doses were collected. Patients were classified as high (TBS <1.23), intermediate (1.23-1.31), or low risk (>1.31), according to the fracture risk based on a recent meta-analysis.

Results: The mean current dose and duration of GC treatment were 3.9 ± 1.9 mg/d and 3.9 ± 4.2 years, respectively. The prevalence of VF was significantly higher in chronic GC users than in controls (20.2% vs 5.1%, $P = .010$), although the prevalence of non-VF was similar (11.2% vs 5.1%). The GC group had significantly lower L1-L4 TBS and femur total BMD than did the controls (all with $P < .01$) without significantly different lumbar BMD. TBS (<1.31) showed a higher sensitivity for patients with VF and FF (83.3% and 81.8%, respectively) than with densitometric osteoporosis in the GC group (61.1% and 59.1%, respectively). Using the receiver operating characteristic curve, TBS <1.31 showed better diagnostic accuracy than TBS <1.23 and BMD in chronic GC users.

Conclusion: TBS is more sensitive than BMD in detecting VF and FF in chronic GC users, even at a lower dose.

KEYWORDS

bone, cancellous bone, fractures, glucocorticoid, osteoporosis, rheumatic diseases, spine

1 | INTRODUCTION

Glucocorticoids (GCs) play an important role in the treatment of chronic inflammatory rheumatic diseases. Although long-term GC treatment is widely used to control inflammation in various rheumatic diseases, GC causes secondary osteoporosis and increases the risk of fracture.[1] High daily and cumulative GC doses increase the risk of fracture, especially vertebral fracture (VF), due to the greater effects of GC on trabecular than on cortical bone.[2] Fracture risk assessment is essential for the management of chronic inflammatory rheumatic diseases treated with GC. A number of studies have reported that fracture occurs in GC-treated subjects with non-osteoporotic T-score values, suggesting the need for other tools to evaluate bone quality.[3-5] The trabecular bone score (TBS) is a texture tool obtained from dual-energy X-ray absorptiometry (DXA) images of the lumbar spine. TBS provides information on bone microarchitecture and has an independent predictive value for fragility fractures.[6] High-resolution peripheral quantitative computed tomography (HRp-QCT) also provides information on the microarchitecture and trabecular network of bone tissue. However, HRp-QCT has the disadvantage of exposing patients to higher doses of radiation than DXA. [7]

TBS is a promising method for the management of osteoporosis, especially in patients with chronic inflammatory rheumatic diseases who have a higher chance of chronic exposure to GC. Even in patients receiving GC at a dosage of <7.5 mg/d, the relative risk of osteoporotic fracture increases by approximately 60%.[8] Another study found that low GC doses, such as 2.5 mg/d of a prednisolone equivalent, increased the risk of fracture.[9] Guidelines for GC-induced osteoporosis (GIOP) emphasize the need for the assessment, prevention, and treatment of osteoporosis and fractures in patients taking ≥2.5 mg/d of prednisone for ≥3 months.[2,10] Florez et al. reported that TBS has a greater discriminative accuracy than bone mineral density (BMD) for fracture risk assessment in adult patients with autoimmune diseases on chronic GC treatment (≥5 mg/d of prednisone or an equivalent, for >3 months).[11] However, in the study by Florez et al., the mean dose of GC was moderate, and there was no control group. A recent meta-analysis on TBS demonstrated the threshold for risk of fracture: high (TBS <1.23), intermediate (TBS 1.23-1.31), and low risk (TBS >1.31).[12] However, this threshold for TBS was not validated in chronic GC users, particularly those who take low-dose GC (prednisolone ≤7.5 mg/d).

The aim of our study was to evaluate the ability of TBS to discriminate VF and fragility fractures in patients with chronic inflammatory rheumatic diseases on long-term GC treatment (≥2.5 mg/d of prednisone or an equivalent for >3 months) and those without exposure to GC.

2 | METHODS

2.1 | Study population

A single-center case-control study was conducted at Soonchunhyang University Seoul Hospital (Seoul, South Korea) between July 2019 and February 2021. We consecutively included Korean adults with chronic inflammatory rheumatic diseases on prolonged GC treatment (≥2.5 mg/d of prednisone or an equivalent, for >3 months) and those without exposure to GC treatment as controls. Exclusion criteria included patients with a premenopausal status, thyroid or parathyroid disorders, chronic renal or liver disease, malabsorption syndrome, chronic obstructive pulmonary disease, malignancy, and/or who underwent gastrectomy or bariatric surgery. The study was approved by the Institutional Review Board for Human Research (2020-10-008) at Soonchunhyang University Seoul Hospital. Written consent was obtained from all of the participants.

2.2 | TBS and BMD assessment

BMD (g/cm^2) of the lumbar spine (L1-L4) and left proximal femur (FN and FT) were evaluated by DXA using Hologic Horizon W (Hologic Inc, Danbury, CT, USA).[13] According to the World Health Organization criteria, osteoporosis is defined as a T-score of ≤ −2.5.[14] Lumbar spine (L1-L4) TBS was calculated using the TBS iNsight software (version 3.0; Med-Imaps, Merignac, France) on the DXA images. All of the measurements were taken by experienced operators using the same machine and standardized procedures for participant positioning. Patients were divided into 3 TBS groups according to the risk of fracture in a recent meta-analysis: high risk, TBS <1.23; intermediate risk, TBS 1.23-1.31; and low risk, TBS above 1.31. [12]

2.3 | Clinical and laboratory evaluation

For each patient, the following clinical data were collected: demographics (age, height, and weight), menopause status, osteoporosis risk factors (smoking status, alcohol consumption, and family history of osteoporotic fracture), disease duration, and diabetes. The daily current and cumulative GC doses, use of immunosuppressive agents, and anti-osteoporosis drugs were documented. The cumulative GC dose for a patient was calculated by multiplying the indicated number of pills, dose per pill, and prednisolone conversion factor per prescription, and then adding the resulting products across all prescriptions. VFs were evaluated using radiographs of the thoracic and lumbar spines. VF was defined as a reduction of 20% or more in the anterior, posterior, and/or middle vertebral height compared with the adjacent, undeformed vertebral body.[15] History of non-VF (excluding fracture of digits, pathological, and non-minimal trauma fractures)[16] was also collected from self-reported questionnaires and medical chart reviews.

Laboratory tests were performed to determine serum 25-hydroxyvitamin D (25[OH]D) levels, bone turnover markers (bone alkaline phosphatase, procollagen type 1 intact N-terminal propeptide, and C-terminal telopeptide of type 1 collagen), erythrocyte sedimentation rate, and C-reactive protein levels.

2.4 | Statistical analysis

Statistical analyses were performed using the SPSS software package (SPSS Inc., Chicago, IL, USA) for Windows v.22.0 (Microsoft Corp., Redmond, WA, USA). Continuous variables were expressed as mean (SD) or median (Q1, Q3), and categorical variables were presented as numbers (%). The results were compared using Pearson's Chi-square test, Fisher's exact test, and the Student's *t* test or Mann-Whitney *U* test, as appropriate. Pearson's correlation test was used to assess the relationship between the TBS and continuous variables. We estimated the sensitivity, specificity, and positive and negative predictive values (NPV) for TBS and BMD to identify VF and fragility fractures (VF + non-VF). The area under the receiver operating characteristic (ROC) curve was calculated to compare diagnostic abilities. The results were considered statistically significant at $P < .05$.

3 | RESULTS

3.1 | Baseline characteristics of the study population

We included 89 patients (88.9% women) with a mean (SD) age of 62.5 (11.0) years on chronic treatment with GC, who had any of the following conditions: rheumatoid arthritis (RA, n = 39), primary Sjögren's syndrome (pSS, n = 28), systemic sclerosis (SSc, n = 21), and inflammatory myopathy (n = 1). A total of 59 patients (84.7% women) with a mean (SD) age of 60.3 (9.6) years were included as controls. The control group had SSc (n = 29), pSS (n = 19), or RA (n = 11). Table 1 summarizes the demographic and clinical characteristics of the study population. The mean duration of GC treatment and cumulative GC doses were 3.9 (4.2) years and 4693.2 mg, respectively. The mean current doses of GC were 3.9 (1.9) mg/d (2.5 mg/d: 50.5% [n = 45], 5 mg/d: 38.2% [n = 34], 7.5 mg/d: 7.9% [n = 7], and 10 mg/d: 3.4% [n = 3]).

In the chronic GC group, 18 patients (20.2%) had VFs (1 VF, n = 12; 2 VFs, n = 2; ≥3 VFs, n = 4) and 10 patients (11.2%) had non-VF (rib, n = 7; radius, n = 2; and metatarsal fracture, n = 1). In the control group, 3 patients had VFs (one VF, n = 2, ≥3 VFs, n = 1), and 3 patients had non-VF (rib, n = 3). The prevalence of VF was significantly higher in the GC group than in the control group ($P = .010$). However, the prevalence of non-VF was similar between both groups.

Patients with inflammatory rheumatic diseases on chronic GC treatment had significantly lower TBS and BMD of the FT than did the controls ($P = .008$ and $P = .006$, respectively). The proportion of patients with a high and intermediate risk of fracture, as per TBS, was significantly higher in the GC group than in the control group. However, no significant difference in lumbar spine BMD and densitometric osteoporosis was observed between both groups.

TBS values correlated negatively with age ($P < .001$, $r = -.574$) and positively correlated with BMD of FT, lumbar, and FN (all with $P < .001$) of both groups. In both groups, no correlation was observed between the TBS and the current and cumulative GC doses (Table 2).

TABLE 1 Characteristics of the study population

	Chronic GC users (n = 89)	Controls (n = 59)	*P* value
Age, y	62.5 (11.0)	60.3 (9.6)	.221
Female, n (%)	79 (88.8)	50 (84.7)	.474
BMI, kg/m^2	22.8 (3.2)	22.6 (2.8)	.773
Disease duration, y	4.1 (4.3)	4.7 (6.0)	.426
Osteoporosis risk factors			
Current smoking	10 (11.2)	5 (8.4)	.820
Alcohol ≥3 U/d, n (%)	5 (5.6)	3 (5.1)	.463
Diabetes mellitus, n (%)	9 (10.1)	3 (5.1)	.369
Family history of osteoporotic facture, n (%)	1 (1.1)	1 (1.7)	1.000
Medications			
Current GC dose at time of BMD, mg/d	3.9 (1.9)		
Cumulative GC dose, mg	4963.2 (3850)		
GC treatment duration, mean (SD), y	3.9 (4.2)		
Hormone replacement therapy, current, n (%)	2 (2.2)	5 (8.4)	.095
Immunosuppressive agents, n (%)	26 (29.2)	13 (22.0)	.332
BP, ever	23 (25.8)	9 (15.3)	.129
BP duration, y			
SERM	16 (18.0)	8 (13.6)	0.484
Vitamin D	49 (55.0)	24 (40.7)	0.078
Calcium	21 (23.6)	10 (16.9)	0.321
Laboratory tests			
ESR, mm/h	47.0 (31.8)	38.0 (17.3)	**0.027**
CRP, mg/dL	0.2 (0.4)	0.4 (1.5)	0.280
Bone ALP, μg/L	12.6 (5.4)	12.3 (3.9)	0.775
25(OH)D, ng/mL	21.8 (12.9)	22.0 (11.4)	0.891
C-telopeptide of collagen type I, ng/mL	0.33 (0.4)	0.2 (0.2)	**0.031**
Procollagen 1 N-terminal propeptide, ng/mL	37.9 (25.2)	50.5 (24.9)	**0.012**
Fractures			
Vertebral fractures (VF), n (%)	18 (20.2)	3 (5.1)	**0.010**
Non-VF, n (%)	10 (11.2)	3 (5.1)	0.196
Fragility fractures (VF + non-VF), n (%)	22 (24.7)	6 (10.2)	**0.027**

(Continues)

TABLE 1 (Continued)

	Chronic GC users (n = 89)	Controls (n = 59)	*P* value
BMD			
Lumbar spine BMD, g/cm^2	0.82 (0.12)	0.84 (0.12)	0.173
T-score lumbar spine	-2.04 (1.04)	-1.88 (1.04)	0.363
Femoral neck BMD, g/cm^2	0.62 (0.10)	0.65 (0.12)	0.060
T-score femoral neck	-2.16 (0.96)	-1.77 (1.09)	**0.027**
Total femur, BMD, g/cm^2	0.72 (0.14)	0.78 (0.13)	**0.006**
T-score femur total	-1.74 (0.96)	-1.35 (1.05)	**0.023**
Densitometric osteoporosis, n (%)	41 (46.1)	19 (32.2)	0.093
TBS			
Lumbar spine TBS	1.33 (0.08)	1.37 (0.07)	**0.008**
TBS <1.23	8 (8.9)	0 (0)	**0.022**
TBS<1.31	51 (57.3)	24 (40.7)	**0.048**

Note: Data are presented as the mean (SD), unless otherwise stated.

Abbreviation: BMI, body mass index; BMD, bone mineral density; GC, glucocorticoid; BP, bisphosphonate; SERM, selective estrogen receptor modulator; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ALP, alkaline phosphatase; 25(OH)D, 25-hydroxy vitamin D3; TBS, trabecular bone score.

3.2 | Comparison of chronic GC users with and without VFs

When comparing patients with and without VF on chronic GC treatment, those with VF were older and had lower FN and FT BMD, and TBS values (Table 3). The number of bisphosphonate users was higher in patients with VF (50%) than in those without VF (19.7%) (*P* =.011). Patients with VF presented TBS scores indicative of intermediate-to-high risk significantly more frequently (TBS value <1.31). However, no significant differences were observed in the lumbar spine BMD and the presence of densitometric osteoporosis and high-risk TBS (TBS value <1.23) between both groups. Indeed, the current and cumulative GC doses and GC treatment durations were found to be similar. There were also no significant differences between the 2 groups with respect to smoking status, alcohol consumption, BMI, gender, disease duration, use of immunosuppressive agents, intake of vitamin D, and intake of calcium supplement.

3.3 | Fracture discrimination using TBS and/or BMD

Intermediate-to-high risk TBS scores (TBS value <1.31) showed a higher sensitivity for detecting patients with VF and fragility fractures than for detecting osteoporosis in BMD in both the GC group (sensitivity 83.3% and 81.8% vs 61.1% and 59.1%, respectively) and controls (sensitivity 66.7% and 50.0% vs 33.3% and 16.7%, respectively) (Table 4). On the other hand, osteoporosis in BMD (T-score ≤ −2.5) showed slightly higher specificity than intermediate-to-high risk TBS scores (TBS value <1.31) for identifying patients with fractures (VF and fragility fractures) in both groups. Remarkably, the NPV for TBS (<1.31) was high for VF and fragility fractures in chronic GC users (92.1% and 89.5%, respectively) and controls (97.1% and 91.4%, respectively). The combined TBS value (<1.31) and BMD (T-score ≤−2.5) increased specificity but decreased sensitivity in the identification of patients with fractures.

Using the ROC curve, TBS <1.31 showed better diagnostic accuracy than TBS <1.23 and lumbar, FN, and FT BMD (T-score ≤2.5) in patients with chronic inflammatory rheumatic diseases on prolonged GC treatment (Figure 1). The area under the ROC curve for identifying VF and fragility fractures was 0.663 (0.532-0.794) and 0.663 (0.538-0.787), respectively. Only TBS <1.31 was significantly different from 0.5, when assessing VF and fragility fractures. In the control group, there was no significant difference in the discriminatory ability of TBS and BMD for VF and fragility fractures (See Supporting Information).

TABLE 2 Correlation between clinical data, inflammatory markers, BMD, and TBS

	Chronic GC users, TBS, L1-L4	Controls, TBS, L1-L4
Age, y	-.574 (<.001)	-.547 (<.001)
BMI, kg/m^2	-.154 (.150)	-.106 (.425)
Current GC doses, mg/d	-.180 (.920)	-
Cumulative GC dose, mg	-.129 (.229)	-
ESR, mm/h	-.174 (.107)	-.124 (.371)
CRP, mg/dL	-.183 (.087)	-.215 (.107)
BMD, g/cm^2		
Lumbar spine	.540 (<.001)	.671 (<.001)
Femoral neck	.399 (<.001)	.553 (<.001)
Total hip	.470 (<.001)	.538 (<.001)

Note: R coefficients (*P* values).

Abbreviations: BMD, bone mineral density; BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GC, glucocorticoid; TBS, trabecular bone score.

4 | DISCUSSION

The present study demonstrated that patients with chronic inflammatory rheumatic diseases on prolonged GC treatment, even at low doses, had a higher prevalence of VF and fragility fractures than those without GC exposure. In the chronic GC treatment groups, patients with VF had lower TBS and BMD of FN and FT than those without VF, but lumbar BMD was similar. Furthermore, TBS (<1.31) showed a higher discriminative ability for identifying patients on GC treatment with VF and fragility fractures than BMD (T-score ≤ -2.5).

TABLE 3 Comparison of chronic GC users with and without vertebral fractures (VF)

	With VF (n = 18)	Without VF (n = 71)	*P* value
Age, y	71 (66, 77)	58.5 (55, 67)	**<.001**
Current smoking	2 (11.1)	8 (11.3)	.554
Current GC dose at time of BMD, mg/d	2.5 (2.5, 5)	2.5 (2.5, 5)	.839
Cumulative GC dose, mg	4705 (2750, 9277)	3795 (1952, 6187)	.088
GC treatment duration	2.6 (0.4, 4.6)	3.0 (1.1, 6.1)	.184
Laboratory tests			
CRP, mg/dL	0.65 (0.35, 0.89)	0.12 (0.03, 0.55)	.295
25(OH)D, ng/mL	18 (15.0, 31.8)	17.8 (12.2, 29.6)	.382
C-telopeptide of collagen type I, ng/mL	0.1 (0.09, 0.27)	0.28 (0.14, 0.50)	.082
Procollagen 1 N-terminal propeptide, ng/mL	20.0 (18, 28)	26 (17, 33)	.099
Fractures			
Nonvertebral fractures, n (%)	6 (33.3)	4 (5.6)	**.004**
BMD			
Lumbar spine BMD, g/cm^2	0.78 (0.72, 0.83)	0.83 (0.73, 0.91)	.128
T-score lumbar spine	-2.4 (−2.9, −1.9)	-1.9 (−2.7, −1.2)	.085
Femoral neck BMD, g/cm^2	0.56 (0.50, 0.59)	0.65 (0.54, 0.70)	**.005**
T-score femoral neck	-2.6 (−3.1, −2.3)	-2.0 (−2.8, −1.4)	**.005**
Total hip BMD, g/cm^2	0.66 (0.61, 0.74)	0.74 (0.64, 0.83)	**.034**
T-score femur total	-2.3 (−2.7, −1.6)	-1.6 (−2.4, −1.0)	**.016**
Densitometric osteoporosis, n (%)	11 (61.1)	30 (42.3)	.152
TBS			
Lumbar spine TBS	1.29 (1.24, 132)	1.34 (1.29, 1.40)	**.004**
TBS <1.23, n (%)	3 (16.7)	5 (7.0)	.202
TBS<1.31, n (%)	15 (83.3)	36 (50.7)	**.012**

Note: Data are presented as the mean (SD), unless otherwise stated.

Abbreviations: 25(OH)D, 25-hydroxy vitamin D3; BMD, bone mineral density; BMI, body mass index; CRP, C-reactive protein; GC, glucocorticoid; TBS, trabecular bone score.

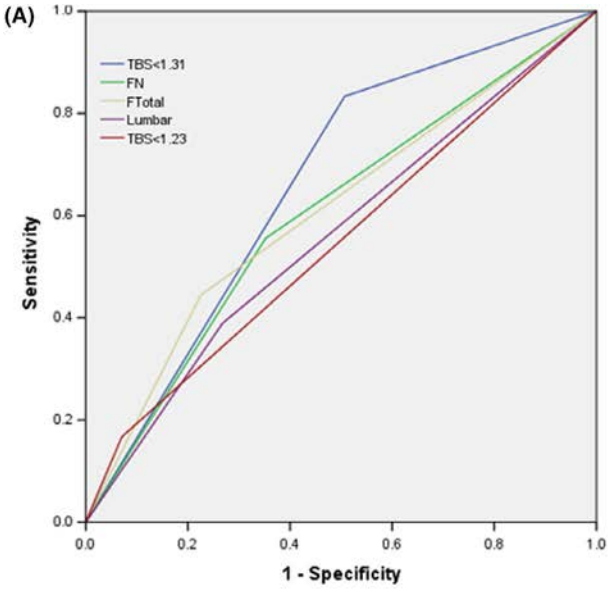
We included patients on chronic GC treatment, defined as ≥2.5 mg/d for >3 months. A recent study evaluated the utility of TBS for fracture risk assessment in adult patients on chronic GC treatment (defined as ≥5 mg/d of prednisone or an equivalent for >3 months), and the mean (SD) current GC dose was 14.5 (14.1) mg/d for a GC treatment duration similar to our study.[11] On the other hand, the mean current GC dose in our study was 3.9 (1.9) mg/d and the proportion of patients with mean current GC doses of >7.5 mg/d were only 3.4% (n = 3). The discrepancy in baseline characteristics could explain the difference in the absolute TBS value and proportion of patients with TBS <1.23, between previous reports (1.220 [0.18] and 52%, respectively) and our study results (1.33 [0.08] and 8.9%, respectively). However, the prevalence of VF and fragility fractures was similar between the 2 studies. Indeed, in our study, there was no difference in the prevalence of VF and fragility fracture, TBS, and BMD among patients treated with current GC doses of 2.5, 5, and ≥7.5 mg/d (data not shown). As such, we did not find a significant correlation between TBS and current/cumulative GC doses, which is consistent with the findings of Florez et al.[11] Our study confirms that long-term treatment with low-dose GC, such as 2.5 mg/d, can induce degraded bone microarchitecture and increase the risk of VF and fragility fracture.

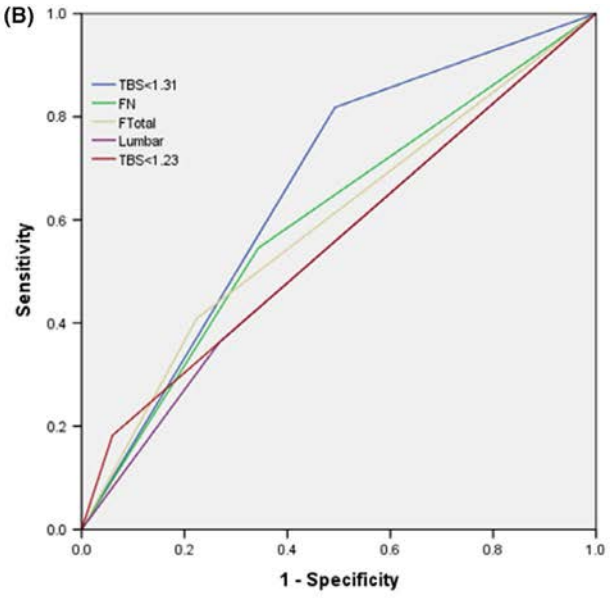
In our study, approximately 40% of chronic GC users with VF had no evidence of osteoporosis based on BMD. Indeed, we showed that TBS had a higher diagnostic accuracy than densitometric measurements. Likewise, previous studies on systemic lupus erythematosus, polymyalgia rheumatica, and rheumatoid arthritis demonstrated that TBS had a higher diagnostic value than BMD for discriminating osteoporotic fracture.[17-19] Most previous studies of TBS on rheumatic diseases were uncontrolled study designs or included healthy subjects as controls.[17-20] However, we enrolled patients with chronic inflammatory rheumatic diseases without GC exposure as controls. Although the small sample size limited our results, we could not detect a discriminatory ability of TBS for fractures in the control groups.[17-20] One study included men and women over the age of 50 years (mean [SD] age: 74.3 [11.7] years) and reported a better area under the curve (AUC) of TBS for the discrimination of VFs than those of lumbar BMD.[21] Another study

TABLE 4 Sensitivity, specificity, and PPV and NPV of TBS and BMD

		Sensitivity	Specificity	PPV	NPV
Chronic GC users					
TBS<1.31	VF	83.3	49.3	29.4	92.1
	Fragility fracture	81.8	50.7	35.3	89.5
Osteoporosis in BMD	VF	61.1	57.7	26.8	85.4
	Fragility fracture	59.1	58.2	31.7	81.3
TBS<1.31 and osteoporosis in BMD	VF	55.6	70.4	32.3	86.2
	Fragility fracture	54.5	71.6	38.7	82.8
Non-GC users					
TBS<1.31	VF	66.7	60.7	8.3	97.1
	Fragility fracture	50.0	60.4	12.5	91.4
Osteoporosis in BMD	VF	33.3	67.9	5.3	95.0
	Fragility fracture	16.7	66.0	5.3	87.5
TBS<1.31 and osteoporosis in BMD	VF	33	75	6.7	95.5
	Fragility fracture	16.7	73.6	6.7	88.6

Abbreviations: BMD, bone mineral density; GC, glucocorticoid; NPV, negative predictive value; PPV, positive predictive value; TBS, trabecular bone score; VF, vertebral fracture.





Vertebral fracture	AUC (95% CI)	P-Value
TBS< 1.23	0.548 (0.392, 0.704)	0.530
TBS< 1.31	0.663 (0.532, 0.794)	**0.033**
Osteoporosis at Lumbar BMD	0.561 (0.408, 0.713)	0.429
Osteoporosis at femur neck BMD	0.602 (0.453, 0.750)	0.184
Osteoporosis at femur total BMD	0.610 (0.457, 0.762)	0.153

Fragility fracture	AUC (95% CI)	P-Value
TBS< 1.23	0.561 (0.392, 0.416)	0.392
TBS< 1.31	0.663 (0.538, 0.787)	**0.022**
Osteoporosis at Lumbar BMD	0.547 (0.406, 0.689)	0.506
Osteoporosis at femur neck BMD	0.601 (0.463, 0.740)	0.156
Osteoporosis at femur total BMD	0.593 (0.450, 0.735)	0.194

FIGURE 1 Area under the curves (AUC) of trabecular bone score (TBS) and bone mineral density (BMD) for (A) vertebral fracture (VF) and (B) fragility fractures in patients with chronic glucocorticoid treatment

was conducted on postmenopausal women with a mean (SD) age of 65 (12) years, and did not show a significant improvement in the ROC-AUC when adding TBS to BMD. A baseline characteristic of our study is that the control group had a mean (SD) age of 60.3 (9.6) years. Because advanced age is a determinant factor for fractures in general,[22] the usefulness of TBS for fracture risk evaluation could

differ according to the age group. Further studies are needed to reveal the discriminative value of TBS in chronic rheumatic diseases without GC treatment. [23]

The occurrence of fracture increases the risk of subsequent fractures, and all low-trauma fractures are associated with increased mortality.[22] Fracture risk assessment is a key component of the management of GC users. A recent meta-analysis of TBS in fracture risk prediction reported that the 2 thresholds corresponded to TBS values of 1.23 and 1.31.[12] When applying the TBS cut-off of 1.23 to our study population, ROC-AUC did not show discriminative accuracy, which was even lower than the lumbar, FN, and FT BMD. Intermediate-to-high TBS (<1.31) showed the largest AUC for VF and fragility fractures in our study results. Our study results suggest that a higher cut-off (1.31) than previous studies can be regarded as degraded microarchitecture of bone tissue in chronic GC users.[11,24] We administered anti-osteoporosis drugs, which could have affected the higher accuracy of the TBS cut-off of 1.31 in our study. The effect of bisphosphonate use on TBS has also been investigated, with results indicating an increase in TBS in patients receiving bisphosphonates.[25,26] In our study, the number of bisphosphonate users was higher in patients with VF than in those without VF. This can be explained by the cross-sectional study design, and high-risk patients, who were already prescribed bisphosphonate due to osteoporosis or fractures, were included in the VF group. To minimize the effects of other drugs while observing the effect of GC on TBS, previous studies usually excluded patients who received osteoporosis treatment, such as bisphosphonates, and calcium and vitamin D replacement.[11,23,27] However, in clinical practice, a number of patients undergoing chronic GC treatment have been treated with osteoporosis drugs. For application of TBS in real clinical practice, further large-scale studies that include chronic GC users with and without osteoporosis drugs are needed to reveal the appropriate cut-off of TBS values.

The present study has some limitations. First, it was a single-center and observational study. Hence, the causative association between TBS and fractures could not be determined, and the number of control patients with fractures was small. Second, the heterogeneity of our study population limited our results. It included patients with various inflammatory rheumatic diseases on diverse anti-inflammatory treatment. Additionally, GC groups and controls were not properly matched. There were patients who shared the same diagnosis but had different histories of GC exposure, which can probably be attributable to differences in disease activity. High disease activity can reflect persistent inflammatory process, which can also play a role in the development of low TBS.[28,29] Further studies focusing on a single disease entity with long-term and low-dose GC exposure are needed to confirm our study results and reduce biases to other factors such as disease activity. Third, we did not include patients undergoing GC treatment (<2.5 mg/d) as current GIOP guidelines focus on patients administered prednisolone or an equivalent medication at a dose of ≥2.5 mg/d for ≥3 months. However, fracture risk in patients receiving daily prednisolone doses of <2.5 mg/d has not been well studied, suggesting the need to expand the inclusion criteria to this patient group. Fourth, the majority of patients were postmenopausal women. Therefore, our data are not applicable to young and menopausal women.

In conclusion, TBS assessment showed poor bone quality in patients with chronic GC treatment, even at low doses. TBS >1.31 is a more sensitive method than densitometric osteoporosis and TBS <1.23 for the detection of VF and fragility fractures in chronic GC users. These data support the use of TBS as a complementary clinical tool to identify the risk of osteoporosis in patients with chronic GC treatment.

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CONFLICTS OF INTEREST

The authors declare they have no 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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Interval between symptom onset and diagnosis among patients with autoimmune rheumatic diseases in a multi-ethnic Asian population

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Abstract

Aim: The interval between symptom onset and diagnosis (pre-diagnosis interval) can at times be longer than is ideal in patients with autoimmune rheumatic diseases (ARDs). In this study, we aimed to characterize this interval and to identify its associated factors.

Method: We characterized pre-diagnosis interval into 4 intervals: Interval #1 between symptom onset and first visit to healthcare professionals; Interval #2 between first visit to healthcare professionals and rheumatology referral; Interval #3 between rheumatology referral and first rheumatology assessment; and Interval #4 between first rheumatology assessment and diagnosis. Median regression models were used to identify factors associated with longer pre-diagnosis interval and Interval #1.

Results: Among 259 patients (median age = 52.0 [41.6-61.9] years, 71% female, rheumatoid arthritis [n = 75], axial spondyloarthritis [axSpA] [n = 40] and psoriatic arthritis [n = 35]), median pre-diagnosis interval was 11.5 (4.7-36.0) months. Interval #1 (median = 4.9 months) was significantly longer than Intervals #2-#4 (median = 0.3, 1.5, and 0.0 months, respectively). Patients with axSpA had significantly longer pre-diagnosis interval (median = 38.7 months) and Interval #1 (median = 26.6 months) than patients with the other ARDs. Median regression suggested that patients referred from specialty care had significantly longer pre-diagnosis interval (median difference = 7.7 months) and Interval #1 (median difference = 6.4 months) compared to those referred from primary care.

Conclusion: A long pre-diagnosis interval was observed among patients with ARDs (especially axSpA), due largely to a long interval between symptom onset and the first visit to healthcare professionals. This highlights the importance of interventions targeting patients prior to their first visit to healthcare professionals in reducing pre-diagnosis interval.

KEYWORDS
delayed diagnosis, rheumatic diseases, rheumatoid arthritis, signs and symptoms, spondylarthritis

1 | INTRODUCTION

Early diagnosis and treatment in autoimmune rheumatic diseases (ARDs) have been shown to significantly improve clinical outcomes such as higher rates of response and remission and less radiographic progression as well as patient-reported outcomes such as better health-related quality of life and lower productivity loss.[1-8] However, recent studies suggest that prolonged interval from symptom onset to diagnosis and treatment remains an unsolved problem in patients with ARDs worldwide.[9-15] For example, in patients with rheumatoid arthritis (RA) where this interval is reported to be relatively shorter compared to other ARDs, a systematic review found that the reported interval remained consistently long over the past 2 decades (4 to 24 months).[13] In patients with axial spondyloarthritis (axSpA) where this interval is reported to be relatively longer compared to other ARDs, a recent study found that the mean interval did not improve from 1996-2005 to 2006-2015 (6.3 vs 7.4 years, respectively).[11]

Two main categories of factors associated with prolonged interval from symptom onset to diagnosis and treatment have been identified in the literature. The first category is patient-related factors such as less favorable socio-demographic status, similarity of clinical manifestations with other conditions, and poor awareness and misperception of ARDs and related symptoms. The second category is physician- and healthcare system-related factors such as lack of disease knowledge among primary care physicians and non-rheumatology specialists, limited access to rheumatology care, and unavailability of diagnostics.[10,13,16-20] While some of these identified factors such as socio-demographics and disease manifestations are universal, others such as awareness and perception of ARDs and their symptoms and access to rheumatology care are specific to a given socio-cultural context and/or healthcare system.[21-23]

We and others have found a lack of awareness and misperception of ARDs and their symptoms among patients prior to their first rheumatology assessment.[17,24-26] However, how long the interval is between symptom onset and diagnosis among patients with various ARDs and how this interval is associated with misperception and other patient-related factors remain unclear. Our aims in this study are thus 2-fold: first, to quantify the interval between symptom onset to diagnosis among an inception cohort of patients with newly diagnosed ARDs, and second, to identify patient-related factors associated with this interval. We hypothesized that patient-related factors such as less favorable socio-demographics and misperception of ARDs and their symptoms were associated with a longer interval.

2 | MATERIALS AND METHODS

This study is part of a larger effort that aimed to facilitate early identification of ARDs by cross-culturally adapting 2 screening questionnaires, the Connective Tissue Disease Screening Questionnaire (CSQ) and Hamilton axSpA screening questionnaire,[27,28] for use in the Singapore population.[26] Singapore, the setting of this study, is a multi-ethnic urban population of Chinese, Malays and Indians, representing 3 major ethnic groups in Asia.[29]

2.1 | Patients

Patients enrolled in the larger study who fulfilled the classification criteria for any ARDs between April 2018 and November 2020 were included in this study. ARDs and their corresponding classification criteria used in this study included: (1) RA, 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria;[30] (2) axSpA, 2009 Assessment of SpondyloArthritis International Society (ASAS) classification criteria;[31] (3) psoriatic arthritis (PsA), 2006 ClASsification criteria for Psoriatic Arthritis (CASPAR) criteria;[32] (4) seronegative inflammatory arthritis (IA), defined as having IA but not fulfilling the classification criteria for RA, axSpA or PsA; (5) Sjögren's syndrome (SjS), 2016 ACR/EULAR criteria;[33] (6) undifferentiated connective tissue disease (UCTD), 1999 Mosca criteria;[34] and (7) other ARDs including systemic lupus erythematosus (SLE), 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria,[35] systemic sclerosis (SSc), 2013 ACR/EULAR criteria,[36] idiopathic inflammatory myopathies (IIM), 2017 EULAR/ACR criteria,[37] palindromic rheumatism (PR), 1992 Guerne and Weisman criteria,[38] and overlap ARDs that fulfilled the classification criteria for more than one ARDs.

Written informed consent was sought from all patients prior to participation. This study was approved by the SingHealth Centralized Institutional Review Board (CIRB reference: 2016/3138).

2.2 | Model to study the interval between symptom onset and diagnosis

There are 2 main routes in the journey of patients with ARDs from symptom onset to assessment by a rheumatologist. In the first route, similar to many other countries, the majority of patients with ARDs in Singapore consult a primary care physician before they are referred to a rheumatologist for further assessment. After assessment by a primary care physician, individuals who are deemed to require further assessment are referred to a specialist in referral centers. In cases

where any findings are suggestive of conditions of other specialities, individuals are referred from one specialist to another. That is, the majority of patients with ARDs in Singapore are referred from primary or specialty care to rheumatology care. In the second route, individuals may bypass the primary care physician and non-rheumatology specialist and consult a rheumatologist directly. That is, these individuals are self-referred to rheumatology care. Individuals referred to rheumatology care are then assessed by a rheumatologist, and if they are diagnosed with any ARDs, provided appropriate treatment.

Several models have been developed to study the interval between symptom onset and diagnosis.[39-41] Among these, Scott's model of pathways to treatment, although being developed in the context of cancer, has been widely used in a variety of diseases including cardiovascular diseases, diabetes, rheumatic diseases and mental disorders. We adapted Scott's model for use in our patients with ARDs (see details in Appendix S1) in light of the different healthcare system (walk-in primary care) and journey of patients with ARDs (the majority of patients are referred from primary and specialty care to rheumatology care) in Singapore. In the adapted model, we defined pre-diagnosis interval as the time from symptom onset to diagnosis by a rheumatologist. We characterized pre-diagnosis interval into 4 individual intervals (Figure 1).

1. Interval #1, the time from symptom onset to the first visit to healthcare professionals in primary care, non-rheumatology specialty care or rheumatology care clinics
2. Interval #2, the time from the first primary care or non-rheumatology specialty care assessment to referral to rheumatology care
3. Interval #3, the time from rheumatology referral to the first rheumatology assessment, and
4. Interval #4, the time from the first rheumatology assessment to diagnosis.

Pre-diagnosis interval has been referred to as diagnostic delay, (time) interval or lag time between symptom onset and diagnosis in published studies.[9-14,16,20,42,43] We did not use the term "delay" because, according to the systematic review by Scott et al., "delay" is not only value-laden but also inaccurate as many patients do seek medical attention promptly after symptom onset.[41] For patients who consult a primary care physician or a non-rheumatology specialist after symptom onset, pre-diagnosis interval is the sum of Intervals #1-#4. For patients who consult a rheumatologist directly, Intervals #2-#3 are not applicable, and pre-diagnosis interval is the sum of Intervals #1 and #4.

We renamed pre-treatment interval (the time from diagnosis to commencement of treatment in Scott's model) Interval #5 to be consistent with the terminology used in our adapted model. Interval #5 may or may not exist in patients with ARDs as treatment may or may not be initiated. If treatment is initiated, it usually commences immediately after, or in some cases, before a diagnosis is made.

2.3 | Data collection

2.3.1 | Data collected form patients

Patients completed a standardized data collection form prior to rheumatology assessment during their first visit to rheumatology clinics. The data collection form in patients' preferred language (English or Chinese) was self-administered or, in cases where patients were unable to read, administered by a caregiver or trained interviewer (if caregiver was not available) who were instructed not to provide any explanation of the items and response options.

The following data were collected from patients: socio-demographics, self-reported symptoms, comorbidities, use of complementary and alternative medicine (CAM), and coping with symptoms. Socio-demographics included age, gender, ethnicity, marital status and years of education. Self-reported symptoms were collected using the cross-culturally adapted CSQ and Hamilton axSpA screening

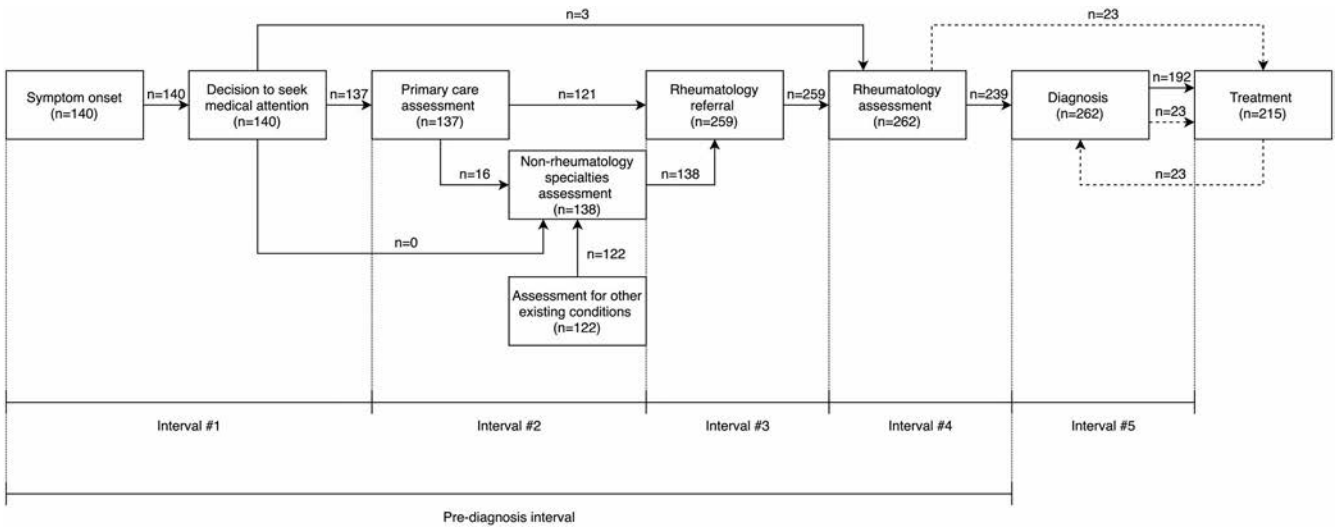

FIGURE 1 Patient flowchart

questionnaire.[24,25] Self-reported comorbidities included diabetes mellitus, hypertension, high cholesterol, heart disease, stroke, asthma or other lung disease, cancer, rheumatism, skin disease, digestive disorders, mental illness, and other illnesses. CAM included traditional Chinese medicine, traditional Malay medicine such as Jamu, traditional Indian medicine such as Ayurveda, and other traditional medicine. Coping with symptoms was assessed by asking patients to rate how well they had been coping with their symptoms/conditions on a 10-point scale (1 = coping extremely poorly, 10 = coping extremely well).

2.3.2 | Data extracted from medical records

Dates of symptom onset, first primary care assessment, first non-rheumatology specialty assessment, rheumatology referral, first rheumatology assessment, diagnosis, and commencement of treatment were extracted from medical records. Commencement of treatment was defined as initiation of ARD-specific therapies such as disease-modifying antirheumatic drugs and biologic agents. Rheumatologist-documented symptoms were extracted from patients' first rheumatology consultation notes.

2.3.3 | Symptom reporting pattern

We compared patients' self-reported symptoms and rheumatologist-documented symptoms (considered as the gold standard) to determine if a patient had under- or over-reported any symptoms. Under-reporting was defined as a symptom not being reported by patients on the self-reported screening questionnaire (administered prior to assessment by the attending rheumatologist) while it was documented by the attending rheumatologist in the first consultation note. Over-reporting was defined as a symptom being reported by a patient on the screening questionnaire while it was not documented by the attending rheumatologist. A patient could therefore under-report one symptom while over-reporting another. We categorized symptom reporting into 4 patterns: (1) only over-reporting; (2) both over- and under-reporting; (3) only under-reporting; and (4) neither over- nor under-reporting.

Qualitative interviews with selected patients who under-reported symptoms were conducted by the first author to understand the reasons for under-reporting. This was based on the hypothesis that, compared to over-reporting, under-reporting is more likely to result in a longer pre-diagnosis interval. Interviews were conducted in patients' preferred language (English or Chinese) in a separate room in rheumatology clinics or over the phone as per patients' preference. Notes were taken during the interview and coded immediately after the interview. Interviews were conducted until data saturation was reached (n = 6).

2.4 | Data analysis

Age, pre-diagnosis interval, Intervals #1-#5 and coping with symptoms were expressed as median (lower and upper quartiles). Gender, ethnicity, marital status, years of education (≤10 vs 11-14 vs ≥15),

TABLE 1 Profile of patients with newly diagnosed autoimmune rheumatic diseases

	Patients referred from primary care (n = 137)	Patients referred from specialty care (n = 122)	Overall (N = 259)	*P* value
Age, y, median (lower and upper quartiles)	51.6 (39.4-59.6)	52.7 (43.0-65.2)	52.0 (41.6-61.9)	.078
Female, n (%)	95 (69.3)	88 (72.1)	183 (70.7)	.623
Ethnicity, n (%)				
Chinese	99 (72.3)	97 (79.5)	196 (75.7)	.435
Malay	12 (8.8)	11 (9.0)	23 (8.9)	
Indian	18 (13.1)	10 (8.2)	28 (10.8)	
Other	8 (5.8)	4 (3.3)	12 (4.6)	
Marital status: married, n (%)	90 (66.2)	79 (64.8)	169 (65.5)	.810
Y of education, n (%)				
≤10	39 (28.7)	45 (37.5)	84 (32.8)	.296
11-14	55 (40.4)	45 (37.5)	100 (39.1)	
≥15	42 (30.9)	30 (25.0)	72 (28.1)	
Comorbidities, n (%)				
0	48 (35.0)	26 (21.3)	74 (28.6)	.015
≥1	89 (65.0)	96 (78.7)	185 (71.4)	
Use of complementary and alternative medicine, n (%)	42 (30.7)	33 (27.1)	75 (29.0)	.523
Coping, median (lower and upper quartiles)	6 (5-8)	6 (5-8)	6 (5-8)	.109

number of comorbidities (0 vs ≥1), use of CAM (yes vs no) and referral source (primary care vs specialty care) were expressed as number (percentage).

We compared Intervals #1-#4 using Wilcoxon's signed rank tests to determine which interval comprised a larger proportion of the pre-diagnosis interval. We compared pre-diagnosis interval and Intervals #1-5 between patients with different ARDs using Kruskal-Wallis tests and, if significant, Mann-Whitney *U* tests for multiple comparisons. We examined the association between pre-diagnosis interval and Interval #1 and the following patient-related factors: (1) socio-demographics; (2) comorbidities; (3) use of CAM; (4) coping with symptoms; (5) symptom reporting pattern; and (6) referral source using median regression tests. All analyses were performed using Stata 15.

3 | RESULTS

3.1 | Patients

Of the 274 newly diagnosed ARD patients who completed the data collection form, 12 with no clear dates of symptom onset documented were excluded as their intervals could not be calculated, and 3 self-referred patients were excluded due to the small number of such patients, leaving 259 patients included in subsequent analyses (Figure 1). Of these 259 patients, 75 were diagnosed with RA, 40 with axSpA (25 with ankylosing spondylitis and 15 with non-radiographic axSpA), 35 with PsA, 21 with seronegative IA, 27 with SjS, 27 with UCTD and 34 with other ARDs (14 with SLE, 9 with SSc, 1 with IIM, 6 with PR, and 4 with overlap ARDs).

These patients were referred from 2 main sources: (1) primary care, including 121 (47%) patients referred to rheumatology care directly and 16 (6%) patients referred to non-rheumatology specialty care and subsequently from non-rheumatology specialty care to rheumatology care; and (2) specialty care, including 122 (47%) patients seeking treatment in non-rheumatology specialty care for other existing conditions.

Socio-demographics of these patients are shown in Table 1. No difference was observed between patients referred from primary care and those referred from specialty care except that a significantly smaller proportion of patients reported at least one comorbidity in the former compared to the latter (65% vs 79%, $P = .015$).

TABLE 2 Under- and over-reporting of symptoms

	Under-reporting[a]		Over-reporting[b]	
	Patients with symptom documented, n	Patients who did not report symptom, n (%)	Patients without symptom documented, n	Patients who reported symptom, n (%)
Joint pain	202	31 (15.4)	57	22 (38.6)
Joint swelling	157	15 (9.6)	102	56 (54.9)
Joint stiffness	138	44 (31.9)	121	41 (33.9)
Swollen fingers	25	5 (20.0)	234	0 (0.0)
Raynaud's phenomenon	14	4 (28.6)	245	22 (9.0)
Digital ulcer	3	3 (100.0)	256	4 (1.6)
Malar rash	4	2 (50.0)	255	6 (2.5)
Telangiectasia	4	3 (75.0)	255	12 (4.7)
Heliotrope rash	0	—	259	6 (2.3)
Gottron's papules/sign	1	0 (0.0)	258	19 (7.4)
Skin thickening	8	6 (75.0)	251	77 (30.7)
Dry eyes	46	14 (30.4)	213	30 (14.1)
Dry mouth	40	8 (20.0)	219	60 (27.4)
Oral/nasal ulcer	22	11 (50.5)	237	21 (8.9)
Loss of hair	32	17 (53.1)	227	22 (9.7)
Seizure	0	—	259	6 (2.3)
Chest pain	6	2 (33.3)	253	21 (8.3)
Muscle weakness	8	4 (50.0)	251	74 (29.5)
Low back pain	75	16 (21.3)	184	60 (32.6)
Heel pain	22	6 (27.3)	237	86 (36.3)
Any symptom	259	109 (42.1)	259	218 (84.2)

[a]A symptom was documented by the attending rheumatologist in the first consultation note but was not reported by patients on the self-reported screening questionnaire (administered prior to assessment by the attending rheumatologist).

[b]A symptom was not documented by the attending rheumatologist but was reported by a patient on the screening questionnaire.

TABLE 3 Patient intervals among patients with autoimmune rheumatic diseases (ARDs)

	Pre-diagnosis interval, mo, median (lower and upper quartiles)	Interval #1, mo, median (lower and upper quartiles)	Interval #2, mo, median (lower and upper quartiles)	Interval #3, mo, median (lower and upper quartiles)	Interval #4, mo, median (lower and upper quartiles)	Interval #5, mo, median (lower and upper quartiles)
Overall, n = 259	11.5 (4.7-36.0)	4.9 (1.0-24.0)	0.3 (0.0-3.9)	1.5 (0.8-1.8)	0.0 (0.0-1.2)	0.0 (0.0-0.7)
RA, n = 75	7.6 (3.1-14.8)	3.5 (1.3-11.6)	0.2 (0.0-2.5)	1.3 (0.6-1.6)	0.0 (0.0-0.2)	0.2 (0.0-0.6)
AxSpA, n = 40	38.7 (9.6-66.7)	26.6 (4.2-56.1)	1.6 (0.0-7.6)	1.6 (1.2-2.3)	0.0 (0.0-2.0)	0.0 (0.0-0.0)
PsA, n = 35	7.0 (3.0-28.4)	2.6 (0.2-11.3)	0.5 (0.2-3.9)	1.6 (0.6-1.7)	0.0 (0.0-0.0)	0.2 (0.0-0.7)
Seronegative IA, n = 21	12.0 (4.7-22.8)	6.4 (1.9-34.4)	0.1 (0.0-4.6)	1.4 (1.3-1.5)	0.0 (0.0-0.8)	0.5 (0.0-1.3)
SjS, n = 27	14.2 (6.0-48.0)	4.6 (0.6-19.0)	0.3 (0.0-3.9)	1.6 (0.9-1.9)	0.8 (0.0-2.3)	0.8 (0.0-1.6)
UCTD, n = 27	15.7 (5.1-39.8)	2.2 (0.7-24.0)	0.8 (0.1-8.1)	1.6 (0.5-1.8)	1.2 (0.0-2.1)	0.0 (0.0-0.9)
Other ARDs, n = 34	8.1 (5.3-36.0)	6.3 (0.9-31.7)	0.2 (0.0-1.1)	1.5 (1.2-1.8)	0.3 (0.0-1.1)	0.0 (0.0-1.1)

Note: Pre-diagnosis interval, the time from symptom onset to diagnosis; Interval #1, the time from symptom onset to the first visit to healthcare professionals; Interval #2, the time from the first primary care or non-rheumatology specialty care assessment to referral to rheumatology care; Interval #3, the time from rheumatology referral to the first rheumatology assessment; Interval #4, the time from the first rheumatology assessment to diagnosis; Interval #5, the time from diagnosis to commencement of treatment.

Abbreviations: axSpA, axial spondyloarthritis; IA, inflammatory arthritis; other ARDs, systemic lupus erythematosus, systemic sclerosis, idiopathic inflammatory myopathies, palindromic rheumatism and overlap syndromes; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SjS, Sjögren's syndrome; UCTD, undifferentiated connective tissue disease.

3.2 | Symptom reporting

All patients had at least one symptom documented by the attending rheumatologist (Table 2). The 3 most commonly documented symptoms were joint pain (n = 202, 78%), joint swelling (n = 157, 61%), and joint stiffness (n = 138, 53%). Nearly 52% (n = 134) of patients only over-reported symptoms, 32% (n = 84) both under- and over-reported symptoms, 10% (n = 25) only under-reported symptoms, and 6% (n = 16) neither under- nor over-reported symptoms.

Through qualitative interviews, 2 main reasons for under-reporting of symptoms were elicited. First, some patients were unaware of their symptoms/bodily changes. For example, one patient mentioned that her knuckles were "big since young" and she did not notice any swelling until being told by her rheumatologist that all her hand joints were swollen. Second, some patients were aware of their symptoms/bodily changes but perceived these symptoms/bodily changes as normal changes in their body instead of manifestations of disease. For example, one patient shared that she noticed her hair started falling after she gave birth but thought the hair falling was normal as "this is what will happen to a woman after she gives birth".

3.3 | Interval between symptom onset and diagnosis

Pre-diagnosis interval and Intervals #1-#5 among patients with different ARDs are shown in Table 3. The median pre-diagnosis interval in our inception cohort of patients was 11.5 months. Interval #1 (median = 4.9 months) was significantly longer than Intervals #2-#4 (median = 0.3, 1.5, and 0.0 months, respectively) in all patients ($P < .001$). Patients with axSpA had significantly longer pre-diagnosis interval (median = 38.7 months) and Interval #1 (median = 26.6 months) compared to patients with the other ARDs ($P < .001$). No differences in pre-diagnosis and Intervals #1-#5 were observed between patients with ankylosing spondylitis and patients with non-radiographic axSpA patients (data not shown).

3.4 | Factors associated with patient intervals

Of all patient-related factors examined, only referral source was significantly associated with pre-diagnosis interval and Interval #1 in univariate analysis among this inception cohort of patients (Table 4). Patients referred from specialty care had significantly longer pre-diagnosis interval (median difference = 7.7 months, $P = .004$) and Interval #1 (median difference = 6.4 months, $P = .014$) compared to those referred from primary care.

Factors associated with pre-diagnosis interval and Interval #1 for patients with different ARDs are detailed in Appendix S2. In brief, referral from specialty care was associated with longer pre-diagnosis interval among patients with UCTD (median difference = 29.7 months, $P = .050$) and longer Interval #1 among patients with RA (median difference = 5.5 months, $P = .038$). Non-Chinese ethnicity (Malay, Indian or others) was associated with longer pre-diagnosis interval

TABLE 4 Factors associated with pre-diagnosis interval and Interval #1

	Pre-diagnosis interval, mo			Interval #1, mo		
	Coefficient	95% CI	*P* value	Coefficient	95% CI	*P* value
Age, y	−0.10	−0.34-0.14	.405	−0.12	−0.30-0.06	.184
Female gender	−0.56	−8.65-7.53	.892	0.82	−4.49-6.13	.761
Non-Chinese ethnicity (reference: Chinese ethnicity)	−4.04	−12.19-4.10	.329	−1.61	−7.07-3.85	.562
Other marital status (reference: married)	1.21	−4.93-7.36	.697	2.24	−3.33-7.80	.429
Years of education (reference: ≤10)						
11-14	0.43	−7.70-8.55	.918	−0.99	−7.28-5.31	.758
≥15	0.82	−7.99-9.63	.854	1.12	−5.56-7.79	.742
Comorbidities[a]	0.21	−2.89-3.32	.892	0.07	−2.04-2.18	.951
Use of complementary and alternative medicine (reference: nil)	1.02	−5.62-7.66	.763	4.90	−0.01-9.81	.050
Coping with symptoms[b]	0.51	−0.95-1.97	.492	−0.07	−1.34-1.21	.919
Reporting of symptom (reference: over-reporting)						
Both over- and under-reporting	−0.79	−8.23-6.65	.835	−1.55	−7.17-4.07	.588
Under-reporting	−3.52	−15.16-8.13	.552	−3.65	−12.34-5.04	.409
Neither over- nor under-reporting	2.14	−12.00-16.27	.766	3.98	−6.73-14.69	.465
Referral from specialty care (reference: primary care)	7.66	2.40-12.92	.004	6.35	1.28-11.41	.014

Note: Pre-diagnosis interval: the time from symptom onset to diagnosis; Interval #1: the time from symptom onset to the first visit to healthcare professionals.

[a]Number of self-reported comorbidities.

[b]Self-reported coping with symptoms was assessed on a 10-point scale (1 = comping extremely poorly, 10 = coping extremely well).

(median difference = 89.5 months, $P = .001$) and Interval #1 (median difference = 55.8 months, $P = .002$) among patients with SjS. Longer years of education was significantly associated with both pre-diagnosis interval (median difference = 64.5 months, $P = .024$) and Interval #1 (median difference = 63.5 months, $P = .026$) among patients with seronegative IA. The association between symptom reporting pattern and Interval #1 was mixed. Patients with UCTD who both over- and under-reported symptoms had significantly shorter Interval #1 (median difference = 23.1 months, $P = .039$) compared to those who only over-reported symptoms, whereas patients with other ARDs who under-reported symptoms had significantly longer Interval #1 (median difference = 53.7 months, $P = .036$) compared to those who over-reported symptoms.

4 | DISCUSSION

In this study, we quantified the pre-diagnosis interval among patients with ARDs and explored possible patient-related factors associated with this interval. The median pre-diagnosis interval was 11.5 months, with Interval #1 comprising a significantly larger proportion compared to Intervals #2-#4. Patients with axSpA had significantly longer pre-diagnosis interval and Interval #1 compared to patients with the other ARDs. Socio-demographics, symptom reporting pattern and referral source were associated with the pre-diagnosis interval and Interval #1 among patients with different ARDs. Findings from this study could provide insights into the development of interventions to reduce the pre-diagnosis interval in the population.

A prolonged pre-diagnosis interval was observed in patients with all ARDs in our study. For example, among patients with RA, less than one-quarter were diagnosed and treated within 3 months of symptom onset, the window of opportunity for treatment to improve patient outcome.[3-6] Interval #1 was significantly longer than Intervals #2-#4 in our study. This is consistent with findings from a systematic review of lag times in RA by Barhamain et al.[13] Our patients with RA had comparable Interval #1 compared to the pooled average in the review (3.5 vs 3.4 months), and relatively shorter Interval #2 (0.2 vs 2.1 months), Interval #3 (1.3 vs 2.9 months), and Interval #4 (0.0 vs 2.1 months).[13] The relatively shorter Intervals #2-#4 in our patients could be explained by the nature of the healthcare system in Singapore and the fact that diagnosis of ARDs has advanced significantly over the past decades.[44,45] It also highlights the importance of interventions targeting patients prior to their first visit to healthcare professionals in reducing the pre-diagnosis interval, as discussed below.

Identification of risk factors for prolonged pre-diagnosis interval and Interval #1 can provide a basis for development of interventions to reduce these intervals. In our study, a variety of factors were identified among patients with different ARDs. This might reflect the fact that, in addition to patient-related factors that we examined, other factors such as physician- and healthcare system-related factors might also have played important roles in affecting

these intervals. Patients referred from specialty care were found to have significantly longer pre-diagnosis interval and Interval #1 compared to those referred from primary care. This could be mainly attributed to 2 reasons. First, patients referred from specialty care were seeking treatment for other existing conditions, to which they might have attributed their ARD symptoms. This was reflected in the significantly higher proportion of patients who reported comorbidities among patients referred from specialty care compared to those referred from primary care. Second, non-rheumatology specialists are obliged to exclude diseases in their specialties before referring a patient to rheumatology care. Hence, these patients might have even longer intervals if the ARD causes for their symptoms were not recognized by non-rheumatology specialists.

The findings that 10% of our patients only under-reported their symptoms and the reasons elicited from qualitative interviews with these patients (unawareness and misperception of symptoms) are of concern. This is because if individuals are not aware of their own symptoms/bodily changes, or attribute their symptoms/bodily changes to reasons other than being ill, they are less likely to seek medical attention or, if they do, seek medical attention early in the course of disease. The awareness and reporting of symptoms have been shown to be dependent on a variety of factors including the relative intensity of symptoms in relation to other information to be processed by an individual at a given time, one's general propensity to attend to own bodily changes, one's knowledge of the symptoms, and cultural beliefs of ARDs.[21-23] For example, individuals who develop low back pain, the main manifestation of axSpA, might not pay sufficient attention to it for one or several of these reasons, especially in the early stage of disease when the pain is mild and insidious in onset. Without any knowledge of axSpA and its symptoms, individuals are less likely to perceive it as a health concern that requires medical attention if low back pain is normally considered as a common and non-severe condition in the general population (cultural beliefs). Interventions to increase the awareness of ARDs and related symptoms could help symptomatic individuals, primary care physicians and non-rheumatology specialists recognize and interpret symptoms and reduce pre-diagnosis interval. For example, public awareness campaigns that improve the knowledge of and correct the false beliefs of ARDs have been shown to promote early axSpA identification in the general population.[42,43,46-48] Self-administered screening questionnaires with illustrations to explain symptoms/signs of ARDs (such as photographs) can prompt symptomatic individuals in the general population to notice their bodily changes and seek medical attention.[48]

There are several limitations in this study. First, self-referred patients were excluded from analysis due to the small number of such patients. We were thus unable to provide a complete picture of intervals among patients with ARDs. We hope to address it in a future study when a larger sample becomes available. Second, we explored only a limited number of patient-related factors associated with patient intervals. There are many other patient-related factors such as severity of symptoms[13] and attitudes toward health professionals,[17] as well as physician- and healthcare system-related factors such as knowledge/familiarity with ARDs among primary care physicians/non-rheumatology specialists,[10,13] access to medical/specialist care[10,13,17] and availability of diagnostics[13,16] that might have contributed to patient intervals in our study. Third, rheumatologist-documented symptoms were used as the gold standard to determine if a patient had under- or over-reported symptoms. It is possible that some rheumatologists might not have documented all symptoms a patient had in the consultation notes, leading to an over-estimation of patients' over-reporting of symptoms. Last, patients in our study were referred from primary and specialty care, where patients might have gained some knowledge of their ARDs and relevant symptoms. Hence, under-reporting of symptoms might have been under-estimated in our study. Ideally, individuals with possible ARD symptoms in the general population should be studied prior to their first visit to healthcare professionals and followed till their diagnosis and commencement of treatment. However, such design is operationally challenging and requires a much larger sample size due to the low prevalence of ARDs in the general population.

5 | CONCLUSION

A long pre-diagnosis interval was observed among patients with ARDs (especially axSpA), due largely to a long interval between symptom onset and the first visit to healthcare professionals. This highlights the importance of interventions targeting patients prior to their first visit to healthcare professionals in reducing pre-diagnosis interval.

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CONFLICT OF INTEREST

Nil.

AUTHOR CONTRIBUTIONS

LX, AHLL, YYL, WF, SWY, TCL, DRK and JT were involved in the conception and study design. LX, AHLL, YYL, WF, MG, SWY and JT were responsible for data analysis and interpretation.

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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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Development and validation of a Behçet's Disease Damage Index for adults with BD: An Explicit, Composite and Rated (ECR) tool

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Abstract

Background: Behçet's disease (BD) is a chronic multisystem variable vessel vasculitis. Disease damage is irreversible and permanent. Validated tools evaluating damage are limited. Enhancements in the clinical treatment of vasculitis will take place from the development of refined and exclusive indices for individual vasculitic syndromes including BD and attempting their international validation.

Objectives: This aim was to develop and validate a simple BD Damage Index (BDI).

Methods: This was a nationwide study including 1252 BD patients. The work consisted of 3 stages. Stage 1: items generation for score content. Stage 2: items selection for the draft score was performed by an expert rheumatologist. Stage 3: the content validity of the draft score was assessed and BDI, Vasculitis Damage Index (VDI), Antineutrophil cytoplasmic antibody-associated Vasculitis Index of Damage (AVID) and Combined Damage Assessment Index (CDAI) were calculated and compared.

Results: The mean age of the BD patients was 36.1 ± 9.9 years. Stages 1 and 2 resulted in a BDI instrument containing 73 items with a maximum score of 100. Stage 3, the VDI, CDAI, AVID, and BDI were 2.9 ± 2.2, 3.1 ± 2.3, 3.1 ± 2.3 and 5.1 ± 2.9, respectively. High correlations ($r = .9$) between comparable damage scores assured acceptable concurrent validity.

Conclusion: The proposed BDI represents a new robust and potentially useful tool when dealing with BD chronic status.

KEYWORDS
Behçet's disease, damage index, derivation, validation

1 | INTRODUCTION

Behçet's disease (BD) is a chronic multisystem variable vessel vasculitis[1] and tends to be more severe in men.[2] A high prevalence is reported in countries along the ancient silk road, stretching from Asia to the Mediterranean countries, but is not confined to these geographic locations.[3] Ocular, vascular, and central nervous system (CNS) involvements are the major causes of BD morbidity and mortality.[2] The long-term outcome of BD involves morbidities from recurrent flares and cumulative damage from preceding disease activities or treatment.[4,5] BD damage is irreversible and not improved by treating disease activity.[1] A comprehensive assessment of patients with BD is critical to distinguish between the disease activity and damage in order to direct the clinician in weighing the management plan.[6]

Specific BD measures exist for the assessment of disease activity, the BD Current Activity Form (BDCAF). At present, the Vasculitis Damage Index (VDI) is the most widely used measure for assessment of BD damage. Despite that, in the original VDI, 8% of the vasculitis patients had BD fulfilling the International Study Group criteria[7] and were considered among other patients with secondary systemic vasculitis. Other generic measures including the Combined Damage Assessment Index (CDAI), and the Antineutrophil cytoplasmic antibody-associated Vasculitis Index of Damage (AVID) have been used in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). Both AVID and VDI weigh all forms of damage equally, thus they do not capture the intuitive sense that some forms of damage exert greater impact on patient health, outcomes and quality of life.[8,9] Because none of these were designed for a BD population, they may contain

irrelevant items and/or lack items that are deemed important to these patients and may be less sensitive than a disease-specific measure.

There was an emerging demand to propose a damage index specific for BD. A recent study published the first tool for describing and measuring organ damage in BD patients, namely the BD Overall Damage Index (BODI) originating from 10 centers in 4 European countries.[10] The BODI showed good to excellent reliability, and significantly correlated with the VDI, and is pending for further validation. With a high prevalence of BD in the Middle Eastern countries and with an increased recognition of geographic diversity in clinical manifestations, for example, cardiac manifestations are less frequent in Caucasians compared to Asian and Middle Eastern patients, while neurological features are more found,[1] there was a need to develop and validate a damage index among Egyptian BD patients.

Taking a step toward the development of refined and exclusive indices for BD, the aim of the study was to develop and validate an explicit, compound and rated BD damage index (BDI) that could function as a measure of BD damage status. The study objectives were extended to allow for comparing the performance of the BDI to the VDI, CDAI and AVID damage scores in patients with BD.

2 | PATIENTS AND METHODS

2.1 | Data sources

Testing the developed index was carried out using data extracted from a large cohort of patients with BD enrolled in the Egyptian College of Rheumatology-Behçet's Disease Study Group (ECR-BDSG). Inclusion criteria were any adults (age ≥18 years) satisfying the diagnostic criteria published by the International Study Group for Behçet's Disease[11] who presented to one of the 26 specialized rheumatology centers around the country, representing 15 major governorates from north to south during 2017-2018. Any patients newly diagnosed (within the first 3 months of the disease course) or with other connective tissue diseases or vasculitis rather than BD were excluded. Any patient diagnosed with juvenile BD (age <18 years) at the time of study recruitment was excluded. Data were collected on a standardized data sheet and stored in an electronic database. The missing data from the various areas were considered. The study conforms to the 1995 Helsinki Declaration and to the ethical standards within the participating university hospitals. Informed consent was obtained from all individual participants included in the study. Study ethics approval was obtained from the University Health Ethics Research Board. More details on ECR-BDSG recruitment and data collection can be found elsewhere.[12]

The patients' files were retrospectively reviewed and analyzed as follows: (1) demographic data, age, gender; (2) clinical data, disease duration, medications, and organs involved; (3) laboratory results, white blood cell count (cells/μL of blood), hemoglobin (Hb: g/dL), platelet, serum creatinine, and lipid panel measures including total cholesterol levels and triglyceride levels (mg/dL). Subjects selected from the ECR-BDSG population for our study (N = 1252) were those with documented evidence in the available medical records (non-missing data). The BD Current Activity Form (BDCAF) was assessed in BD patients.[13] Juvenile-onset cases were defined as those adult patients with an age at disease onset before 18 years.

2.2 | BDI development and validation

The work consisted of 3 stages: (1) the derivation of items for index content; (2) the selection of items for the draft index; and (3) the assessment of the draft index for content validity.

2.2.1 | Stage 1: Derivation of items for score content

Items were derived from the VDI which was carefully revised by 5 rheumatology experts and professors (SF, NN, HF, NH and TG) and the less relevant items to BD were removed. Items of importance to the BD were carefully added.

2.2.2 | Stage 2: Selection of items for the draft score

Item selection for the draft index was conducted manually. Each item was carefully revised to ensure that it related specifically to BD. These items were organized into groups (systems). A panel (highly qualified professors and consultants) met to consider each potential item in order to further modify it. Decisions regarding the combination of items are included in this stage. A maximum score of 100 was considered in order to add to the easiness of interpretation and follow up of the index.

2.2.3 | Stage 3: Assessment of the draft score for content validity

In order to properly test whether the suggested index determines what it is meant to measure and ensures that it is meaningful to patients with BD, the BDI was measured in a cohort of patients with BD through expert rheumatologists. Any damage scored had to be present following the onset of vasculitis and be present for at least 3 months. The total VDI score and total CDAI are each represented by the cumulative number of items recorded. All forms were completed in English. Written instructions on how to complete the assessment were provided and the feedback response of each expert was highly considered.

2.3 | Statistical analysis

Data were analyzed to determine the normality distribution using the Kolmogorov-Smirnov test. Continuous variables with normal distribution presented as mean ± SD, or as proportions for categorical variables. Correlation analysis was performed using Spearman correlation analysis. Multiple regression models were fitted with suspected covariates to identify factors independently associated with the BDI score. A *P* value of less than .05 was considered to be statistically significant. All statistical analyses were performed using Statistical Package for Social Science (SPSS) program version 22.

TABLE 1 Characteristics of the Behçet's disease patients

Feature, mean ± SD or n (%)	Behçet's disease (N = 1252)
Age, y	36.1 ± 9.9
Gender M:F	2.6:1
Age at onset, y	29.4 ± 8.6
Disease duration, y	6.8 ± 5.2
BMI	28.1 ± 5.5
Diabetes	212 (16.9)
Hypertension	347 (27.7)
Oral ulcers	1246 (99.5)
Genital ulcers	1044 (83.4)
Cutaneous	660 (52.7)
Ocular	923 (73.7)
Arthralgia/arthritis	630 (50.3)
Neuropsychiatric	254 (20.3)
Cardiovascular	321 (25.6)
Vascular	344 (27.5)
Pulmonary	119 (9.5)
Renal	48 (3.8)
GIT	139 (11.1)
ENT	35 (2.8)
BDCAF	4.8 ± 4.4
ESR, mm/1st h	29.6 ± 20.3
Hb, g/dL	13.1 ± 5.4
TLC, $\times 10^3/mm^3$	8.5 ± 13.1
Platelets	262.8 ± 78.8
ALT, U/L	29.5 ± 82.6
Serum creatinine, mg/dL	0.81 ± 0.79
Cholesterol, mg/dL	180.8 ± 47
Triglycerides, mg/dL	120.9 ± 74.2
SUA, mg/dL	4.8 ± 1.5

Abbreviations: ALT, alanine-aminotransferase; BDCAF, Behçet's disease current activity form; BMI, body mass index; ENT, ear/nose/throat; ESR, erythrocyte sedimentation rate; GIT, gastrointestinal tract; Hb, hemoglobin; SUA, serum uric acid; TLC, total leucocytic count.

3 | RESULTS

3.1 | Sociodemographic and clinical characteristics of the study population

The study included 1252 BD patients with complete records and disease duration more than 3 months. The patients' characteristics are presented in Table 1. There were 394/1252 (31.5%) who were smokers, mostly males. Fifty-seven patients had juvenile-onset BD. The mean ± SD of the BDCAF was 4.8 ± 4.4. All patients received steroids at any time of their disease period, 1023 (81.7%) received colchicine, 275 received cyclosporine A (22%), azathioprine in 431 (34.4%), cyclophosphamide in 213 (17%), oral anticoagulants in 203 (16.2%), methotrexate in 94 (7.5%), chlorambucil in 8 and biologic therapy in 82 (6.5%).

Derivation and selection of items for draft score (stages 1 and 2). A potential pool of 98 items was generated. From the panel's discussions, it was agreed that 73 items in 11 categories should form the draft index. Rating certain items was important to allow for a weighted index that considers the more serious morbidities and items as heavier. A maximum BDI score of 100 was generated. The final version of the BDI is shown in Figure 1, and the definitions of the individual items are presented online (Supplement 1).

Assessment of the draft index for content validity (stage 3). Total and individual item score differences between BDI and the VDI, CDAI and AVID in patients with BD are presented in Supplement 2. For the studied patients with BD, the VDI, CDAI, AVID and BDI were 2.9 ± 2.2, 3.1 ± 2.3, 3.1 ± 2.3 and 5.1 ± 2.9 respectively, Figure 2. The BDI significantly correlated with the VDI ($r = .95$, $P < .001$), CDAI and AVID ($r = .97$, $P < .001$).

3.2 | Variation of BDI across demographic groups

There was a significant increase in the assessed damage indices in females compared to males in BD patients (VDI 3.3 ± 2.5 vs 2.7 ± 2.1, $P < .0001$; CDAI/AVID 3.5 ± 2.6 vs 2.9 ± 2.1, $P < .001$; BDI 5.5 ± 3.2 vs 4.9 ± 2.7, $P = .002$). The BDI significantly correlated with the patient's age ($r = .16$, $P < .0001$), and age at onset ($r = .15$, $P < .0001$). Among the governorates of the participating centers, there is a significant difference ($P < .0001$) in the average BDI score as shown in Figure 3. There was a tendency to a north-south gradient of BDI, being higher in the northern and central provinces compared to southern.

3.3 | Relationship with clinical and laboratory manifestations

The BDI significantly correlated with disease duration ($r = .17$, $P < .0001$). A comparison of the BDI after dividing the BD patients according to their disease duration was conducted. The mean BDI score was significantly higher in patients with longer disease duration (>5 years) (5.4 ± 2.9 years) compared to those with ≤5 years

Behçet's disease Damage Index (BDI) Date: (d/m/y) / /

Visit number: Gender: M / F Patient's ID number:
Disease onset: (d/m/y) / / Age (years): Patient's name code:

Record positive items (1point each unless specified) persistent (>3 months) unless initially irreversible
Complete BDI at baseline visit (3 months after disease onset) and every 3 months thereafter

1. Skin/mucous membrane	/2
NONE ○	
Scarring/Refractory Ulcer	○
Refractory PG-like or EN-like	○
2. Ocular	/20
NONE ○	
Cataract *(either eye)*	○○
Synechiae *(either eye)*	○○
Glaucoma *(either eye)*	○○
Optic atrophy *(either eye)*	○○
Visual field defect *(either eye)*	○○
Visual impairment, Diplopia *(either eye)*	○○
Retinal vein occlusion *(either eye)*	○○
Retinal artery occlusion *(either eye)*	○○
Retinal changes (retinopathy)*(either eye)*	○○
Blindness(score 2 if one eye/ 4 if both)*	○○
3. Ear/Nose	/2
NONE ○	
Sensorineural hearing loss (SNHL)	○
Vestibular disorder (dizziness/vertigo)	○
4. Musculoskeletal	/4
NONE ○	
Arthritis	○
Sacroiliitis	○
Enthesopathy	○
Avascular necrosis	○
5. Neuropsychiatric	/11
NONE ○	
Epileptic seizures	○
Transverse myelitis	○
Cranial nerve lesion #	○
Stroke (CVA)/2nd CVA	○○
Peripheral neuropathy	○
Brain stem or Δ tract lesion	○
ICHy (headache/papilledema)	○
Cerebellar or extra Δ disorder	○
Cognitive dysfunction	○
Psychosis (hallucination/delusion)	○
6. Cardiovascular	/11
NONE ○	
Angina	○
Hypertension	○
Cardiomyopathy	○
Cardiac thrombus	○
Left ventricular dysfunction	○
Pericarditis or pericardiectomy	○
Myocardial infarction/*Subsequent*	○○
Endocarditis (valvular heart disease)	○
Percutaneous coronary intervention	○
Coronary bypass graft or angioplasty	○

7. Vascular Disease	/14
NONE ○	
Claudication	○
Minor tissue loss/*Subsequent*	○○
Major tissue loss/*Subsequent*	○○
DVT (one side/both sides)	○○
DS-T /IJV–T (score 2 if both)	○○
SVC-T/IVC-T (score 2 if both)	○○
Cerebral or vertebral artery lesion	○
Aortic/carotid lesion (score 2 if both)	○○
8. Pulmonary	/11
NONE ○	
Pulmonary artery aneurysm/*Subsequent*	○○
Pulmonary artery thrombosis/*Subsequent*	○○
Pulmonary artery hypertension/*Subsequent*	○○
Pulmonary embolism, infarction/*Subsequent*	○○
Alveolar hemorrhage	○
Impaired PFT/ *Progressive* PFT impairment	○○
9. Renal	/4
NONE ○	
CKD: GFR <60 ml/min/1.73m²	○
Proteinuria ≥ 0.5 g/d or Cr >2mg/dl	○
ESRD: GFR <15 ml/min/1.73m² ‡	○
Dialysis/Transplant	○○
10. Gastrointestinal	/4
NONE ○	
Persistent GIT ulcers	○
Budd-Chiari syndrome	○
Intestinal perforation	○
Intestinal ischemia, Infarction	○
11. Other Damages	/17
NONE ○	
Association Co-morbidity	
Amyloidosis	○
Fibromyalgia	○
Gonadal failure	○
Diabetes mellitus	○
Chronic infection	○
Neurogenic bladder	○
BM failure (refractory cytopenia)	○
Hematological malignancy/*Progressive*	○○
Solid malignant neoplasm/ *Progressive*	○○
Management-related (Irreversible AE):	
Medications	○
Surgery	○
Overlap Variant$	
One overlap/ ≥ two overlaps	○○
Other features	○○
BDI total score	%

Clinic: Clinic Coordinator ID: Physician ID:
Signature *Signature*

The BDI is for recording irreversible organ damage that has occurred in Behçet's disease patients since disease onset due to the disease itself, newly associated conditions or its management. Events should persist for >3 months to be scored unless initially irreversible. It is anticipated that the score is steady or increases over time. Associated conditions or co-morbidities present before the disease onset should not be scored. It should not be used to assess the disease activity; *visual impairment should not be scored if the corresponding eye is blind; # cranial neuropathy excluding optic (II) and vestibulocochlear (VIII) nerves; ‡CKD should not be scored if the patient has ESRD. $*Overlap and variants include*: MAGIC: mouth and genital ulcers with inflamed cartilage, VHS: Vogt-Koyanagi-Harada syndrome, Sweet syndrome, IBD: inflammatory bowel disease and SpA: spondyloarthritis.AE: adverse events, BM: bone marrow, CKD: chronic kidney disease, CVA: cerebrovascular accident, DS-T: dural sinus thrombosis, DVT: deep venous thrombosis, EN: erythema nodosum, ESRD: end stage renal disease, GIT: gastrointestinal tract, GFR: glomerular filtration rate, ICHy: Intracranial hypertension, IJV-T: internal jugular vein thrombosis, IVC-T: inferior vena caval thrombosis, PFT: pulmonary function test, PG: pyoderma gangrenosum, SVC-T: superior vena caval thrombosis, Δ: pyramidal.

FIGURE 1 The Behçet's Disease Damage Index (BDI)

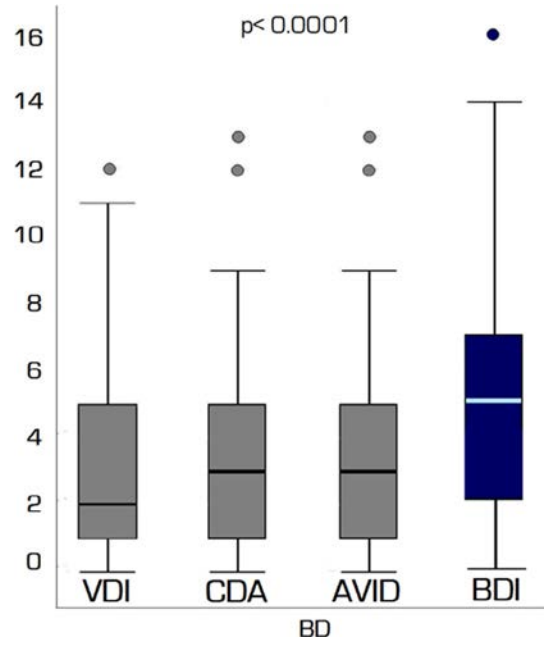

FIGURE 2 Disease damage scores in Behçet's disease (BD) patients

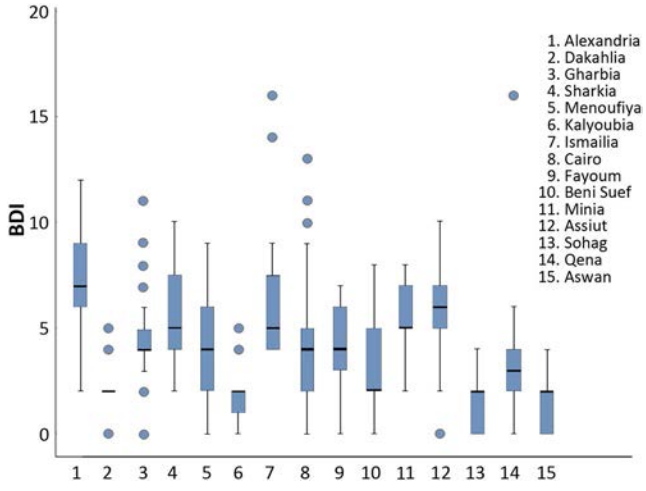

FIGURE 3 The Behçet's Disease Damage Index (BDI) among the Egyptian governorates ordered according to the north-south gradient in Behçet's disease patients

disease duration (4.8 ± 2.8 years, $P < .001$). The BDI significantly correlated with serum cholesterol level ($r = .15$, $P = .012$); however, there was no significant correlation of the BDI with the erythrocyte sedimentation rate, Hb, total leukocyte count, platelets, triglycerides, serum creatinine and serum uric acid ($P > .05$). Additionally, there was no significant correlation of the BDI score with the BDCAF ($r = .008$, $P = .9$). In the multivariable regression analysis, male gender ($\beta = -.104$, $P < .001$), disease duration ($\beta = .066$, $P = .017$), CNS involvement ($\beta = .186$, $P < .001$), vascular involvement ($\beta = .271$, $P < .001$), treatment with glucocorticoids ever ($\beta = .496$, $P < .001$), and cyclophosphamide use ever ($\beta = -.300$, $P < .001$) were independently associated with a higher BDI score.

4 | DISCUSSION

In the present study, we have developed a new BD damage index aimed at determining the specific damage events occurring in BD patients. Furthermore, BDI shows a good discriminative validity compared to the other vasculitis damage indices in patients with BD. Development and validation of an efficient, responsive and reliable damage index, to properly measure the chronic damage status, is a priority in BD. The BDI was derived from an authenticated vasculitis damage score and literature review with expert feedback throughout, then was validated on an independent national multicenter cohort. This index demonstrates a high predictive accuracy for disease severity, and shows a strong association with the presence of certain clinical manifestations.

The final BDI score consists of 73 items in 11 categories and a total score of 100 points, making it short and practical to use in research studies and clinical practice. There is a potential influence of the demographic parameters and geographic distributions on BD severity nationwide. The BDI varied with age and women tended to have severer index. In contrast, the previous review demonstrated that the disease could have a more severe course and an overall significantly higher mortality rate among young male patients.[14] Various factors may explain these differences such as clinical features of BD and treatment adherence that may vary between the genders and influence the outcomes.

The DBI varied across geographic areas where there was a higher tendency toward a north-south gradient. In-depth level and capabilities of investigation among the different governorates may explain the differences in the BDI and also the limited number of contributions from certain areas. Additionally, severity appears to be influenced by age of onset, older age at diagnosis tends to be accompanied by a high degree of overall damage compared to juvenile-onset BD. Yet, the longer the disease duration, the more the damage. Literature on severity of organs of BD according to the age is conflicting, which may be the result of differences in geographic and ethnic origin of the patients, use of different age criteria, and variations in the study design. Because our data were collected from different centers, we assume that the results are homogenized. Interestingly, previous studies[15,16] from Turkey revealed that the frequency of disease manifestations was not different between juvenile- and adult-onset BD, except neurologic and gastrointestinal involvement, which were higher in juvenile cases; Alpsoy et al.[16] reported that serious organ involvement such as neurologic, large-vessel, and gastrointestinal involvement had later onset during the course of the disease. They mentioned that BD usually starts with relatively milder manifestations, and severe involvements, in general, appear later which may explain the underestimation of damage at early onset when the organ injury is easily overlooked. Among the reported limitations of the Turkish studies was that the BD patients were applying to dermatology and venerology departments, thus giving reason for the milder speculated spectrum of the disease. Also, in an Egyptian study, VDI was significantly related to the diagnostic delay in BD.[17]

Majority of the patients in this cohort had mucocutaneous manifestations, followed by ocular and musculoskeletal systems in that order and the number of patients with renal involvement was low. This is comparatively similar to the previously report that renal involvement in BD patients varies in different studies but is not common. End-stage renal failure can be seen in very rare cases.[18] Those with hypertension and diabetes mellitus were associated with higher BDI score. Association of comorbid conditions is consistent with increased morbidity in BD and hypertension increases the risk of cardiovascular events,[19] contributing to organ damage in BD patients.

Literature that implies a difference in the severity of organ involvement between male and female BD patients is limited. In contrast to our finding, the BODI score was significantly higher in men.[10] This may be attributed to the gender difference in male to female ratio according to ethnicity. The BODI study was conducted on 228 patients from 10 southern European centers with a male : female ratio of 0.97:1. Moreover, a recent study from Cagliari, Italy reported a male : female ratio of 0.5:1,[20] while in this study the male : female ratio was 2.6:1. Furthermore, a possible cultural reluctance among women from Eastern countries to visit a physician for genital ulcers leading to delayed diagnosis of BD with the development of more severe organ involvement may partly serve as another cause. In concert, in a US registry on 498 patients with axial spondyloarthritis (axSpA), women had higher disease burden than men[21] in part because they have a longer delay in diagnosis.[21,22] Improved awareness of gender differences in the presentation of axSpA may aid physicians in earlier identification and improved disease management.[21] X-linked genetic factors, hormonal factors, different subjective experience and response to treatment could all influence the severity of an autoimmune disease.[23] The prevalence of the disease, the frequency of specific clinical findings, and the mortality rate have distinct geographical and ethnic variation. Further research is required to replicate and clarify the gender differences of damage in BD.

Disease activity is the presence of any ongoing expression of vasculitis that is not caused by disease damage, comorbidity, or treatment and is usually graded as low, medium, or high.[6] For BD, the BDCAF is a convenient option.[24] The BDI score is not correlated with BDCAF; however, this does not make it invalid. The BDI measure would be expected to discriminate between levels of disease activity and damage. Rather, lack of correlation may sometimes be desirable because BDI is typically conceptualized as being independent of clinical activity status. Furthermore, it may be possible to provide a clinical explanation for this result. Some damage is amenable to treatment (eg, cataracts), whereas some damage is related to being longstanding which is different from the current disease status. In a Romanian study, the VDI was assessed in BD patients and found to be significantly correlating with the BVAS.[25]

Lacking validated and unequivocal instruments for an accurate assessment of the damage events occurring during the course of BD makes the other non-specific damage scores still the most used tools. There are currently few validated vasculitis damage scales published in the literature: VDI, CDAI, and AVID.[26] The VDI is an organ-based system that is scored after 3 months; however, there are concerns about its ability to determine the full spectrum of damage in patients with vasculitis of small- and medium-sized vessels.[27] In accordance, the AVID was created to obtain a "score" that represents the total burden of damage uniquely in AAV.[28] Both AVID and VDI weigh all forms of damage equally so that the damage score does not capture the intuitive sense that some forms of damage exert a greater impact on patients' health, outcomes and quality of life.[8,9] The CDAI is a damage tool based on the VDI and was constructed to record additional special items for GPA.[9,26,28] The CDAI has been tested in comparison to the original VDI but found to be too cumbersome for practical use and did not add any value to the existing damage index;[25] however, there are ongoing attempts to exercise the CDAI in BD patients.[28] The VDI and the CDAI measure the damage that has occurred since the onset of vasculitis; pre-existing comorbidity is not counted.[26] The VDI performance in determining the damage in BD has never been critically assessed until a preliminary BD-specific damage index was proposed by a Turkish team of investigators and was presented in the 2016 European League Against Rheumatism congress.[29] They considered the VDI items and added new BD-specific damage items including detailed cardiovascular, ocular, and vascular involvement, particularly venous disease-related damages to provide a more comprehensive assessment of damage in BD. A recent study focused on the development and validation of the first tool for describing and measuring organ damage in BD patients, namely the BODI.[10] The BODI includes 9 domains, 34 unweighted items and 12 subitems with a total score of 46. It showed good to excellent reliability, and significantly correlated with the VDI (construct validity) but had greater sensitivity in identifying major organ damage and did not correlate with disease activity measures discriminating damage from the major confounding factor. The instrument was deemed credible (face validity), complete (content validity) and feasible. In line, the Outcome Measures in Rheumatology (OMERACT) vasculitis working group developed a core set of domains for BD that includes damage as one of them to be assessed in clinical trials.[30]

The BDI demonstrates good concurrent validity with the comparable domains of the VDI; however, they show differences in the scores of certain domains which is corresponding to the disease-specific comorbidities. The 2 indices have been derived from distinct sets of available parameters and neither was intended to be all similar. When compared with other vasculitis damage scores, BDI demonstrated good performance in terms of sensitivity, specificity, positive predictive value and negative predictive value. Encompassing more precise and focused items of relevance to the BD makes it unsurprising that the range and mean of the damage score were higher than that calculated for the VDI or CDA. Ocular and neuropsychiatric manifestations weighed higher on BDI, while ear/nose and pulmonary weighed more on VDI consistent with previous literatures of the severity of these organs affected in BD.[31-33] Although the item scores of skin lesions and musculoskeletal were comparable in both, the domains within the items were not similar in their representation.

Notably both indices, BDI and CDAI, heavily weighed cardiovascular involvement equally, reflecting the well-known prognostic

significance of cardiovascular involvement in medium and small-vessel vasculitis cohorts.[34] The number and description of the items within the BDI are not unified to other vasculitis damage scores to allow for a reasonable comparison of the individual systems within each index. There are a few unique components in BDI that are more likely to exist in BD, the vascular diseases such as arterial lesions, for example, aortic, carotid and pulmonary artery aneurysm. Budd-Chiari syndrome, a rare portal venous thrombosis lesion, is known to be associated with BD in reported cases[35] and exists in the BDI.

The literature focused on long-term damage and remission is limited. BD is characterized by a relapsing and remitting course.[36] If untreated, morbidity and mortality are considerably high in patients with major organ involvement.[37] The main goal of management is to suppress inflammation rapidly for major organ involvement that may cause damage and even be fatal.[33] Although complete remission is the current target in rheumatological diseases, it is fairly difficult to achieve complete remission in BD with current approved therapeutic regimens.[38] Remission of BD in patients with major organ involvement can be achieved after anti-tumor necrosis factor (anti-TNF) treatment. Drug-free, long-term remission after withdrawal of successful anti-TNF treatment is feasible in patients with severe BD.[39] Prospective studies should examine whether anti-TNF agents should be used as first-line treatment for the induction of remission in patient with major organ involvement.

One of the innovative aspects of our study comprise the inclusion of new items into the BD damage score, corresponding to a careful evaluation of the number of potential items that could contribute to the damage process. A second important contribution is the assignment of a numerical score to each selected item and its corresponding subheading, yielding a global score ranging from 0 to 100. Furthermore, each system damage assessment is corresponding to a separate score that allows the accurate follow up assessment of the specific system. Despite that, the present study has some limitations. A first critical point consists of potential patient bias since they were recruited from tertiary centers and we could have selected those patients with more severe disease, not fully representing the entire spectrum of the disease. A second limitation of this study was that some items of the damage index, in particular the rare ones, were not collected in the retrospective BD patients' cohort. Linked to this point, a third limitation depends on the low number of some rare renal events retrieved in the study cohorts, making the results not fully generalizable to all the renal events included in BD. Finally, patients asymptomatic for particular systems were not considered for further investigations of the involved system which may lead to an underestimated frequency of organ/system damage involvement. Further prospective studies are needed to undermine the extended relation of disease age at onset to the BD damage score.

An important future research agenda would be to further validate the performance of the BDI in other populations and it could be refined, and revised versions considered later on with changes to improve the score. It would be interesting to compare the BDI with BODI among our BD patients in the future ECR work. It is recommended that a long-term damage follow up is considered in future work as a part of a wider international initiative to determine the disease outcome. Well-designed longitudinal studies in a BD cohort could be useful in validating the BDI, especially if they provide independent assessments of changes in disease status over time.

In conclusion, the newly developed and validated BD damage index could potentially determine the damage events occurring in BD patients. The BDI represents a new robust and potentially useful tool that can be used for research and also assist clinician in dealing with BD chronic states. It may further enable the identification of the events most contributing to disease morbidity. Hopefully, this initiative presents a great start to foster quality assessment and management of BD.

CONFLICT OF INTEREST

All authors declare no 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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Familial aggregation of juvenile idiopathic arthritis with other autoimmune diseases: Impact on clinical characteristics, disease activity status and disease damage

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Abstract

Objectives: To evaluate the impact of family history of autoimmune diseases (FHADs) on the clinical characteristics and outcome of juvenile idiopathic arthritis (JIA).

Methods: We retrospectively reviewed children with JIA seen in 7 pediatric rheumatology clinics from 6 Arab countries. All included patients met the International League of Associations for Rheumatology classification criteria for JIA and had a disease duration greater than 1 year. Data were collected at the last follow-up visit and comprised clinical findings, including FHADs. Disease activity and disease damage were assessed by Juvenile Arthritis Multidimensional Assessment Report, and juvenile arthritis damage index (JADI) respectively. Disease activity was categorized as remission off treatment, remission on treatment, or active disease.

Results: A total of 349 (224 females) JIA patients with a disease duration of 5 (interquartile range 2.9-7.5) years were included. The most frequent JIA categories were polyarticular JIA and oligoarticular JIA, followed by systemic JIA. There were 189 patients with FHADs and 160 patients without FHADs. The most frequent FHADs were diabetes mellitus (21.2%), JIA (18.5%), rheumatoid arthritis (12.7%). Among patients with FHADs, 140/189 (74.1%) achieved clinical remission, while 131/160 (81.9%) patients without FHDs had clinical remission (odds ratio [OR] = 1.2, 95% CI 0.97-1.5). Rate of consanguinity, enthesitis-related arthritis (ERA) and psoriatic arthritis were higher in patients with FHADs (OR = 0.6, 95% CI 0.4-0.9 and OR = 1.2, 95% CI 1.1-1.4). Also, articular JADI correlated significantly with presence of FHADs (OR = 1.1, 95% CI 1.0-1.1).

Conclusion: This study shows that autoimmune diseases cluster within families of patients with JIA with a high proportion of ERA and psoriatic arthritis. JIA patients with FHADs are likely to have more disease damage.

KEYWORDS

consanguinity, familial arthritis, familial autoimmune diseases, juvenile arthritis damage index, juvenile arthritis disease activity score, juvenile idiopathic arthritis

1 | INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common chronic childhood idiopathic rheumatic disease, and to date has largely been regarded as a polygenic disorder. However, the exact etiology and pathogenesis of JIA are not well-defined.[1,2] Like other autoimmune and autoinflammatory disorders, the interactions of epigenetic and environmental factors contribute and influence the disease susceptibility and expression.[3,4] The observational studies confirm that co-occurrence of more than 1 autoimmune disease in individuals and within families because of sharing common genetic susceptibility factors support the concept of genetic influence in the pathogenesis of JIA. The consanguineous marriage in Arab countries reaches 50%-75% of all marriages, which might underscore the overall prevalence of autoimmune diseases and the influencing role of genetic factors in the disease phenotype and outcome.[5] Predicting JIA outcome is challenging; most of the studies related the long-term outcomes to various predictors, including JIA category, diagnostic delay and late initiation of the appropriate treatment.[6,7] Also, other factors such as biomarkers could add value to the clinical variables.[8] A high aggregation of autoimmunity including familial JIA is observed among first- and second-degree relatives of patients with JIA; however, the knowledge about this observation is limited.[9-12] Of note, recently, familial JIA gained more attention, and might be associated with guarded outcome.[13-15] Most studies that have looked at the familial aggregation of autoimmunity in JIA patients and their relatives, and have focused on the association and the estimated risk of autoimmune diseases among individuals and their relatives. However, the impact of clustering of autoimmunity has not been adequately studied.[9,16,17] There are scarce data published on JIA long-term outcome in JIA patients with relatives with autoimmune diseases.[18] Our study aimed to determine whether the family history of autoimmune diseases (FHADs) affects the clinical characteristics and outcome of JIA in a highly consanguineous population.

2 | METHODS

2.1 | Study population

This is a multi-center retrospective, observational cohort analysis. Data were collected from 7 tertiary pediatric rheumatology clinics from 6 Arab countries between July 2010 and July 2019. We only included patients if they were younger than 14 years of age and met the International League of Association for Rheumatology criteria for classification for JIA and had a disease duration greater than 1 year with a least 2 follow-up visits.[19] It is worth mentioning that, as per our hospital policy, those who exceed 14 years of age are transferred to adult rheumatology care to provide comprehensive clinical care. Patients with chronic arthritis other than JIA were not included. The study compared JIA patients with and without familial autoimmunity which was defined based on positive family history of any autoimmune diseases, including inflammatory and non-inflammatory, among their first- or second-degree relatives. Enrolled patients were assigned to 2 groups, patients with FHADs and those without FHADs. Data were collected at the last follow-up visit including age, gender, JIA category, age at disease onset and at diagnosis, disease duration, consanguinity, FHADs. The study participants were asked whether their relatives had been diagnosed with autoimmune disease. Laboratory variables such as rheumatoid factor (RF), anticyclic citrullinated peptide (anti-CCP), anti-nuclear antibody (ANA) and human leukocyte antigen B-27 (HLA-B27) results were interpreted according to the cut-off values of the local laboratories. Also, the provided treatment focusing on conventional synthetic and biologic disease-modifying anti-rheumatic drugs (DMARDs) was reviewed.

2.2 | Outcomes

Included patients were assessed every 3-6 months with a detailed musculoskeletal examination, laboratory evaluation, and therapy was adjusted accordingly. At the last follow-up, patients were evaluated for disease activity status and disease damage. The disease activity was assessed using Juvenile Arthritis Multidimensional Assessment Report (JAMAR).[20,21] The main disease activity categories were inactive disease; either off or on treatment and active disease. Also, the damage was calculated using juvenile arthritis damage index (JADI) for both articular damage (JADI-A) and extra-articular damage (JADI-E).[22] Disease damage was only scored when it was not due to active disease and present for at least 6 months.

Completed data sheets were sent back to the principal investigator at King Faisal Specialist Hospital and Research Center (KFSH-RC), Riyadh, for analysis.

2.3 | Statistical analysis

All statistical analyses were performed using the SAS software package, version 9.4 (Statistical Analysis System, SAS Institute Inc.). Descriptive statistics for continuous variables were reported as median and interquartile range (IQR), and mean ± SD was used when deemed necessary. Categorical variables were summarized as frequencies and percentages. The strength of the correlation between FHADs and JIA characteristics and outcome was measured as odds ratio (OR) calculated from the coefficient of a binary logistic regression. The level of significance was set at P value <.05.

2.4 | Ethical considerations

These patients were previously enrolled in a cumulative damage study among Arab children with JIA under the approval of the Ethics Committee of the Research Affairs Council (RAC) at KFSH-RC (RAC#2191 110 on 19 December 2019). The study was conducted in accordance with the Declaration of Helsinki. All the collected data resulted from routine medical assessments. All data were collected

anonymously, and the confidentiality of the patients was protected. Ethical approval was also obtained by all the participating centers by their institutional research ethics committees.

3 | RESULTS

The study cohort constituted 349 (224 females) JIA patients with a median age of 11.3 (IQR 8.0-15.0) years. The median age at onset was 5 (IQR 2.0-9.0) years and the median disease duration was 5 (IQR 2.9-7.5) years. The most frequent JIA categories were polyarticular JIA (31.2%) and oligoarticular JIA (30.7%), followed by systemic JIA (22.6%). Overall, there were 189 (54.2%) patients with at least 1 relative with FHADs and 160 (45.8%) patients without FHADs. There were 175 familial aggregations of autoimmune diseases. Table 1 shows the frequency of autoimmune diseases among families of JIA patients. Three main clusters of autoimmune diseases were identified. Diabetes mellitus (21.2%), JIA (18.5%), rheumatoid arthritis (12.7%) and autoimmune thyroid disease (7.4%) were the most frequent familial autoimmunities. Table 2 shows the comparison of the demographics, features and provided treatment of JIA patients with and without familial autoimmune diseases. The consanguinity rate was more noticeable in JIA patients with FHADs, while patients without FHADs had a younger age at onset. The 2 groups (patients with and without FHADs) were largely similar in clinical characteristics. However, RF negative polyarticular category, psoriatic arthritis and enthesitis-related arthritis (ERA) were more frequent in patients with FHADs. Interestingly, patients with psoriatic and ERA had a polyarticular course. The differences between ANA, RF, anti-CCP and HLA-B27 in the 2 groups were not significant. Most of the patients received methotrexate and various biologic DMARDs. Of note, anti-tumor necrosis factor blockade was the most frequent biologic DMARD prescribed, followed by tocilizumab. However, the frequency was comparable in the 2 groups.

TABLE 1 Frequency of autoimmune diseases among families of juvenile idiopathic arthritis patients

Autoimmune disease	Frequency
Diabetes mellitus	40 (21.2%)
Juvenile idiopathic arthritis	35 (18.5%)
Rheumatoid arthritis	24 (12.7%)
Autoimmune thyroid disease	14 (7.4%)
Psoriasis	12 (6.3%)
Systemic lupus erythematosus	11 (5.8%)
Vitiligo	9 (4.8%)
Celiac disease	9 (4.8%)
Inflammatory bowel disease	7 (3.7%)
Psoriatic arthritis	3 (1.6%)
Dermatomyositis	2 (1.1%)
Behçet's disease	2 (1.1%)
Gout	2 (1.1%)
Enthesitis-related arthritis	1 (0.5%)
Scleroderma	1 (0.5%)
Myasthenia gravis	1 (0.5%)
Multiple sclerosis	1 (0.5%)
Uveitis	1 (0.5%)

3.1 | Outcomes

One hundred and thirty (81.3%) patients without FHADs had inactive disease status while 140 (74.1%) patients with FHADs showed inactive disease with nearly one-third of them were in complete remission without treatment. Furthermore, 30 (18.8%) patients without FHADs and 49 (25.9%) patients with FHADs showed active disease despite intensive treatment. Overall, 107 (30.7%) patients suffered from joint damage with a mean JADI-A score of 2.1 ± 5.1, while 81 (23.2%) patients had extra-articular damage with a mean JADI-E score of 0.5 ± 1.1. The frequency of damage and the cumulative extra-articular damage were comparable between the 2 groups. However, patients with FHADs had a greater cumulative articular damage. Table 3 shows the comparison of disease status and damage between JIA patients with and without FHADs.

3.2 | Correlation and prediction

There was a significant difference between the 2 groups in favor of patients with FHADs regarding high rate of consanguinity and predisposition of clinically distinct JIA phenotypes. The probabilities of impact of FHADs on the disease characteristics and outcome are shown in Table 4. Considering only FHADs, the rate of consanguinity (OR = 0.6, 95% CI 0.4-0.9, P = .02), and certain JIA categories namely, RF negative polyarticular JIA, ERA and psoriatic arthritis (OR = 1.2, 95% CI 1.1-1.4, P = .001) were higher in patients with FHADs. Patients with familial aggregation of autoimmune diseases had more cumulative articular damage (OR = 1.1, 95% CI 1.0-1.1, P = .005). There was a correlation between FHADs and continuation of active disease. However, it was not statistically significant. In contrast, FHADs did not show significant influence on gender, age of disease onset, or cumulative extra-articular damage.

4 | DISCUSSION

Using standardized and validated outcome tools and measurements is inconsistent among JIA studies. Various clinical and laboratory variables have been suggested as predictors for disease damage and quality of life of patients with JIA.[6,23,24] Data about the disease activity status and disease damage of JIA are increasingly reported worldwide. However, there is scant data from Arab countries. Of note, the prevalence of JIA categories and disease course and prognosis are variable among different ethnicities.[7,15,25] High rate of consanguinity might influence the

TABLE 2 Demographics and clinical features of JIA patients with and without familial autoimmune diseases

	Total	JIA with familial autoimmune diseases	JIA without familial autoimmune diseases
Number of patients	349	189	160
Gender, M:F ratio	1:1.8	1:1.7	1:1.9
Current age, y (median)	11.3 (8.0-15)	12 (8.0-15)	12 (8.0-13)
Age at onset, y (median)	5.0 (2.0-9.0)	5.8 (3.0-9.0)	4.2 (2.0-8.5)
Disease duration, y (median)	5.0 (2.9- 7.5)	5.0 (3.0-7.6)	5.0 (2.4-7.4)
Consanguinity (%)	51.3	57.1	44.4
JIA categories			
Oligo persistent	88 (25.2%)	35 (18.5%)	53 (33.1%)
Oligo extended	19 (5.4%)	12 (6.4%)	7 (4.4%)
Poly RF+	27 (7.7%)	11 (5.8%)	16 (10%)
Ploy RF−	82 (23.5%)	51 (26.9%)	31 (19.4%)
Systemic	79 (22.6%)	39 (20.6%)	40 (25%)
Psoriatic	23 (6.6%)	18 (9.5%)	5 (3.1%)
ERA	24 (6.9%)	17 (8.9%)	7 (4.4%)
Undifferentiated	7 (2%)	6 (3.2%)	1 (0.6%)
ANA	102/337	52/181	50/156
RF	27/298	15/165	12/133
Anti-CCP	16/182	10/103	6/79
HLA-B27	8/135	6/87	2/48
Methotrexate	240	130 (54.2%)	110 (45.8%)
Adalimumab	87	47 (54%)	40 (46%)
Etanercept	82	47 (57.3%)	35 (42.7%)
Tocilizumab	74	44 (59.5%)	30 (40.5%)
Infliximab	14	9 (64.3%)	5 (35.7%)
Anakinra	13	7 (53.8%)	6 (46.2%)
Abatacept	8	6 (75%)	2 (25%)

Abbreviations: Anti-CCP, anticyclic citrullinated peptide; ERA, enthesitis-related arthritis; F, female; HLA, human leukocyte antigen; JADI-A, juvenile arthritis damage index-articular; JADI-E, juvenile arthritis damage index-extra-articular; JIA, juvenile idiopathic arthritis; M, male; RF, rheumatoid factor.

phenotype and outcome. Recently, we demonstrated a positive correlation of family history of JIA with cumulative disease damage and continuation of active disease in JIA patients, denoting that familial JIA might be a potential predictive factor for disease outcome.[26] Autoimmune diseases are a heterogenous group with a wide spectrum of clinical manifestations and predisposition to clustering of autoimmunity. The importance of FHADs was previously recognized; the co-existence of selected autoimmune diseases in relatives of JIA patients with autoimmune disease has been observed. Evidence revealed a higher prevalence of diabetes mellitus, chronic arthritis and autoimmune thyroid disease within JIA families than in control families.[9,27,28] However, it is unclear whether familial aggregation of autoimmunity represents a burden in the long-term outcome of JIA patients. Khani et al.[18] found that 16 JIA patients with FHADs were mostly of the polyarticular category and younger at onset, with likely persistent active disease. However, they did not report the cumulative disease damage. In the current study, we reported the influence of familial aggregation of autoimmunity in the phenotype and outcome of a large multi-center cohort of JIA patients in a highly consanguineous population. Our study showed variability in the frequency of JIA; patients of relatives with familial aggregation of autoimmunity had a higher frequency of RF negative polyarticular JIA, ERA and psoriatic arthritis with polyarticular course. The exact explanation for this discrepancy among JIA categories compared to other studies remains unclear. However, genetic factors might influence the phenotypic variability. There was a positive correlation between active disease and articular damage and familial aggregation of autoimmunity, showing that the presence of FHADs might be considered as a likely predictive factor for the disease status and

TABLE 3 Disease activity status and disease-related damage at the last follow-up visit of JIA patients with and without familial autoimmune diseases

	Total	JIA with familial autoimmune diseases	JIA without familial autoimmune diseases
Number of patients	349	189	160
Inactive disease			
Off treatment, n (%)	85	41	44
On treatment, n (%)	186	99	86
Active disease, n (%)	78	49	30
JADI-A, ≥1, n (%)	107 (30.7)	60 (31.7)	47 (29.3)
Mean ± SD	2.1 ± 5.1	2.6 ± 6.1	1.4 ± 3.5
JADI-E, ≥1, n (%)	81 (23.2)	45 (23.8)	36 (22.5)
Mean ± SD	0.5 ± 1.1	0.5 ± 1.2	0.5 ± 1.1

Abbreviations: ERA, enthesitis-related arthritis; JADI-A, juvenile arthritis damage index-articular; JADI-E, juvenile arthritis damage index- extra-articular; JIA, juvenile idiopathic arthritis.

TABLE 4 Models obtained by a binary logistic regression analysis to estimate the correlation between family history of autoimmune diseases and JIA characteristics and outcome

	JIA with familial autoimmune diseases	JIA without familial autoimmune diseases		
	189	160	OR (95% CI)	*P* value
Gender, M:F	70:119	55:105	0.9 (0.6-1.4)	.6
Consanguinity	108 (57.1%)	71 (44.4%)	0.6 (0.4-0.9)	.02
Disease categories				
Poly RF−	51 (26.9%)	31 (19.4%)	1.2 (1.1-1.4)	.001
ERA	17 (8.9%)	7 (4.4%)		
Psoriatic arthritis	18 (9.5%)	5 (3.1%)		
Age at onset; y, median (IQR)	5.8 (3.0-9.0)	4.2 (2.0-8.5)	1.0 (0.9-1.01)	.1
Disease status				
Inactive, n (%)	140 (74.1)	130 (81.3)	1.2 (0.97-1.5)	.08
Active, n (%)	49 (25.9)	30 (18.8)		
JADI-A, ≥1, n (%)	60 (31.7)	47 (29.3)	1.1 (1.0-1.1)	.05
JADI-E, ≥1, n (%)	45 (23.8)	36 (22.5)	1.0 (0.8-1.2)	.9

Abbreviations: CI, coefficient intervals; ERA, enthesitis-related arthritis; F, female; JADI-A, juvenile arthritis damage index-articular; JADI-E, juvenile arthritis damage index- extra-articular; JIA, juvenile idiopathic arthritis; M, male; OR, odds ratio; RF, rheumatoid factor.

outcome. Our results showed some similarity to the observations from other studies.[18] Most previous studies of familial JIA have either ignored the familial aggregation of autoimmunity in JIA or have not reported long-term disease outcome.[9,10,18]

This study has its limitations, and results should be interpreted carefully. Sample size was not calculated, given the rarity of this disease and the nature of this work (retrospective observational study), in particular the sample taken from an abnormally distributed population. We are aware of the analytical challenges that arise without power analysis. Also, data were collected retrospectively for patients diagnosed over a long period with variations in management and availability of medications. Information about the relative's autoimmune disease was obtained from the participants without confirmatory review of their records. Additionally, molecular genetic studies were not considered in this work. There was no healthy matched control group. Accordingly, the prevalence rate of familial autoimmunity was not calculated.

5 | CONCLUSION

This study shows that autoimmune diseases cluster within families of patients with JIA with a high proportion of ERA and psoriatic arthritis. JIA patients with FHADs are likely to have more disease damage. To the best of our knowledge, this is the largest study showing the impact of familial aggregation of autoimmunity on long-term prediction of a highly consanguineous population-based JIA cohort. Our findings might be useful in guiding decisions about JIA management

and family counseling. Hence, FHADs should be systematically considered in the assessment of children with JIA. However, further studies are needed to assess the impact of familial autoimmunity in patients with JIA before this is incorporated as an established risk.

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ORIGINAL ARTICLE

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Sjögren's syndrome complicated with membranous nephropathy, a cause or coincidence?

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Abstract

Background: Sjögren's syndrome (SS) has been a well-documented cause of secondary membranous nephropathy (MN); however, the prevalence is quite low. Since primary MN is also a common disease in middle age, whether MN is secondary to SS or just coincidence remains uncertain. The detection of phospholipase A2 receptor (PLA2R), which is most often positive in idiopathic MN, has been rarely reported in such cases.

Methods: We retrospectively studied 13 cases diagnosed with MN and SS in Huashan Hospital between 2009 and 2020, and performed PLA2R detection. We also review the literature by searching in the PubMed database.

Results: Among the 13 patients, 8 were found to be PLA2R-positive and 5 were negative. Nine patients were female. All but 1 patients had normal renal function at the time of biopsy. All patients showed positive anti-nuclear antibody and anti-SSA. Two of the 8 PLA2R-positive patients showed positive anti-SSB and 1/8 had mild hypocomplementemia, while all 5 PLA2R-negative patients had positive anti-SSB and 3 showed hypocomplementemia. Renal biopsy revealed focal MN and markedly mesangial hyperplasia in PLA2R-negative patients. Mesangial electron deposits were observed in 1 PLA2R-positive patients in small amounts, and in 3 PLA2R-negative patients with 2 in large amounts. During follow-up, 2 patients in the PLA2R-negative group presented with progressively decreasing serum complement levels, and another one was diagnosed with systemic lupus erythematosus (SLE).

Conclusions: In patients with both MN and SS, PLA2R-negative MN should be considered as a secondary form. Careful screen for SLE is necessary in these patients during follow-up.

KEYWORDS

membranous nephropathy, phospholipase A2 receptor, primary Sjögren's syndrome, secondary

Ruiying Chen and Jia Wang contributed equally to this work.

1 | INTRODUCTION

Sjögren's syndrome (SS), also known as sicca syndrome, is a chronic autoimmune inflammatory process presenting either as a primary disorder or in a secondary form that complicates other rheumatic conditions like systemic lupus erythematosus (SLE) and rheumatoid arthritis.[1] Primary SS usually occurs in middle age, with an incidence of 0.3%-0.5% in the general population aged 40 years or older, and with a predilection for women in a female-to-male ratio of 9.[2,3] Diminished lacrimal and salivary gland function is the dominant characteristics of SS, which results in xerostomia and xerophthalmia. Further, extra-glandular manifestations can also be seen, such as cutaneous vasculitis, interstitial lung disease and nephritis.[4] The prevalence of renal involvement reported in primary SS varies from 1% to >30%, depending on the study population and diagnostic criteria.[5] Among cases undergoing kidney biopsy, tubular-interstitial nephritis (TIN) is the most common type, which is characterized by plasma cells and lymphocytes surrounding the tubules, reminiscent of those seen around the ducts in inflamed salivary glands. By contrast, glomerulonephritis is relatively rare and mostly attributed to the deposition of immune complexes. Pathologically, it is predominantly manifest mesangioproliferative glomerulonephritis (MPGN) associated with cryoglobulins, as well as membranous nephropathy (MN).[5]

MN, characterized by the thickening of glomerular basement membrane due to the presence of subepithelial immune deposits, is among the most common causes of nephrotic syndrome in nondiabetic adults. It is most often idiopathic, but also found to be associated with autoimmune diseases, infections, malignancies and the use of certain drugs in about one-quarter of cases.[6] SS has been a well-documented cause of secondary MN since the 1970s when MN associated with SS was first described in a case report. However, the prevalence is quite low, which is <2% in most reported studies, and even 0 in a study involving 471 primary SS patients.[7-10] Studies describing the concurrence of SS and MN are sparse, most of which are case reports, usually with confounding diseases like lymphoma and other autoimmune diseases. Recently, 2 studies showed that MN accounted for 36% and 50% of renal lesions respectively in SS patients who had undergone renal biopsy, being the leading pattern of glomerulonephritis in primary SS.[9,11] However, since primary MN is also a common disease in middle age, whether MN is secondary to SS or just coincident remains uncertain in both studies.

M-type phospholipase A2 receptor (PLA2R) has been considered as a promising diagnostic biomarker for idiopathic MN since its discovery.[12] In this study, we present 13 MN cases concomitant with primary SS and perform PLA2R detection, so as to explore the value of PLA2R examination in determination of MN secondary to SS. We also review the literature regarding the renal pathological pattern of primary SS and case reports of SS with the presence of MN, in an attempt to determine the association between MN and SS.

2 | MATERIALS AND METHODS

2.1 | Patients

We screened the clinical and pathological data of 545 consecutive patients with biopsy-proven MN but no SLE, hepatitis or malignancies at the time of kidney biopsy in Division of Nephrology, Huashan Hospital, Fudan University between January 1, 2009 and March 31, 2020. We found 13 patients who fulfilled the 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for SS which requires patients to have at least 1 symptom of ocular or oral dryness and have a total score of ≥4, derived from the sum of the weights assigned to the following positive items: (a) focal lymphocytic sialadenitis with focus score ≥1 (score 3); (b) anti-SSA/Ro positivity (score 3); (c) ocular staining score ≥5 or van Bijsterveld score ≥4 in at least 1 eye (score 1); (d) Schirmer's test result ≤5 mm/5 min in at least 1 eye (score 1); (e) unstimulated whole saliva flow rate ≤0.1 mL/min (score 1). None of them fulfilled the 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria for SLE at the time of kidney biopsy. This study was approved by ethics committee of Huashan Hospital, Fudan University (KY2016-394). Informed consent was obtained from all patients.

2.2 | Clinical data

Clinical information at the time of biopsy were collected, including demographic data, medical history, clinical presentation and laboratory results. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease-Epidemiology Collaboration 4-level race equation. Follow-up data comprising intervening therapy and renal outcome were also collected. Remission was defined as urinary protein excretion <3.5 g/24 h and a ≥ 50% reduction from peak value, accompanied by an improvement or normalization of serum albumin and stable serum creatinine.

2.3 | Renal pathological procedure

A standard renal biopsy procedure including light, immunofluorescence (IF) and electron microscopy was used for renal pathological diagnosis. The extent of glomerular sclerosis, mesangial proliferation, and tubular-interstitial damage was assessed and scored semi-quantitatively. Immunoglobulin G (IgG), IgA, IgM, C3, C4 and C1q were measured by direct IF on frozen sections of fresh tissue and scaled from 0 to 4+. Dense deposits were quantified under electron microscopy.

2.4 | Renal PLA2R and serum PLA2R antibodies (PLA2R-Ab) detection

Renal PLA2R detection was performed on paraffin-embedded renal biopsy samples, using heat retrieval in citrate buffer (pH 6)

and anti-PLA2R-Ab (Sigma, HPA012657) with a 1:500 dilution. PLA2R positivity was characterized as granular staining along the capillary loops. Negative control (secondary antibody only) was used in every case to exclude the cross-reactions between secondary antibody and human IgG. Serum PLA2R-Ab was detected by indirect IF. The procedure has been described in our previous report.[13] In brief, serum samples were diluted at 1:10, 1:100 and 1:1000 in phosphate-buffered saline, applied to the fixed cells expressing the full length PLA2R DNA. A fluorescein isothiocyanate (FITC)-conjugated anti-human IgG antibody from mice with 1:100 dilution was used as secondary antibody. Transfected cells with specific cytomembrane fluorescence were considered to be positive. Commercial anti-PLA2R-Ab was used for positive control, and normal serum and secondary antibodies were used for negative control.

2.5 | Literature search

A search of MEDLINE (PubMed) was performed for all published reports using the MESH terms: Sjögren syndrome; sicca syndrome; glomerulonephritis. All case reports with complete data and articles accessing renal involvement in primary SS by renal biopsy were included, while those without renal biopsy results were excluded.

3 | RESULTS

3.1 | Clinical characteristics

In our previous research, 84% of primary MN patients and 64% of hepatitis B virus-associated MN patients were PLA2R-positive respectively, while only 2.6% of class V lupus nephritis were PLA2R-positive.[13] Of the 13 patients with MN and SS included in this study, 8 were PLA2R-positive. According to the PLA2R, these 13 patients were classified into 2 groups. Among the 8 patients with PLA2R positivity, 5 were female, and 7 were 40-70 years old while 1 (Patient 2) was 30 years old. Five patients were diagnosed with SS and MN concurrently, while 1 (Patient 4) had SS years before the onset of MN and another 2 (Patients 2 and 6) had SS after MN. Six of the 8 patients presented with nephrotic syndrome. Among the 5 patients with PLA2R negativity, 4 were female and only 1 was male, aged from 33 to 68. They were diagnosed with SS and MN concurrently, with 2 exhibiting nephrotic syndrome. Twelve of the 13 patients had normal renal function at the time of renal biopsy while the other 1 (Patient 5) experienced acute kidney injury and temporary hemodialysis, who recovered 1 month after the treatment. All patients had positive anti-nuclear antibodies and anti-SSA. Two of the 8 PLA2R-positive patients showed positive anti-SSB and 1/8 had slightly decreased serum complement level. By contrast, all 5 PLA2R-negative patients had positive anti-SSB and 3 of them showed hypocomplementemia (Table 1).

3.2 | Histological features

Biopsies showed typical features of MN in all cases, including thick-appearing capillary loops and spike-like formation under light microscope, granular IgG deposits along the capillary wall by IF, and subepithelial electron-dense deposits under electronic microscope. To be noted, the membranous lesion only involved partial glomeruli (focal) in Patient 10 whose PLA2R was negative. C3 was positive in 11 out of 13 patients. IgA was positive in only 1 patient whose PLA2R was positive, and IgM was all negative. C1q was positive in 1/8 PLA2R-positive patient and 2/5 negative patients. In the PLA2R-positive group, mild mesangial hypercellularity was observed. Acute interstitial nephritis was diagnosed in 1 patient (Patient 2) and acute tubular necrosis was diagnosed in another (Patient 5). Small amounts of mesangial deposits were detected under electron microscopy in only 1 patient. In the PLA2R-negative group, only mild tubulointerstitial lesions could be noted. Markedly mesangial hyperplasia was found in 1 patient (Patient 12) and mesangial deposits were identified in 3 of the 5 patients, 2 of which were in large amounts (Table 2).

3.3 | Treatment and outcome

In the PLA2R-positive group, 4 patients with severe nephrotic syndrome received prednisone plus immunosuppressant and 1 used prednisone monotherapy. The other 3 received non-immunosuppressive treatment alone. Among the 6/8 with follow-up data available, 5 patients achieved remission and 1 patient showed no relief of the disease after immunosuppressive therapy during the 15-month follow-up. In the PLA2R-negative group, 1 patient (Patient 12) was diagnosed with SLE 20 months later with increasing anti-double-stranded DNA levels, and 2 (Patient 11 and Patient 13) presented with progressively decreasing serum complement levels. Therefore, they were given prednisone plus immunosuppressant or hydroxychloroquine, and all achieved remission. The other 2 patients were given non-immunosuppressive therapy, 1 of which showed improved proteinuria during 24-month follow-up while the other was lost. All 10 patients with follow-up data available maintained stable renal function during 26 months (range 8-120 months) of median follow-up (Table 1).

3.4 | Literature review

Fifteen articles reporting the prevalence of glomerulonephritis in primary SS with renal biopsy data available were identified from the database (Table 3).[7-11,14-23] Among the total 483 cases, 203 (42.0%) demonstrated changes compatible with glomerulonephritis. MN was the leading type, which accounted for 36.5%, followed by mesangial proliferative glomerulonephritis (20.7%), MPGN (18.2%) and FSGS (13%). However, apart from the 2 recent studies of Yang et al. and Luo et al. from China where the prevalence of MN dramatically increased, MN was seldom reported in SS, which

TABLE 1 Baseline clinical characteristics and follow-up of patients with Sjögren's syndrome and membranous nephropathy

Patient	Age	Gender	PLA2R[a]	eGFR (mL/min*1.73 m^2)	UPro (g/24 h)	ALB (g/L)	ANA	Anti-SSA	Anti-SSB	C3 (g/L)	C4 (g/L)	Follow-up (mo)	Treatment	Renal outcome
1	60	F	+	98.5	2.16	37	1:3200	+	-	1	0.25	8	ARB	Remission
2	30	F	+	90.7	8.46	22	1:10 000	+	+	1.03	0.73	/	ARB	Loss to follow-up
3	64	F	+	97.6	1.18	30	1:320	+	-	**0.85**	0.22	/	ARB	Loss to follow-up
4	54	M	+	93.0	7.23	22	1:1000	+	+	1.04	0.31	120	P + HCQ +CTX P + FK506 (relapse)	Remission
5	50	M	+	**6.7**[b]	10.09	15	1:1000	+	-	1.04	0.47	81	Urgent HD, P + CTX	Remission and restoration of normal renal function
6	68	M	+	79.6	18.86	13	1:1000	+	-	1.09	0.24	15	P + CTX	No remission after treating for 15 months
7	65	F	+	85.7	5.65	26	1:3200	+	-	1.27	0.27	46	P +HCQ	Remission
8	70	F	+	71.9	15.16	24	1:100	+	-	1.47	0.28	17	P + CTX	Remission
9	45	F	-	110.8	2.38	33	1:1000	+	+	0.95	0.24	/	ARB	Loss to follow-up
10	33	F	-	124.6	1.58	38	1:1000	+	+	**0.67**	**0.08**	24	ARB	Decreasing urine protein after treating for 3 months
11	50	F	-	105.0	0.84	37	1:320	+	+	**0.66**	0.15	60	P + CTX	Remission; progressively decreasing serum complement level after 3 months
12	46	F	-	108.7	3.78	28	1:1000	+	+	**0.56**	0.14	28	P + HCQ +ARB	Remission; progressively increasing anti-double-stranded DNA level after 20 months
13	68	M	-	88.6	13.41	15	1:10 000	±	+	1.04[c]	0.39	16	P + FK506; P + CTX (relapse)	Remission but relapse after 5 months with decreasing C3 level

Bold indicates hypocomplementemia in Patient 3, 10, 11 and 12.

Abbreviations: ALB, serum albumin; ANA, anti-nuclear antibody; CTX, cyclophosphamide; HCQ, hydroxychloroquine; HD, hemodialysis; ND, no data. ARB, angiotensin receptor blocker; P, prednisone; PLA2R, phospholipase A2 receptor; UPro, urinary protein.

[a]Patients 4 and 6 were found to be PLA2R-positive with serum PLA2R-Ab positivity at a titer of 1:1000, and others were classified into PLA2R-positive or negative groups according to the renal PLA2R detection results.

[b]Patient 5 experienced acute kidney injury at the onset of the disease.

[c]Patient 13 presented decreased serum complement level during follow-up.

TABLE 2 Histological features of patients with Sjögren's syndrome and MN

	Immunofluorescence								
Patient	IgG	C3	IgA	IgM	C1q	PLA2R	Mesangial EDs	Tubulointerstitial lesion	Diagnosis
1	3+	+	-	-	-	+	None	Focal infiltration of lymphocytes and tubular atrophy; mild interstitial fibrosis	MN
2	3+	+	-	-	-	+	**Small amounts**	Interstitial infiltrate with lymphocytes, accompanied by a few tubules atrophic	MN; AIN
3	3+	+	-	-	-	+	None	None	MN
4	+	+	-	-	-	ND	ND	None	MN
5	3+	+	-	-	-	+	None	Focal infiltration of lymphocytes and interstitial fibrosis	MN; ATN
6	+	-	-	-	+	ND	ND	Focal infiltration of lymphocytes, tubular atrophy, and interstitial fibrosis	MN
7	3+	+	±	-	-	+	None	Vacuolar degeneration in a few tubular epithelial cells; occasional tubular atrophy	MN
8	3+	+	-	-	-	+	None	Occasional tubular atrophy and mild interstitial fibrosis	MN
9	3+	+	-	-	-	-	**Small amounts**	None	MN
10	+	-	-	-	-	-	None	None	Focal MN
11	2+	+	-	-	-	-	None	None	MN
12	3+	2+	-	-	±	-	**Large amounts**	Phosphate deposition within the lumen of some tubules; mild interstitial fibrosis with a few lymphocyte infiltrate; occasional interstitial foam cells	MN
13	3+	2+	-	-	+	-	**Large amounts**	Occasional tubular atrophy and mild interstitial fibrosis	MN

Bold indicates mesangial eletronic deposits in Patient 2, 9, 12, 13.

Abbreviations: AIN, acute interstitial nephritis; ATN, acute tubular necrosis; EDs, electron-dense deposits; MN, membranous nephropathy; ND, no data; PLA2R, phospholipase A2 receptor.

seems less than MPGN (22 vs 34 cases) in the other 13 studies. In 8 reports with available total number of primary SS patients, the prevalence of MN was reported ranging from 0% to 3.46%.

In addition, 19 case reports including 22 patients with SS and MN were also obtained (Table 4).[24-42] The medium age of these patients was 55 years and 76.2% were female. Sixteen cases had other concurrent diseases, with 3 lymphomas and 13 autoimmune disorders such as rheumatoid arthritis, thyroiditis, Graves' disease, primary biliary cirrhosis and so on. Among the other 6 patients, one had MN mixed with proliferative glomerulonephritis, and another with crescentic glomerulonephritis as well. Only 4 patients had primary SS complicating MN. The treatment of these cases was various. Sixteen patients received prednisone with or without other therapies such as plasma exchange and immunosuppressant. Nine out of 21 patients achieved remission of the renal diseases.

4 | DISCUSSION

Although renal involvement in SS was first described with reports of typical tubular defects in the 1960s,[43] it was found heterogeneous by later studies, ranging from isolated electrolyte disturbances and nephrolithiasis, to glomerulonephritis and TIN in both acute and chronic forms. Glomerulonephritis accounts for about 42% of patients with renal biopsy data available,[8,44] of which the majority is immune complex-mediated, with the leading type being MPGN associated with cryoglobulins and subsequent MN. Recently, MN was found even more significantly than MPGN in 2 studies from China.[10,11] As both primary MN and primary SS are frequent diseases in middle-aged people, and the incidence of primary MN has dramatically increased during the past 2 decades in China,[45] coincidence of the 2 diseases could be common.

It is important to distinguish MN secondary to SS from concomitant MN and SS as the management could be quite different. In patients with SS who present with sicca symptoms alone but no glandular enlargement or other organ involvement, systemic immunosuppressive therapy will not be required. Instead, when renal involvement like secondary MN presents, glucocorticoids and/or immunosuppressants should be considered. So far, there is no consistent treatment recommendation for SS-associated glomerulonephritis. Most patients were treated with steroids alone or combined with immunosuppressant or plasma exchange based on histological lesions.[8,18,44] For MN secondary to SS, limited data have showed a reasonable response to steroids, so that a course of corticosteroids was suggested as first-line treatment.[8,19,24,30,44] This is different from the treatment of primary MN, in which non-immunosuppressive therapy

TABLE 3 Pathological spectrum of renal involvement in primary Sjögren's syndrome (SS) patients

References	Year	Total primary SS (No.)	Renal involvement (No.)	Renal biopsy (No.)	TIN (No.)	GN (No.)	MN (No.)	MPGN (No.)	MesPGN (No.)	FSGS (No.)	Others (No.)
Siamopoulos KC, et al.[14]	1992	36	-	9	5	2	1	1	0	0	0
Yang J, et al.[10]	1997	-	26	26	18	14	4	1	1	8	0
Goules A, et al.[7]	2000	471	20	18	10	9	0	5	4	0	0
Bossini N, et al.[15]	2001	60	16	9	6	3	1	1	1	0	0
Ren H, et al.[16]	2005	-	103	30	20	10	1	0	5	2	2
Wu M, et al.[17]	2007	-	-	16	8	11	2	1	6	2	0
Maripuri S, et al.[8]	2009	7276	-	24	19	8	1	2	0	3	2
Goules AV, et al.[18]	2013	715	35	33	16	21	2	10	8	1	0
Kidder D, et al.[19]	2015	-	-	25	15	9	1	6	1[a]	0	1
Ichikawa K, et al.[20]	2017	-	-	35	29	6	2	0	2	0	2
Jasiek M, et al.[21]	2017	-	-	95	93	22	4	9	0	5	4
Yang HX, et al.[9]	2017	2096	-	103	53	50	37	0	6[b]	3	4
Jain A, et al.[22]	2018	70	35	17	9	6	0	0	3[a]	1	2
Carrillo-Perez DL, et al.[23]	2018	-	-	13	6	7	3	1	0	1	2
Luo J, et al.[11]	2018	434	217	30	9	25	15	0	5[c]	1	4
Total		-	-	483	316	203	74	37	42	27	23

Abbreviations: FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; MesPGN, mesangial proliferative glomerulonephritis; MN, membranous nephropathy; MPGN, membranoproliferative glomerulonephritis; TIN, tubulointerstitial nephritis.

[a]Immunoglobulin A (IgA) nephropathy.

[b]Half of the patients were diagnosed with IgA nephropathy.

[c]Four of the 5 patients were diagnosed with IgA nephropathy.

TABLE 4 Cases of Sjögren's syndrome (SS) complicating membranous nephropathy (MN)

References	Year	Age	Gender	Complicated diseases	Treatment	Renal outcome
Moutsopoulos HM, et al.[24]	1978	72	M	None	P	Remission
Schwartzberg M, et al.[25]	1979	ND	F	Rheumatoid arthritis	Chrysotherapy	No increase of proteinuria
Font J, et al.[26]	1989	34	M	Mixed membranous and proliferative glomerulonephritis; cryoglobulinemia	P + CTX; PE	Died of septicemia
Ogawa N, et al.[27]	1990	53	F	Progressive systemic sclerosis; Use of bucillamine	Discontinued bucillamine	No remission
Kosugi E, et al.[28]	1996	50	F	None	P	Decreasing proteinuria
Yoshida K, et al.[29]	1996	13	M	None	P + MZR	Remission
Tatsumi H, et al.[30]	1998	64	F	None	PE; P	Remission
Abe H, et al.[31]	2004	75	F	TTP, crescentic glomerulonephritis	PE; P	Unresponsive and died
Laraki R, et al.[32]	2005	40	F	Polyarthritis, immune-type lymphadenopathy, Hashimoto thyroiditis	P + CTX	Remission
Baba A, et al.[33]	2005	40/62/63	F/F/F	PBC, autoimmune thyroiditis / PBC, autoimmune thyroiditis / Interstitial pneumonia	P ± CTX	Decreasing proteinuria in 2 patients
Vos P, et al.[34]	2006	59	F	Marginal zone B-cell lymphoma	P + chlorambucil	No remission
Iwanaga N, et al.[35]	2007	62	F	Non-Hodgkin's lymphoma of the tongue	CHOP chemotherapy; radiation	Remission
Makino M, et al.[36]	2008	40s	F	Graves' disease, renal necrosis, catastrophic thrombosis	Right nephrectomy; P	Remission
Stefanidis I, et al.[37]	2008	43	F	Polymyositis, autoimmune hepatitis	ND	ND
Prasad D, et al.[38]	2009	77	M	Celiac disease	P + CTX	Died of MI
Kim CS, et al.[39]	2012	52	F	EBV-positive diffuse large B-cell lymphoma	P + HCQ	Remission
Yabuuchi J, et al.[40]	2016	19	F	Progressing to MPGN with cryoglobulinemia; Graves' disease	P + CsA; thyroidectomy	Remission
Tam WK, et al.[41]	2018	57	F	AVB	HCQ +ARB; permanent pacemaker implantation	Decreasing proteinuria
Gupta N, et al.[42]	2020	56	M	CIDP	P + (IVIG→rituximab→CTX)	Remission

Abbreviations: ARB, angiotensin II receptor blocker; AVB, atrioventricular block; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CIDP, chronic inflammatory demyelinating polyneuropathy; CsA, cyclosporine A; CTX, cyclophosphamide; HCQ, hydroxychloroquine; IVIG, intravenous immunoglobulin; MPGN, membranoproliferative glomerulonephritis; MZR, mizoribine; ND, no data; P, prednisone; PBC, primary biliary cirrhosis; PE, plasma exchange; TTP, thrombotic-thrombocytopenic purpura.

is recommended at first unless there is damaged renal function or life-threatening complications, while corticosteroid monotherapy is recommended not to be used.[46] In this case series, the treatment is heterogeneous with regimens from non-immunosuppressive therapy to corticosteroids plus hydroxychloroquine and/or cyclophosphamide, as a result of the uncertain relationship between MN and primary SS. Therefore, a definitive diagnosis regarding such relationship before initiating therapies will be important.

PLA2R was first discovered as a target autoantigen in 70% of patients with primary MN by Beck et al. in 2009.[12] Although PLA2R (renal PLA2R or serum PLA2R antibodies) has also been found positive in some secondary MN with different rates, it is usually negative in rheumatic disease-associated MN, especially in type V lupus nephritis. To date, only 1 study has concerned PLA2R detection in MN secondary to SS and reported positive in 1 out of 6 patients.[47] SS is characterized by the presence of various antibodies, such as anti-nuclear antibodies, anti-SSA, anti-SSB or others, which is similar with SLE, so that the mechanism by which SS causes MN may also resemble type V lupus nephritis, which is due to immune complex deposition. In this regard, it is probable that PLA2R positivity would be also rare in SS-associated MN. In our study, nearly all PLA2R-positive patients had normal serum complement levels without evidence of any other systemic autoimmune diseases except SS, and had few secondary signs like the presence of additional immunoglobulin, C1q or mesangial proliferation in pathology. Moreover, 2 of them had been diagnosed with MN years before SS, which was inconsistent with the finding that renal symptoms usually presented 2-7 years after the initial diagnosis of SS.[15,18,44] By contrast, patients with negative PLA2R presented with more hypocomplementemia, as well as atypical MN pathological features. All these findings suggest that PLA2R-positive MN in SS may be coincident occurrence and PLA2R detection is useful for differential diagnosis.

Evidence for MN secondary to SS is extremely limited. Renal biopsy data regarding spectrum of renal involvement in primary SS showed only a handful of cases with MN pattern, no more than 4 in each report, except the recent 2 studies from China, in both of which the testing for PLA2R was not performed, as listed in Table 3. These two studies had also mentioned that whether MN is a distinct entity overlapping with SS or a unique subset of SS-related renal involvement cannot be determined in their studies.[9,11] Although some case reports also presented patients with both MN and SS, as listed in Table 4, most of them had another concomitant autoimmune disease, such as rheumatoid arthritis, systemic sclerosis, polymyositis, autoimmune thyroiditis and primary biliary cirrhosis and so on, or lymphoma. In our study, 4 of the 5 PLA2R-negative patients were female and most of them presented with hypocomplementemia. Three showed marked mesangial hyperplasia and electronic dense deposit, and 1 showed focal MN pathologically. During the follow-up, 1 patient was diagnosed as having SLE with elevated anti-double-stranded DNA level and 2 patients probably developed SLE with decreased serum complement level, indicating lupus as an underlying etiology of MN. Considering that the causal relationship is weak between SS and MN but robust between SLE and MN, and SS could occur in a secondary form as a part of SLE, lupus should be screened carefully during the follow-up in the case of SS complicated with MN.

To sum up, in patients with both MN and primary SS, the association of the 2 diseases needs to be confirmed, in which a detection of PLA2R may be helpful. While PLA2R-positive MN is more likely to be a coincident disease, the negative pattern should be considered for the secondary form. Careful screen for SLE is necessary in these PLA2R-negative patients during follow-up.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Chen R, Wang J, and Xie Q contributed to the data collection while Wang J and Xie Q performed the experiments. Chen R analyzed the data and was the main contributor in writing the manuscript. Xie Q critically reviewed and revised the manuscript. All authors were involved in the design, interpretation of data, and final approval of the manuscript.

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ANNOUNCEMENT

Vale Dr Christina Ann Boros (1966-2021)

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It is with sadness that we record the passing on 31 May, 2021 of our distinguished Associate Editor for *Paediatric Rheumatology*, Dr Christina Boros,

After completing her fellowship at The Hospital for Sick Children in Toronto, Canada, Christina returned to Australia to become the Head of Pediatric Rheumatology at The Women's and Children's Hospital in Adelaide and also a clinical academic at The University of Adelaide. She faced many challenges in this role, not least because she single-handedly provided a state-wide clinical service for over a decade, while also maintaining an academic career. Christina will be remembered for her tireless advocacy for her patients and for the South Australian pediatric rheumatology service as a whole. She was an excellent clinician who demanded the best for her patients, from both herself and others. This dedication was impressive and awe-inspiring to us all. She showed a great passion for teaching and research and was well respected in national and international circles. Such was Christina's dedication to her research that she even completed manuscripts for publication in the days just before her death. She will be missed by her collaborators and friends both locally and overseas.

Christina was an important and active member of the close-knit community of pediatric rheumatology in Australia and New Zealand. She served as the Chair of the Australian Paediatric Rheumatology Group, had been the pediatric representative on the Advanced Training Committee, as well as many other important committees. There is no doubt that Christina was not only a talented clinician but she was also willing to put in the extra effort to advance our specialty.

She lived her life to the fullest and never missed an opportunity for travel or new learning. She played the harp to a professional standard and had to choose between a career in medicine or one in music. She tried her hand at many sports including competition swimming, meeting with great success in recent years. In the last decade of her life, she had become proficient at artisan glass blowing and had planned to retire one day into her studio, teaching this art to others.

"Trust" 77 × 61 Oil on canvas by Avril Thomas – Dr. Christina Boros – Senior Lecturer, School of Paediatrics and Reproductive Health.

As a young medical student Christina was diagnosed with acute lymphoblastic leukemia but was determined that this would not define her life. With her typical tenacity and bravery, she not only beat the disease but went on to forge her very successful career. Sadly, 30 years on she was again confronted by malignancy and

this time even Christina's strength and resolve were not enough to conquer the disease.

Christina is survived by her sisters Elizabeth and Julia, of whom she was always outwardly proud. Pediatric rheumatology is a small community in Australia and New Zealand and we will all miss Christina, with her mirth and intelligence which was taken from us prematurely.

International Journal of Rheumatic Diseases thanks her close friend and colleague, Dr Davinder Singh-Grewal for this fitting tribute to Christina.

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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.