ISSN 1756-1841 VOLUME 24 NUMBER 5 2021

International Journal of Rheumatic Diseases

Official journal of the Asia Pacific League of Associations for Rheumatology (APLAR)

WILEY

Indexed by ISI (SCI-E), MEDLINE

International Journal of Rheumatic Diseases

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For submission instructions, subscription and all other information visit http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1756-185X

View this journal online at wileyonlinelibrary.com/journal/apl

International Journal of Rheumatic Diseases

Volume 24 | Number 5 | May 2021

Contents

Received: 3 December 2020 | Accepted: 5 December 2020

DOI: 10.1111/1756-185X.14045

International Journal of Rheumatic Diseases

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EDITORIAL

Should we aim for personalized prevention in individuals at risk of rheumatoid arthritis?

Progress in our understanding of preclinical rheumatoid arthritis (RA) has been driven largely by clinical and laboratory data from prospective cohorts of at-risk individuals. These include large, well-characterized populations of relatives of RA patients and also auto-antibody-positive individuals with musculoskeletal symptoms.[1,2] More than a decade of observational data from these cohorts has identified risk factors and biomarkers which are associated with progression to clinical arthritis and RA. Consequently, risk stratification is now feasible, enabling case selection for preventative intervention.

How to approach RA prevention is a major contemporary challenge in rheumatology, with important implications for the other autoimmune diseases. A logical strategy in RA is to extend the "early arthritis model", where prompt immunotherapy can induce drug-free remission in patients with early clinical synovitis.[3] As such, use of immunotherapies in high risk individuals without clinical synovitis, are being tested. However, some individuals, especially those with a lower absolute risk of arthritis development may be reluctant to take immunosuppressive drugs, for fear of over-treatment or side-effects. An alternative and perhaps complementary paradigm would be to target specific risk factors with more conservative interventions, thus personalizing prevention according to the biological drivers of disease in any given at-risk individual.

One advantage of this more nuanced approach is that different reversible risk factors for anti-citrullinated protein antibodies (ACPA) and disease progression can already be identified and targeted. Such risk factors may be influenced by lifestyle modification, and non-pharmacological health intervention. For example, cigarette smoking is strongly associated with ACPA and the development of RA; it drives periodontal disease and may also influence the initiation of RA autoimmunity at the lung. Periodontal disease is itself more prevalent in at-risk individuals (both ACPA positive individuals without clinical synovitis and first-degree relatives of RA patients), independently of smoking status.[4,5] Periodontal bacteria such as *Porphyromonas gingivalis* are capable of citrullination and triggering ACPA production.[6,7] Furthermore, periodontal inflammation and lung inflammation are detectable in at-risk individuals who have not yet developed joint inflammation.[4,8] As such, focusing on smoking cessation and/or periodontal intervention in at-risk individuals who have these risk factors may have multiple benefits; both may directly prevent disease progression as well as being associated with broader health benefits to the individual, without the associated risks of pharmacotherapy. Similarly, elevated body mass index and dyslipidemia have been identified as independent risk factors for arthritis development in cohorts of at-risk individuals. Addressing these risk factors should also provide broader systemic health benefits. However, achieving behavioral change in the busy clinic environment is associated with its own set of challenges. Bespoke multidisciplinary clinical pathways for at-risk individuals are likely to be required.

Clinical trials will be required to test whether such personalized approaches to prevention will be acceptable to at-risk individuals and if so, whether they effectively modulate disease progression, either alone, or in combination with immunotherapy. The most relevant outcomes for such trials are also a matter for debate; trial end-points should not just be restricted to the development of clinical arthritis but could also include other important end-points such as absolute risk reduction or improvement in symptoms or quality of life.

A future strategy may be to comprehensively assess a panel of risk factors (including reversible ones) in all at-risk individuals, as well as the overall absolute risk of arthritis in the short and medium term. In those with low absolute risk of arthritis, targeted risk factor modification in the short and medium term alone, with close observation, may be the preferred strategy. Absolute risk reduction, the progression of disease (eg, development of joint involvement on imaging) and/or improvement of symptoms and quality of life may be the right outcomes. Conversely, in those at high risk (often subclinical joint involvement already present) risk factor modification with additional immunotherapy may be more appropriate, with the objective of short-term arthritis prevention. Advances in the treatment of RA have taught us that "one size does not fit all" and personalized treatment is now the agreed goal. This lesson should be applied to RA prevention.

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Received: 7 December 2020 | Revised: 13 February 2021 | Accepted: 15 February 2021

DOI: 10.1111/1756-185X.14092

REVIEW ARTICLE

International Journal of Rheumatic Diseases | WILEY

Prevalence and risk factors for falls in patients with spondyloarthritis: A systematic review

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Abstract

Objective: Patients with spondyloarthritis (SpA) may have a greater risk of falling due to poor postural balance and decreased mobility. To our best knowledge, there are no published reviews that study falls in patients with SpA. Therefore, we aim to systematically review the literature and identify the prevalence and risk factors of falls in patients with SpA.

Methods: We conducted a systematic review using 5 electronic databases: PubMed, EMBASE, Scopus, Web of Science and Google Scholar using controlled vocabulary terms (eg MeSH terms) in the search strategy for the concepts: falls, fall risk, SpA and its subtypes.

Results: We identified 7279 articles, of which 3 studies with a total of 441 patients were included. Prevalence of falls ranged from 13% to 25%. We identified 16 main factors across 5 categories. Under socio-demographic factors, functional limitation, decreased quality of life, advanced age and job loss were associated with an increased risk of falls. Poor balance and mobility and fear of falling were associated with increased risk of falls. Active disease and symptoms of SpA were medical factors that were associated with increased risk of falls. Medication factors including polypharmacy, myorelaxants and antidepressants were not associated with increased fall risk.

Conclusion: We identified potentially modifiable risk factors associated with increased risk of falls in patients with SpA, including functional limitation, poor balance and mobility, fear of falling and active disease. Clinicians should recognize these factors and address them in the holistic management of patients with SpA, thereby reducing falls and their complications.

KEYWORDS

ankylosing spondylitis, balance, fall, fall prevention, spondyloarthritis, systematic review

1 | INTRODUCTION

Falls are a significant public health issue and pose a huge burden for healthcare resources around the world.[1] The World Health Organization (WHO) reported that "falls are the second leading cause of accidental or unintentional injury deaths worldwide".[2] Complications of falls include severe injuries such as hip fractures[3] which have been shown to be associated with significant functional decline and mortality.[4]

Spondyloarthritis (SpA) refers to a group of inflammatory rheumatic conditions, including ankylosing spondylitis, psoriatic arthritis, inflammatory bowel-related arthritis and reactive arthritis.[5] While these are

closely related subtypes, they each have distinct clinical characteristics including spinal (axial) involvement, peripheral joint involvement, enthesopathies and extra-articular manifestations.[5] Prevalence of SpA ranges from 0.20% to 1.61% globally.[6] In a meta-regression analysis, it was found the mean age of SpA patients was 49.4 years, with a larger proportion of males.[6] Although falls are more common among the elderly,[2] the prevalence of falls in patients with SpA was found to be 32.9% and 34.7% in patients with ankylosing spondylitis,[7] despite patients with SpA being generally younger. This highlights the importance of studying falls in SpA patients, regardless of age.

Susceptibility to falls are multifactorial - involving the interaction of physiological, behavioral and environmental factors.[8] Patients with SpA may have a greater risk of falling than the non-SpA population.[9-11] Their increased fall risk could be attributed to various SpA disease-related factors, including poor postural balance[9,10,12] and reduced mobility.[9-11] Patient-related factors such as the fear of falling can also increase fall risk in SpA patients.[11] Falls and their associated complications can be debilitating, some of which include immobility, activity limitation and the fear of falling. These sequelae have been proven to result in a significant decline in one's quality of life.[13] Hence, awareness of falls and their risk factors are crucial in the management of SpA patients to prevent complications resulting from falls. Identification of reversible risk factors can allow for targeted falls prevention interventions.

Although there are other reviews that have looked at risk factors of falls, these mostly focused on the elderly,[8,13,14] and factors identified could be different in patients with SpA as patients tend to be younger.[6] Specific to falls in rheumatologic conditions, there has only been one review which addressed risk of falls in patients with rheumatoid arthritis,[15] and risk factors identified might not be applicable to patients with axial spondyloarthritis, who mainly have spinal involvement. To our best knowledge, there are no published reviews that study the prevalence and risk of falls in patients with SpA. Therefore, we aim to systematically review the literature and identify the prevalence and risk factors of falls in patients with SpA.

2 | MATERIALS AND METHODS

2.1 | Search strategy

We conducted a systematic review on the available medical literature on falls in patients with SpA. We searched 5 electronic databases (PubMed, EMBASE, Scopus, Web of Science and Google Scholar) using the keywords (spondyloarthr* OR ankylosing spondy* OR psoriatic arthritis OR reactive arthritis OR Reiter's syndrome OR axial spondyloarthr* OR Marie Strumpell spondylitis OR Bechterew syndrome OR inflammatory bowel disease* OR ulcerative colitis OR Crohn*) AND (fall* OR slip* OR trip*). While other systematic reviews on falls restricted their search terms to "fall*", we included the additional terms "trip*" and "slip*" in accordance with the UK National Institute for Health and Care Excellence (NICE) clinical guidelines on falls.[16] We applied the search terms to title and/or abstract and completed all searches on 6 April 2020 with no restriction of articles' start date. In addition, we conducted hand-searches of the references from the articles included. We conducted the study in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[17]

2.2 | Study selection, inclusion and exclusion criteria

Two independent reviewers (GRSL, CHN) screened the search results for eligible articles based on the inclusion criteria and discussed when discrepancies occurred. When discrepancies could not be resolved, further discussion with a third independent reviewer (WF) was made. We obtained full manuscripts for the eligible articles and reviewed them again according to the inclusion criteria.

We included studies with full text, original articles published in English peer-reviewed journals with a primary or secondary outcome measure of falls in patients aged 16 years and above with diagnosed SpA. We excluded conference abstracts, case reports, case series, book chapters, notes, letters, errata and other systematic reviews.

2.3 | Data extraction

Two independent reviewers (GRSL, CHN) extracted details from the eligible articles and compared them to check for consistency. This included general information (year and location of study, year of publication), study design and aim, population characteristics (sample size, mean age, gender distribution), disease characteristics, diagnostic criteria and fall characteristics (fall definition, prevalence, risk factors). We extracted 95% confidence intervals (CIs), *P* values and correlation coefficients for the identified fall risk factors. Factors identified were then classified according to the fall risk factors classification proposed by Lord et al.[18] These categories are: socio-demographic factors, balance and mobility factors, sensory and neuromuscular factors, psychological factors, medical factors, medication factors and environmental factors.[18]

2.4 | Study quality assessment

We assessed the quality of the studies included using the Kmet et al. (2004) standard quality assessment criteria for evaluating primary research papers,[19] specifically according to the checklist for assessing quality of quantitative studies. The checklist included 10 domains; namely, research question and objective, study design, study context, theoretical framework, sampling strategy, data collection, data analysis, verification procedure, conclusion and reflexivity. The scores for each domain ranged from 0 to 2, with a maximum total score of 28. We calculated the summary score for each study by dividing the total score by the total possible sum after excluding the non-applicable domains. Kmet et al. used 0.55 as the inclusion

threshold for studies in their systematic review.[19] For this review, we considered studies with a summary score of > 0.55 to be of adequate quality. Two independent reviewers (GRSL, CHN) critically assessed the quality of the included studies. Any discrepancies were resolved by consensus through discussion.

3 | RESULTS

3.1 | Results of search strategy

Through the electronic search, we identified 7279 articles, of which 3382 were excluded as duplicates using EndNote. We screened the remaining 3897 articles for eligibility based on their titles and abstracts. Of these, we included 7 articles and screened their full texts for eligibility. We excluded 4 articles as 3 did not record falls as an outcome measure, while 1 did not focus on falls as the aim of their study. This resulted in the remaining 3 studies that we included in this review (Figure 1).

3.2 | Key characteristics of included studies

Table 1 presents a summary of the key characteristics of the included studies. There were 3 cross-sectional studies. Studies were conducted in 2 countries – Turkey (n = 2)[10,11] and Brazil (n = 1).[9] The number of patients across the 3 studies was 441 and ranged from 55[9] to 306.[11] The mean age of patients ranged from 37.2[10] to 47.8 years.[9] All 3 studies included patients with a diagnosis of ankylosing spondylitis (AS), where it was the sole focus for 2 studies.[10,11] In addition to AS, Mewes et al. studied patients with axial SpA as well.

3.3 | Quality assessment of studies

Table 2 presents the results of the quality assessment using the Kmet et al. (2004) standard quality assessment criteria for evaluating primary research papers.[19] According to the inclusion threshold score of > 0.55, all 3 studies were of an adequate quality. All studies had well-described research questions, appropriate study designs and systematically described data collection and analysis. All the studies fared poorly in the domain of sampling strategy, as they used convenience samples.

3.4 | Falls prevalence

All 3 studies collected the retrospective 12-month fall history of patients, through the use of verbal interviews.[9-11] One study clearly stated their definition of a fall to be "An unintentional coming to the ground or other lower level without sustaining a violent blow, loss of consciousness, or sudden onset of paralysis, as occurs with a stroke or epileptic seizure",[10] while the remaining 2 studies did not include a fall definition.[9,11]

Falls prevalence was calculated based on the number and percentage of patients who fell at least once[9-11] during the 12-month

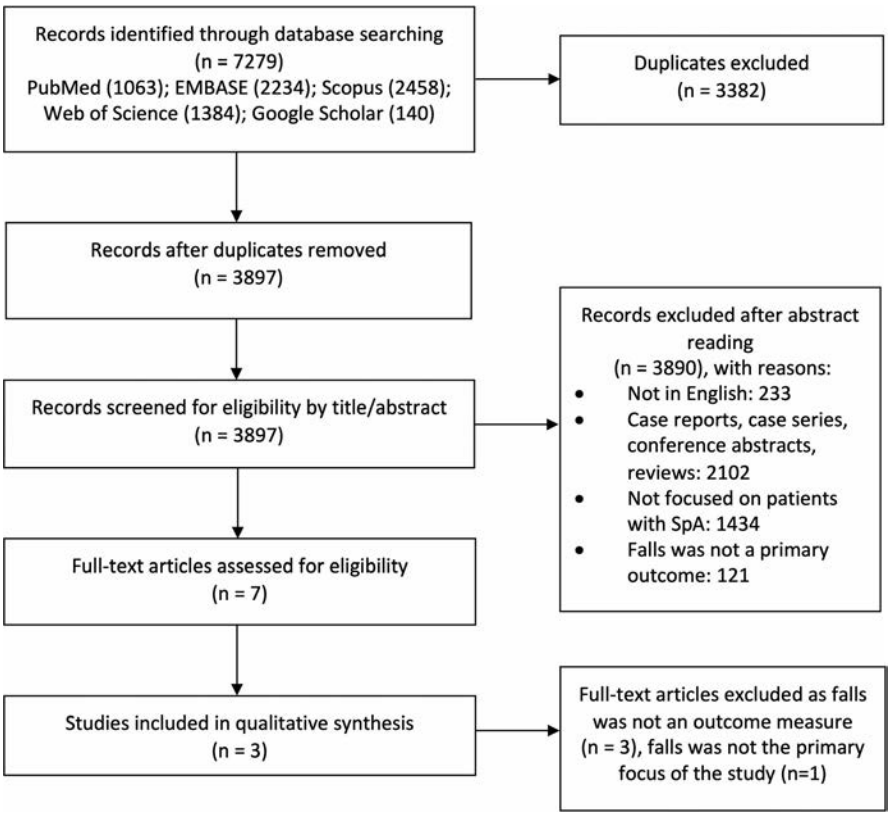

FIGURE 1 PRISMA flow chart of our systematic review

TABLE 1 Summary of characteristics of studies included in our review

Characteristics	Mewes et al.[9]	Alkan et al.[10]	Dursun et al.[11]
Study design	Cross-sectional study	Cross-sectional study	Cross-sectional study
Country	Brazil	Turkey	Turkey
Patients (male : female)	55 (39:16)	Cases: 40 (28:12) Controls: 40 (28:12)	306 (217:89)
Age, y, mean (SD or 95% CI)	47.8 (11)	Cases: 37.2 (9.92) Controls: 37.5 (9.63)	40.1 (11.5)
Diagnosis	Ankylosing spondylitis	Ankylosing spondylitis	Axial spondyloarthritis
Criteria for diagnosis	Classification criteria for axial spondyloarthritis according to Assessment of Spondyloarthritis International Society (ASAS)	Modified New York Criteria	Modified New York Criteria
Method of falls data attainment	Falls in preceding 12 mo via interview	Falls in preceding 12 mo via interview	Verbal questioning, "How many falls did you have in the last year?"
Fall definition	None	An unintentional coming to the ground or other lower level without sustaining a violent blow, loss of consciousness, or sudden onset of paralysis, as occurs with a stroke or epileptic seizure."	None
Method of assessment of falls risk	Berg Balance Scale (BBS): Scores ≤ 45 indicate that individuals may have a greater fall risk and values of < 40 are associated with an almost 100% risk of falling	Tetrax Interactive Balance System: a fall index is obtained from the balance parameters established by this device. A score (from 0 to 100) is produced which estimates an individual's fall risk	
Fall prevalence	Fall history: 25.4% fell at least once in the past 12 mo	Fall history: 20% (cases) fell at least once in the past 12 mo	Fall history: 13% had at least 1 fall in the past 12 mo

duration prior to the individual study durations. Fall prevalence ranged from 13%[11] to 25.4%[9] across all 3 studies.

3.5 | Measurement of fall risk

The methods of assessing fall risk in patients varied across the studies. The study by Alkan et al. employed the use of the Tetrax Interactive Balance System (Sunlight Medical Ltd., Tel Aviv, Israel) on all patients to assess balance and posture control.[10] The balance parameters obtained by the system were used to generate a fall index, which involved a score from 0 to 100, where a higher score indicated greater fall risk. The study by Mewes et al. tested patients using the Berg Balance Scale (BBS) which consists of 14 tasks that assessed the participant's balance.[9] The score obtained from the BBS predicted the participant's fall risk, where scores ≤ 45 indicated an increased fall risk and scores < 40 were associated with a near 100% fall risk. Dursun et al. investigated the fall risk of patients based on number of falls.[11]

3.6 | Risk factors for falls

We identified the fall risk factors that were studied and classified them according to the classification proposed by Lord et al.[18] We classified the risk factors into the following: socio-demographic factors, balance and mobility factors, psychological factors, medical factors and medication factors. We identified both statistically significant and non-significant associations between the factors and risk of falls with their respective results separated and categorized.

3.7 | Socio-demographic risk factors

Table 3 presents the socio-demographic risk factors and their association with risk of falls in patients with SpA. A total of 6 main factors were identified across 3 studies [9-11] under the category of socio-demographic factors. These risk factors included age, gender, body mass index, occupation-related factors, functional limitation and quality of life (QoL). Increased functional limitation, as measured by the Bath Ankylosing Spondylitis Functional Impairment (BASFI),[9-11] was associated with increased risk of falls across all 3 studies. Decreased quality of life, as measured by the AS-QoL, was found to be associated with increased risk of falls.[9,10] Three studies assessed advanced age as a risk factor for falls,[9-11] where only 2 studies found significant associations with risk of falls.[9,11] Occupation-related factors included AS-related job loss and days of sick leave,[11] with only job loss due to AS being associated with an increased risk of falls.[11] Gender and body mass index were not significantly associated with risk of falls.[10,11]

TABLE 2 Quality assessment scores from Kmet et al. 2004 standard quality assessment criteria for evaluating primary research papers

Criteria		Mewes et al.[9]	Alkan et al.[10]	Dursun et al.[11]
1	Question/objective sufficiently described?	2	2	2
2	Study design evident and appropriate?	2	2	2
3	Method of subject/comparison group selection or source of information/input variables described and appropriate?	1	1	1
4	Subject (and comparison group, if applicable) characteristics sufficiently described?	2	2	2
5	If interventional and random allocation was possible, was it described?	N/A	N/A	N/A
6	If interventional and blinding of investigators was possible, was it reported?	N/A	N/A	N/A
7	If interventional and blinding of subjects was possible, was it possible?	N/A	N/A	N/A
8	Outcome and (if applicable) exposure measure(s) well defined and robust to measurement/misclassification bias? Means of assessment reported?	2	2	2
9	Sample size appropriate?	1	1	2
10	Analytic methods described/justified and appropriate?	2	2	1
11	Some estimate of variance is reported for the main results?	2	2	2
12	Controlled for confounding?	N/A	N/A	N/A
13	Results reported in sufficient detail?	2	2	2
14	Conclusions supported by the results?	1	2	1
Summary score = total sum/(28 − [number of "N/A"*2])		0.85	0.90	0.85

Note: All items scored using the following scale: yes = 2, partial = 1, no = 0, unable to determine = N/A.

3.8 | Psychological risk factors

Table 3 presents a psychological risk factor and its association with risk of falls in patients with SpA. We identified only 1 main factor by 1 study[11] under the category of psychological factors. This risk factor of interest was fear of falling, which was significantly associated with increased risk of falls.[11]

3.9 | Balance and mobility risk factors

Table 3 presents the balance and mobility risk factors and their association with risk of falls in patients with SpA. We identified a total of 2 main factors across 3 studies.[9-11] These risk factors included spinal mobility and lower limb function and balance. Three studies assessed spinal mobility using the Bath AS Metrology Index (BASMI), where raised scores, indicating decreased spinal mobility, were associated with increased risk of falls.[9-11] Lower limb function and balance were measured using the Short Physical Performance Battery (SPPB), where decreased SPPB scores were associated with increased risk of falls.[11]

3.10 | Medical risk factors

Table 3 presents the medical risk factors and their association with risk of falls in patients with SpA. We identified a total of 4 main factors across 3 studies[9-11] under the category of medical or disease-related factors. These risk factors included disease status, disease duration, patterns and symptoms of disease and biochemical markers of disease activity. Disease status of SpA was measured using the Bath AS Disease Activity Index (BASDAI).[9-11] Raised scores indicated increased disease activity, which was found to be associated with increased risk of falls[9,10] in all studies except one.[11] One study found

TABLE 3 Factors affecting risk of falls in patients with spondyloarthritis

Risk factor	Studies which found that factor was positively associated with increased risk of falls	Results	*P* value	Studies which found that factor had no significant association with increased risk of falls	Results	*P* value
Socio-demographic factors						
Advanced age	Mewes et al.[9]	r = −0.50[a]	<.0001	Alkan et al.[10]	r = −0.021	.898
	Dursun et al.[11]	r = 0.117	.041			
Gender				Alkan et al.[10]	r = −0.062	.706
				Dursun et al.[11]	NR	>.05
Increasing body mass index				Alkan et al.[10]	r = 0.055	.736
Occupation:						
AS-related job lost	Dursun et al.[11]	r = 0.140	.014			
AS- related days of leave				Dursun et al.[11]	NR	>.05
Functional limitation:						
Increasing BASFI	Mewes et al.[9]	r = −0.71[a]	<.0001			
	Alkan et al.[10]	r = 0.751	<.001			
	Dursun et al.[11]	r = 0.244	.000			
Quality of life:						
Increasing AS-QoL	Mewes et al.[9]	r = −0.56[a]	<.0001			
	Alkan et al.[10]	r = 0.627	<.0001			
Psychological factors						
Fear of falling	Dursun et al.[11]	r = 0.316	.000			
Balance and mobility factors						
Spinal mobility:						
Increasing BASMI	Mewes et al.[9]	r=−0.80[a]	<.0001			
	Alkan et al.[10]	r = 0.751	<.001			
	Dursun et al.[11]	r = 0.234	.000			
Lower limb function and balance:						
Decreasing SPPB	Dursun et al.[11]	r = 0.183	.006			
Medical factors						
Disease status of spondyloarthritis:						
Increasing BASDAI	Mewes et al.[9]	r = −0.28[a]	.03	Dursun et al.[11]	NR	>.05
	Alkan et al.[10]	r = 0.713	<.001			
Increasing ASDAS-CRP	Mewes et al.[9]	r = −0.33[a]	.01			
Increasing ASDAS-ESR	Mewes et al.[9]	r = −0.32[a]	.01			
Longer disease duration of spondyloarthritis	Dursun et al.[11]	r = 0.160	.005	Mewes et al.[9]	r = −0.10[a]	.44
				Alkan et al.[10]	r = 0.276	.085
Disease pattern and symptoms of spondyloarthritis:						
Hip involvement	Dursun et al.[11]	r = 0.112	.05			
Knee involvement				Dursun et al.[11]	NR	>.05
Ankle involvement				Dursun et al.[11]	NR	>.05
Morning stiffness				Dursun et al.[11]	NR	>.05
Biochemical markers of disease activity:						
Increasing CRP				Dursun et al.[11]	NR	>.05
Increasing ESR				Dursun et al [11]	NR	>.05
Increasing 25 (OH) Vitamin D				Dursun et al.[11]	NR	>.05

(Continues)

TABLE 3 (Continued)

Risk factor	Studies which found that factor was positively associated with increased risk of falls	Results	*P* value	Studies which found that factor had no significant association with increased risk of falls	Results	*P* value
Medication factors						
Use of antidepressants				Dursun et al.[11]	NR	>.05
Use of myorelaxants				Dursun et al.[11]	NR	>.05
Polypharmacy				Dursun et al.[11]	NR	>.05

Abbreviation: AS, ankylosing spondylitis; ASDAS, Ankylosing Spondylitis Disease Activity Score; AS-QoL, Ankylosing Spondylitis-Quality of Life; BASDAI, Bath Ankylosing Spondylitis Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; NR, not recorded; SPPB, Short Physical Performance Battery.

[a]Values are negative as Mewes et al. evaluated risk of falls with a flipped scale, the Berg Balance Scale (BBS), where lower scores indicate increased risk of falls.

a positive correlation between disease duration of SpA and risk of falls,[11] while 2 other studies did not find significant associations.[9,10] Hip involvement was positively correlated with risk of falls.[11] Knee involvement, ankle involvement and morning stiffness were not significantly associated with risk of falls.[11] Biochemical markers of disease activity including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and 25-hydroxy-vitamin D were not significantly associated with risk of falls.[11]

3.11 | Medication risk factors

Table 3 presents the medication risk factors and their association with risk of falls in patients with SpA. The use of antidepressants, myorelaxants and polypharmacy was not associated with increased risk of falls.[11]

4 | DISCUSSION

To our best knowledge, this is the first systematic review on the risk factors of falls in SpA. Fall prevalence in patients with SpA ranged between 13%[11] to 25.4%[9] across the 3 studies, and appears to be largely similar to patients with rheumatoid arthritis (RA) (range between 10% to 43%).[15,20] Patients with RA often have peripheral joint arthritis, and this can affect their mobility and impose postural instability,[20] which can similarly affect SpA patients with peripheral arthritis, which has been known to affect 30% of patients with SpA.[10] Despite patients with SpA being generally younger, prevalence of falls in patients with SpA was also largely similar to that in the older adult population which ranged from 17.2% in Singapore[21] to 42.4% in the UK.[22] This is an interesting finding as the age distribution of patients with SpA in our included studies was much younger. One possible reason for this is that despite being younger, patients with SpA have been shown to have poorer lower limb function and balance,[11] thus resulting in more falls than elderly in the general population.

The factors for fall risk were classified according to the classification system proposed by Lord et al.[18] Among the socio-demographic factors, our study found that increasing ages of patients with SpA increased their risk of falls, which was similar to other studies in older adults.[23,24] Aging brings about physiological declines in various systems, including the musculoskeletal, vestibular and cardiovascular systems,[25] and these could all lead to increased falls.

In addition, increasing functional limitation in daily activities such as dressing, standing up and reaching for items was also associated with an increased risk of falls. This was consistent with other studies on RA patients.[26,27] A possible explanation is that functional limitations reduced the ability of patients to engage in physical activity[28] which has been shown to improve muscle strengthening and result in better postural control thereby reducing risk of falls.[29] Hence, physicians should aim to minimize the degree of functional limitation in patients. Recommended interventions would include elastic resistance exercise to improve muscle strength[30] and supervised aerobic exercise[31] as they have both been shown to reduce the risk of functional limitations.

Reduced spinal mobility was associated with increased risk of falls, and this was similar to patients with Parkinson's disease.[32] Hence, physicians could encourage exercises targeted at preserving axial mobility and spinal flexibility to reduce risk of falls.[33] Poor balance was also identified as a risk factor for falls, and patients with SpA should be referred to the physiotherapist for gait and balance rehabilitation.[34] Physicians could consider adopting interventions similar to those in the "Flexibility/Balance/Function" (FBF) exercise program for patients with Parkinson's disease, as these exercises have a similar aim of improving spinal mobility and balance.[35]

Fear of falling was found to have a positive correlation with increased risk of falls. This is important, as fear of falling is not only an outcome of falls, but also a risk factor for future falls.[23,36] Although fear of falling is more common in patients of an older age group,[36] Dursun et al. found that a large proportion of the patients had fear of falling, even though the patients were relatively young. Dursun et al. postulate that progression of the disease, chronic inflammation and functional limitation likely contributed to fear of falling. Teaching patients methods of fall prevention and how to "break" their fall by occupational therapists might help to alleviate their fear of falling.[37]

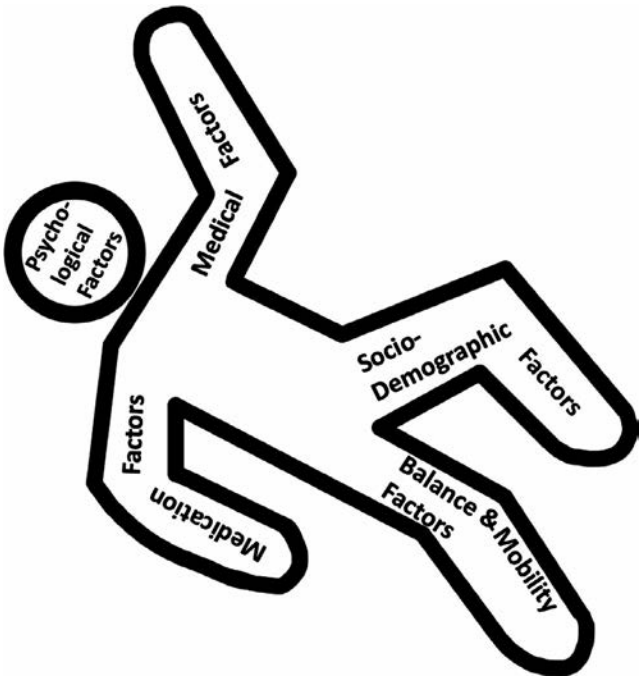

FIGURE 2 Illustration presenting an overview of the risk factors for falls in patients with spa according to the classification recommended by Lord et al.[18]

Among the medical factors, increasing BASDAI or ASDAS scores were positively correlated with increased risk of falls. This could be attributed to the relationship between disease activity and structural bone damage in the spine, which was highlighted in a 12-year longitudinal study by Ramiro et al. The study by Ramiro et al. showed that disease activity of AS contributed longitudinally to the radiographic progression of structural bone damage in the spine.[38] Hence, as Mewes et al. propose, treating inflammation aggressively to reduce disease activity may be a useful falls prevention measure.[9] Interestingly, Dursun et al. found that hip involvement was significantly associated with an increased risk of falls in SpA patients.[11] Hip involvement is a frequent clinical manifestation in SpA and was found to have a prevalence of 18% in 275 SpA patients in a single-center observational study.[39] Hip replacement in this group of patients might be important to reduce their risk of falls.

Among the medication factors, Dursun et al. found that use of antidepressants was not associated with risk of falls. This finding is different from several studies in the literature that have reported antidepressants as a risk factor for falls in older adults due to their medication side effects.[40,41] This difference could be attributed to the younger age distribution of the patients in the study by Dursun et al., as younger patients are less prone to the side effects of antidepressant medications,[42] and hence may not have an increased risk of falls.

In comparison to studies on falls in older adults,[22,23] our included studies did not identify poor vision, impaired cognition or postural hypotension as risk factors for falls in patients with SpA. A possible reason for this difference may be because these factors are a result of physiological declines due to aging, which will be seen more commonly in the older adults as compared to the younger population of SpA patients.

In this review, we included the 5 broad categories of risk factors for falls in SpA patients in an illustration (Figure 2). This illustration can be used by clinicians as a quick overview of the risk factors that should be considered when managing patients with SpA. Several of these factors, such as poor physical function, mobility and balance and active disease, are potentially modifiable, and measures should be taken to reduce these risk factors for falls in patients with SpA, thereby reducing complications resulting from falls.

There are a few limitations to our study. First, there were only 3 studies included in our review. This also highlights the gap in existing medical literature on falls in SpA patients. Second, the studies included were all cross-sectional in nature, and retrospective fall history was used. This could result in underestimation or underreporting of falls when using retrospective fall history, as it has been found that patients tend to forget their falls.[43] Future studies can consider conducting prospective studies instead, to minimize recall bias. However, all studies collected a retrospective fall history of the patients in the preceding 12 months, which has been found to be the most reliable timeframe for obtaining self-reported falls.[44] Third, since this was a systematic review and not a meta-analysis of the factors that influence risk of falls in patients, the aggregate magnitude of each factor was not assessed.

5 | CONCLUSION

In conclusion, the prevalence of falls ranged from 13% to 35%, and 16 factors were found to be associated with an increased risk of falls in patients with SpA. Clinicians should recognize modifiable risk factors, such as functional limitation, poor balance and mobility, fear of falling, active disease and symptoms of SpA, and address them in the holistic management of patients with SpA, thereby reducing falls and their complications.

ACKNOWLEDGEMENTS

The authors would like to thank Ms Wong Suei Nee and Ms Winnifred Wong from the Medical Library of the National University of Singapore for their advice and guidance on our search strategy, without which this study would not have been possible.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

AUTHOR CONTRIBUTION

All listed authors have contributed significantly in all of the following: (1) substantial contributions to the conception or design of the work, or the acquisition, analysis, or interpretation of data for the work; (2) drafting the work or revising it critically for important intellectual content; (3) final approval of the version to be published; (4) agreement to be accountable for all aspects of the work in ensuring

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How to cite this article: Lim GRS, Ng CH, Kwan YH, Fong W. Prevalence and risk factors for falls in patients with spondyloarthritis: A systematic review. *Int J Rheum Dis*. 2021;24:623–632. https://doi.org/10.1111/1756-185X.14092

Received: 28 October 2020 | Revised: 18 January 2021 | Accepted: 15 February 2021

DOI: 10.1111/1756-185X.14093

REVIEW ARTICLE

International Journal of Rheumatic Diseases | WILEY

Diagnostic value of anti-citrullinated α-enolase peptide 1 antibody in patients with rheumatoid arthritis: A systematic review and meta-analysis

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Funding information
the Chinese Academy of Medical Sciences (CAMS) Initiative for Innovative Medicine, Grant/Award Number: 2017-I2M-B&R-01; Chinese Academy of Medical Sciences (CAMS) Initiative for Innovative Medicine, Grant/Award Number: 2017-I2M-3-001; National Natural Science Foundation of China, Grant/Award Number: 81671618 and 81871302; Beijing Key Clinical Specialty for Laboratory Medicine - Excellent Project, Grant/Award Number: ZK201000

Abstract

Aim: To evaluate the diagnostic value of anti-citrullinated α-enolase peptide 1 (anti-CEP 1) antibody in patients with rheumatoid arthritis (RA) by conducting a systematic review and meta-analysis.

Methods: The PubMed, Web of Science, Embase, Scopus, and Cochrane Library databases were searched for relevant studies published until September 23, 2020. A bivariate mixed-effects model was used to calculate the diagnostic indices from primary data of eligible studies. We performed meta-regression and subgroup analysis to explore the sources of heterogeneity.

Results: Twenty-four articles, with a total of 17 380 patients with RA and 7505 control participants, met the criteria for inclusion in the meta-analysis. The pooled sensitivity, specificity, and positive and negative likelihood ratios for the anti-CEP 1 antibody were 44% (95% CI: 38%-51%), 97% (95% CI: 96%-98%), and 14.81 (95% CI: 10.66-20.57) and 0.57 (95% CI: 0.52-0.64), respectively. The pooled positive and negative predictive values were 0.96 (95% CI: 0.95-0.97) and 0.53 (95% CI: 0.43-0.63), respectively. The area under the summary receiver operating characteristic curve was 0.86. Meta-regression indicated that the anti-CEP 1 antibody detection method may be a source of heterogeneity. The subgroup analysis of the group in which the anti-CEP 1 antibody was detected by using a commercial enzyme-linked immunosorbent assay (ELISA) kit had a sensitivity of 59% (95% CI: 50%-68%) and a specificity of 93% (95% CI: 85%-97%).

Conclusions: The anti-CEP 1 antibody had moderate RA diagnostic value with relatively low sensitivity and high specificity. An ELISA may increase the RA diagnostic sensitivity.

KEYWORDS

anti-citrullinated protein antibodies, anti-citrullinated α-enolase peptide 1 antibody, autoantibody, diagnosis, meta-analysis, rheumatoid arthritis

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder characterized by irreversible joint erosion, articular cartilage destruction, and synovial inflammation.[1] Additionally, patients with RA may have coexisting extra-articular manifestations, such as cardiovascular events, lung disease, and neurological involvement,[2,3] which could seriously affect the quality of life in RA patients. However, early diagnosis of RA and intervention can help achieve remission and reduce the possibility of RA-related disabilities.[4]

Autoantibodies are the hallmark of RA, of which anti-cyclic citrullinated peptide (anti-CCP) antibody and rheumatoid factor (RF) are routinely used to diagnose RA.[5] They are also recommended by the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) the criteria of which are used to diagnose RA. Nevertheless, using only anti-CCP antibody and RF is insufficient to identify some potential patients with early stage RA who are negative for anti-CCP antibody and RF.[6] Therefore, more novel autoantibodies are needed to allow identification of seronegative RA patients. Anti-citrullinated protein antibodies (ACPAs) have an important role in diagnosing RA. ACPAs interact with different citrullinated proteins as target antigens, including fibrinogen, type II collagen, vimentin, and α-enolase.[7] In particular, α-enolase, one of the key enzymes for glycolysis, is involved in the pathogenesis of RA.[8] In 2005, Kinloch et al.[9] first reported that citrullinated α-enolase peptide (CEP) was specific for RA, and they observed that CEP can be detected in the synovial fluid of patients with RA and that the anti-CEP 1 antibody had a higher level in synovial fluid than in serum.[10] These findings suggest that the anti-CEP 1 antibody may be produced from joint tissue, and it may better reflect the pathological changes involved in RA than the anti-CCP antibody that targets synthetic antigen but not physiological proteins. Additionally, anti-CEP 1 antibody can be detected in patients with seronegative RA,[11,12] suggesting that it helps with early diagnosis of RA.

Several studies have investigated the diagnostic value of anti-CEP 1 antibody for RA. However, the results from different studies have been inconsistent, and no published systematic review or meta-analysis has evaluated the diagnostic value of anti-CEP 1 antibody for RA. Therefore, we conducted this systematic review and meta-analysis to assess the RA diagnostic performance of the anti-CEP 1 antibody and identify factors that may affect its performance.

2 | METHODS

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[13] The PRISMA checklist is shown in the supplementary files (see Appendix S1).

2.1 | Search strategy

The following 5 electronic databases were searched to retrieve relevant studies: PubMed, Web of Science, Embase, Scopus, and Cochrane Library. All studies were published prior to September 23, 2020, and we applied no language restriction. To construct the search strategy, the index terms were used as follows: autoantibody to citrullinated alpha enolase peptides 1, autoantibody to citrullinated α-enolase peptide 1, autoantibody to CEP-1, anti-CEP-1 antibody, rheumatoid arthritis, and RA. The detailed search strategy is presented in the supplementary files (see Appendix S2B).

2.2 | Study inclusion and exclusion criteria

Two investigators independently screened all the articles searched in the electronic databases. We included studies fulfilling the following inclusion criteria: (a) the diagnostic accuracy of anti-CEP 1 antibodies in RA was evaluated; (b) necessary data including sensitivity, specificity, false positives, and false negatives could be obtained or calculated from the study; (c) healthy donors or non-RA disease patients were enrolled in the study; (d) the diagnosis of patients with RA was based on the ACR or EULAR diagnostic criteria.

The following exclusion criteria were adopted: (a) studies without enough data to construct 2 × 2 contingency tables; (b) studies examining the diagnostic accuracy of the anti-CEP 1 antibody for future RA; (c) patient numbers with RA < 50; (d) tested samples were not in serum or plasma. (e) the study included duplicate data; (f) animal experiments.

2.3 | Data extraction

The process of data extraction was independently conducted by 2 investigators, and we extracted the essential information presented in the eligible articles, including the first author, published year, age, country where the study was performed, type of article, study design, method, plate and antibody used in enzyme-linked immunosorbent assay (ELISA), CEP-1 peptide sequence, diagnostic standard for RA, age, RA number, non-RA number, a cut-off of the method, diagnostic index, and anti-CEP 1 positive rate in patients with RA who were anti-CCP negative or positive. Any disagreements were resolved by reaching a consensus.

2.4 | Quality assessment

According to the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool,[14] 2 investigators independently assessed the quality of all eligible literature. QUADAS-2 evaluates 2 sections: risk of bias and concerns regarding applicability. Evaluation of the risk of bias comprised patient selection, index

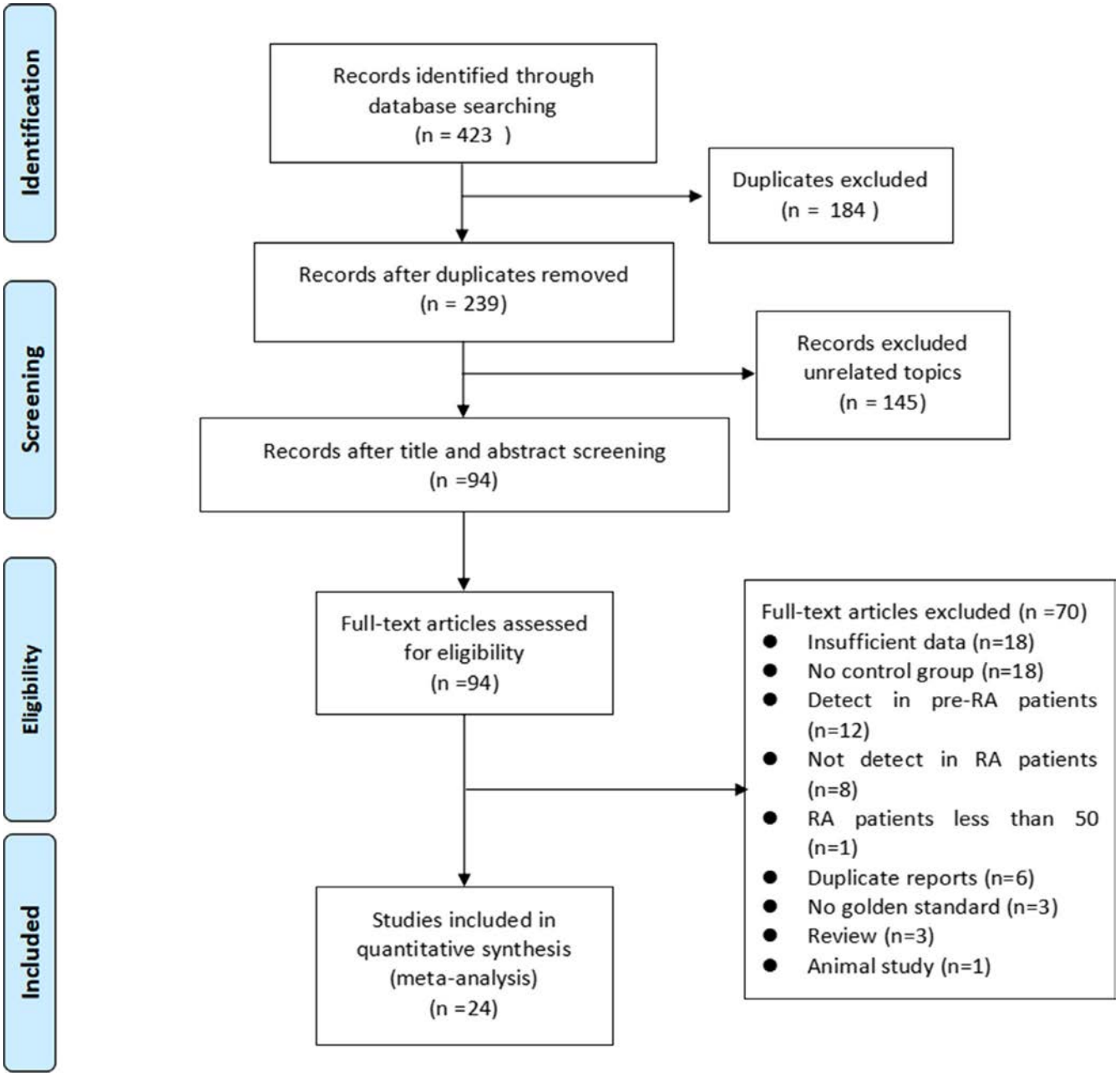

FIGURE 1 Flow diagram for screening studies and results

test, reference standard, flow and timing. The evaluation of the concerns regarding applicability included patient selection, index test, and reference standards. The risk was scored as high, low, or unclear according to the evaluating results of each section. Two investigators performed the quality assessment, and when there were inconsistent evaluation results, we resolved the disagreement through discussion.

2.5 | Statistical analysis

We used STATA 15 (Stata Corp, College Station, TX, USA), Meta-DiSc V.1.4 (Unit of Clinical Biostatistics team of the Ramony Cajal Hospital), and RevMan 5.3 (the Nordic Cochrane Center) software to perform the meta-analysis. A bivariate mixed-effects model was used to calculate the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), positive predictive value (PPV), negative predictive value (NPV), and diagnostic odds ratio (DOR). We established a summary receiver operator characteristic (SROC) curve and calculated the area under the SROC curve (AUC) to evaluate the overall performance of the anti-CEP 1 antibody in patients with RA. The I^2 statistical test, which is an index for assessing heterogeneity, was used to detect heterogeneity within studies; a value of I^2 >50% indicates substantial heterogeneity. We tested for threshold effects, which could lead to heterogeneity of results due to inconsistent cut-off values applied in various studies,

TABLE 1 Some characteristics of 24 eligible studies in this meta-analysis

Author	Published y	Country	Type of article	Study design	Method	Plate and antibody brands of ELISA/ Microarray brand	CEP-1 peptide sequence	Diagnostic standard for RA
Zhou et al[15]	2019	China	Journal article	Case-control	ELISA	Commercial ELISA kit: Euroimmun	NR	2010 ACR/EULAR criteria
Ponikowska et al[16]	2019	Poland	Journal article	Case-control	ELISA	Commercial ELISA kit: Euroimmun	NR	2010 ACR/EULAR criteria
Liu et al[17]	2019	China	Journal article	Case-control	ELISA	Commercial ELISA kit: Euroimmun	NR	1987 ACR criteria
Rönnelid et al[18]	2018	Sweden	Journal article	Case-control	Microarray	Phadia AB	CKIHA(cit)EIFDS(cit) GNPTVEC (cyclic)	1987 ACR criteria
Meyer et al[19]	2018	South Africa	Journal article	Case-control	ELISA	Commercial ELISA kit: Euroimmun	NR	1987 ACR criteria
Jonsson et al[20]	2018	Norway	Journal article	Cross-sectional	Microarray	Phadia AB	CKIHA(cit)EIFDS(cit) GNPTVEC (cyclic)	2010 ACR/EULAR criteria
Alunno et al[21]	2018	Italy	Journal article	Case-control	ELISA	Commercial ELISA kit: Euroimmun	NR	1987 ACR criteria
Alunno et al[22]	2018	Italy	Journal article	Cross-sectional	ELISA	Commercial ELISA kit: Euroimmun	NR	2010 ACR/EULAR criteria
Too et al[23]	2017	Malaysia/ Sweden	Journal article	Case-control	Microarray	Phadia AB	NR	1987 ACR criteria
Schwenzer et al[24]	2017	United states	Journal article	Cross-sectional	ELISA	Plate: NR Antibody: Stratech	KIHA(cit)EIFDS(cit)GNPTVE	1987 ACR criteria
Cabrera-Villalba et al[25]	2017	Spain	Journal article	Cross-sectional	ELISA	Plate: NR Antibody: Jackson Immunoresearch	CKIHA(cit)EIFDS(cit) GNPTVEC (cyclic)	1987 ACR criteria
Brink et al[26]	2017	Sweden	Meeting abstract	Prospective study	Microarray	Phadia AB	NR	1987 ACR criteria
Reed et al[27]	2016	Sweden	Journal article	Cross-sectional	Microarray	Phadia AB	CKIHA(cit)EIFDS(cit) GNPTVEC (cyclic)	1987 ACR criteria
Choi et al[28]	2016	Korea	Journal article	Cross-sectional	ELISA	Plate: MaxiSorp antibody: Millipore	KIHA(cit)EIFDS(cit)GNPTVE	1987 ACR criteria
Quirke et al[29]	2015	United Kingdom	Journal article	Cross-sectional	ELISA	Plate: NR Antibody: Hybridoma Reagent Laboratory	KIHA(cit)EIFDS(cit)GNPTVE	2010 ACR/EULAR criteria
Kokkonen et al[30]	2015	Sweden	Journal article	Case-control	Microarray	Phadia AB	CKIHA(cit)EIFDS(cit) GNPTVEC (cyclic)	1987 ACR criteria
Umeda et al[31]	2013	Japan	Journal article	Case-control	ELISA	Plate: MaxiSorp Antibody: American Qualex International	CKIHA(cit)EIFDS(cit) GNPTVEC (cyclic)	1987 ACR criteria
Goules et al[32]	2013	Greece	Journal article	Case-control	ELISA	Plate: Costar Antibody: Jackson Immunoresearch	KIHA(cit)EIFDS(cit)GNPTVE	1987 ACR criteria

(Continues)

TABLE 1 (Continued)

Author	Published y	Country	Type of article	Study design	Method	Plate and antibody brands of ELISA/ Microarray brand	CEP-1 peptide sequence	Diagnostic standard for RA
Montes et al[33]	2012	Spain	Journal article	Case-control	ELISA	Plate: Pierce Antibody: NR	CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic)	1987 ACR criteria
Hansson et al[34]	2012	Sweden	Journal article	Case-control	Microarray	Phadia Multiplexing Diagnostics GmbH	CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic)	1987 ACR criteria
Montes et al[35]	2011	Spain	Journal article	Case-control	ELISA	Plate: NR Antibody: NR	CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic)	1987 ACR criteria
Lundberg et al[36]	2011	Sweden	Meeting abstract	Case-control	ELISA	Plate: NR Antibody: NR	NR	1987 ACR criteria
Snir et al[37]	2009	Sweden	Journal article	Case-control	ELISA	Plate: Nunc, Antibody: Jackson ImmunoResearch Laboratories	CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic)	1987 ACR criteria
Lundberg[38]	2008	United Kingdom	Journal article	Case-control	ELISA	Plate: MaxiSorp Antibody: Hybridoma Reagent Laboratory	CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic)	1987 ACR criteria

Abbreviations: CEP-1, citrullinated α-enolase peptide 1; cit, citrulline; NR, not reported.

and *P* values <.05 were considered to be indicative of the presence of a threshold effect. Meta-regression and subgroup analysis were performed to detect the potential source of heterogeneity, and *P* values <.05 were taken to be indicative of statistical significance. To detect publication bias, a Deek's funnel plot was employed, and *P* values <.10 were considered to be indicative of existing publication bias.

3 | RESULTS

3.1 | Search results

A total of 423 articles were searched in PubMed, Web of Science, Embase, Scopus, and Cochrane Library databases, of which 184 studies were duplicates and 145 were excluded after the title and abstract screening (Figure 1). Then, 94 studies underwent full-text review, of which 18 did not provide enough data to construct 2 × 2 contingency tables, 18 did not set up a control group, 12 reported that they included pre-RA patients who developed RA in the future, 8 did not detect anti-CEP 1 antibody in patients with RA, 6 were reported as meeting abstracts that had been officially published as papers, 3 reported that the patients with RA were not diagnosed according to the ACR or EULAR criteria, 3 were reviews, 1 reported that the number of included RA patients was <50, and 1 was an animal study. Therefore, 70 studies were excluded after full-text review. Finally, 24 articles met the criteria for inclusion in the meta-analysis.[15-38]

3.2 | Study characteristics and literature quality assessment

Tables 1 and 2 present the main characteristics of the 24 eligible studies. There were 17 380 RA patients, 1231 non-RA patients as the disease control group, and 6274 participants as the health control group. Among the 24 studies, 14 only used health donors as a control group,[15,18,20,21,23,26-28,30,33-37] 1 study only used non-RA patients as a control group,[24] and the other studies mixed health donors and non-RA patients as the control group.[16,17,19,22,25,29,31,32,38] Two studies reported as meeting abstracts,[26,36] and the other studies were reported as journal articles.[15-25,27-35,37,38] Most of the studies used ELISAs to detect the anti-CEP 1 antibody by commercial ELISA kits or a self-established ELISA method.[15-17,19,22,24,25,28,29,31-33,35-38] Two types of CEP-1 peptide sequences were used as coating antigens in the self-established ELISA methods: one was a CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic) with C-terminal and N-terminal cysteines to facilitate cyclization in 6 included studies,[25,31,33,35,37,38] and another was a KIHA(cit)EIFDS(cit)GNPTVE without cysteine in 4 of the studies.[24,28,29,32] Detailed information about the self-established ELISA methods used in the included studies is presented in the supplementary files (see Table S1. [Appendix S2C]).

TABLE 2 The other characteristics of 24 eligible studies in this meta-analysis

Author	Published y	Age, y	RA number (F/M)	NRA (F/M)	Cut-off	TP	FP	TN	FN	SEN	SPE	RA duration	Anti-CEP^{+}/anti-CCP−[a]/anti-CEP^{+}/anti-CCP+[b]
Zhou et al[15]	2019	RA: 16-89 HC: 25-40	219/63	HC: 61/59	20 U/mL	184	16	104	98	65.2%	86.7%	NR	20.8% (10/48)/74.4% (174/234)
Ponikowska et al[16]	2019	RA: 49.0 ± 16.4 DC: 46.0 ± 19.3 HC: NR	35/16	DC:19/4 HC: 20	20 RU/mL	36	2	41	15	70.6%	95.3%	5.7 ± 3.5 mo	41.2% (7/17)/85.3% (29/34)
Liu et al[17]	2019	RA: 45-62 DC: 36-64 HC: 40-57	75/26	DC: 27/19 HC: 51/28	20 RU/mL	62	11	114	39	61.4%	91.2%	MT:7 y (2, 16)	20.8% (5/24)/74.0% (57/77)
Rönnelid et al[18]	2018	RA: 18-70 HC: NR	2825	HC:551	A specificity of 98.0% of healthy control	1332	11	540	1493	47.2%	98.0%	<1 y	NR
Meyer et al[19]	2018	RA: 48 DC: 43-45 HC: 46	61/14	DC: 46 HC: 19/10	46 RU/mL	54	18	57	21	72.0%	76.0%	MT:9 mo (12)	NR
Jonsson et al[20]	2018	RA: 51.5 ± 13.6 HC: 52.1 ± 9.2	131/86	HC: 57/37	A specificity of 98.0% of healthy control	140	1	93	77	64.5%	98.9%	<2 y	7.7% (3/39)/77.0% (137/178)
Alunno et al[21]	2018	RA: 62 ± 2 HC: NR	100	HC: 50	NR	45	1	49	55	45.0%	98.0%	12 ± 3 y	20.0% (7/35)/58.5% (38/65)
Alunno et al[22]	2018	RA: 61.7 ± 0.8 DC: NR HC: NR	196/56	DC: 97 HC: 50	20 U/mL	112	3	144	140	44.4%	98.0%	12.6 ± 0.6 y	17.6% (15/85)/58.1% (97/167)
Too et al (Malaysia)[23]	2017	RA: 18-70 HC: NR	1231	HC: 1625	A specificity of 98.0% of healthy control	283	33	1592	948	23.0%	98.0%	1.1 ± 1.8 y	NR
Too et al (Sweden)[23]	2017	RA:18-70 HC:NR	2858	HC: 578	A specificity of 98.0% of healthy control	1429	12	566	1429	50.0%	98.0%	<1 y	NR
Schwenzer et al[24]	2017	RA: NR DC: NR	287	DC: 330	A specificity of 98.0% of disease control	95	7	323	192	33.1%	97.9%	>10 y	NR
Cabrera-Villalba et al[25]	2017	RA: 54.7 ± 11.8 DC: 51.2 ± 11.3 HC: NR	34/20	DC: 34/20 HC: 64	A specificity of 98.0% of healthy control	22	18	100	32	40.7%	84.7%	3 years	NR
Brink et al[26]	2017	RA: 56.7 ± 14.0 HC: NR	692/330	HC: 477	A specificity of 98.0% of healthy control	549	10	467	473	53.7%	97.9%	<1 y	NR
Reed et al[27]	2016	RA:NR HC: NR	2836	HC: 373	A specificity of 98.0% of healthy control	1171	7	366	1665	41.3%	98.1%	NR	NR

(Continues)

TABLE 2 (Continued)

Author	Published y	Age, y	RA number (F/M)	NRA (F/M)	Cut-off	TP	FP	TN	FN	SEN	SPE	RA duration	Anti-CEP^{+}/anti-CCP−[a]/anti-CEP^{+}/anti-CCP+[b]
Choi et al[28]	2016	RA:58.2 ± 12.0 HC: 58.2 ± 11.6	231/33	HC: 77/11	A specificity of 95.0% of healthy control	46	4	84	218	17.4%	95.5%	13.8 ± 9.8 y	NR
Quirke et al[29]	2015	RA: NR DC: NR HC: NR	72/28	DC: 148/61 HC: 58/21	A specificity of 95.0% of healthy control	42	10	278	58	42.0%	96.5%	NR	NR
Kokkonen et al[30]	2015	RA: 56.5 HC: 50.3	199	HC: 574	A specificity of 97.0% of healthy control	134	35	539	65	67.3%	93.9%	MT:7.2 mo (4.7, 10.6)	NR
Umeda et al[31]	2013	RA: 16-84 DC: 15-84 HC: 18-55	158/50	DC:187/15 HC: 84/90	Mean + 3SD	92	4	372	116	44.2%	98.9%	NR	10.3% (3/29)/49.7% (89/179)
Goules et al[32]	2013	RA: NR DC: NR HC: NR	141	DC: 114 HC: 100	Mean + 2SD	53	9	205	88	37.6%	95.8%	NR	NR
Montes et al[33]	2012	RA: >55 HC: >55	404/117	HC: 173	A specificity of 98.0% of healthy control	117	3	170	404	22.5%	98.2%	MT: 18 y (10, 25)	NR
Hansson et al[34]	2012	RA: 18-70 HC: NR	927	HC: 461	A specificity of 98.0% of healthy control	365	10	451	562	39.4%	97.8%	<1 y	0% (0/526)/91.0% (365/401)
Montes et al[35]	2011	RA: ≥55 HC: ≥55	451	HC: 173	Mean + 3 SD	121	0	173	330	26.8%	100.0%	NR	12.5% (16/128)/32.6% (103/316)
Lundberg et al[36]	2011	RA: NR HC: NR	1985	HC: 150	A specificity of 98.0% of healthy control	714	3	147	1271	36.0%	98.0%	<1 y	NR
Snir et al[37]	2009	RA: 21-86 HC: 24-82	238/53	HC: 81/19	A specificity of 99.0% of healthy control	120	1	99	171	41.2%	99.0%	NR	1.2% (1/81)/56.7% (119/210)
Lundberg et al[38]	2008	RA: NR DC:NR HC:NR	102	DC: 110 HC: 92	OD values above 0.1	37	5	197	65	36.3%	97.5%	NR	23.3% (7/30)/41.7% (30/72)

Abbreviations: 2SD/3SD, the cut-off for a positive response as the mean plus 2/3 times the SD of the specificity anti-CEP 1 reactivity of the healthy control; Anti-CCP, anti-cyclic citrullinated peptide; Anti-CEP 1, anti-citrullinated α-enolase peptide 1; DC, disease control; F, female; FN, false negative; FP, false positive; HC, healthy control; M, male; MT, median time; NR, not reported; NRA, non-RA patients; OD, optical density; RA, rheumatoid arthritis; SEN, sensitivity; SPE, specificity; TN, true negative; TP, true positive.

[a]The prevalence of anti-CEP 1 antibody in anti-CCP antibody negative RA patients.

[b]The prevalence of anti-CEP 1 antibody in anti-CCP antibody-positive RA patients.

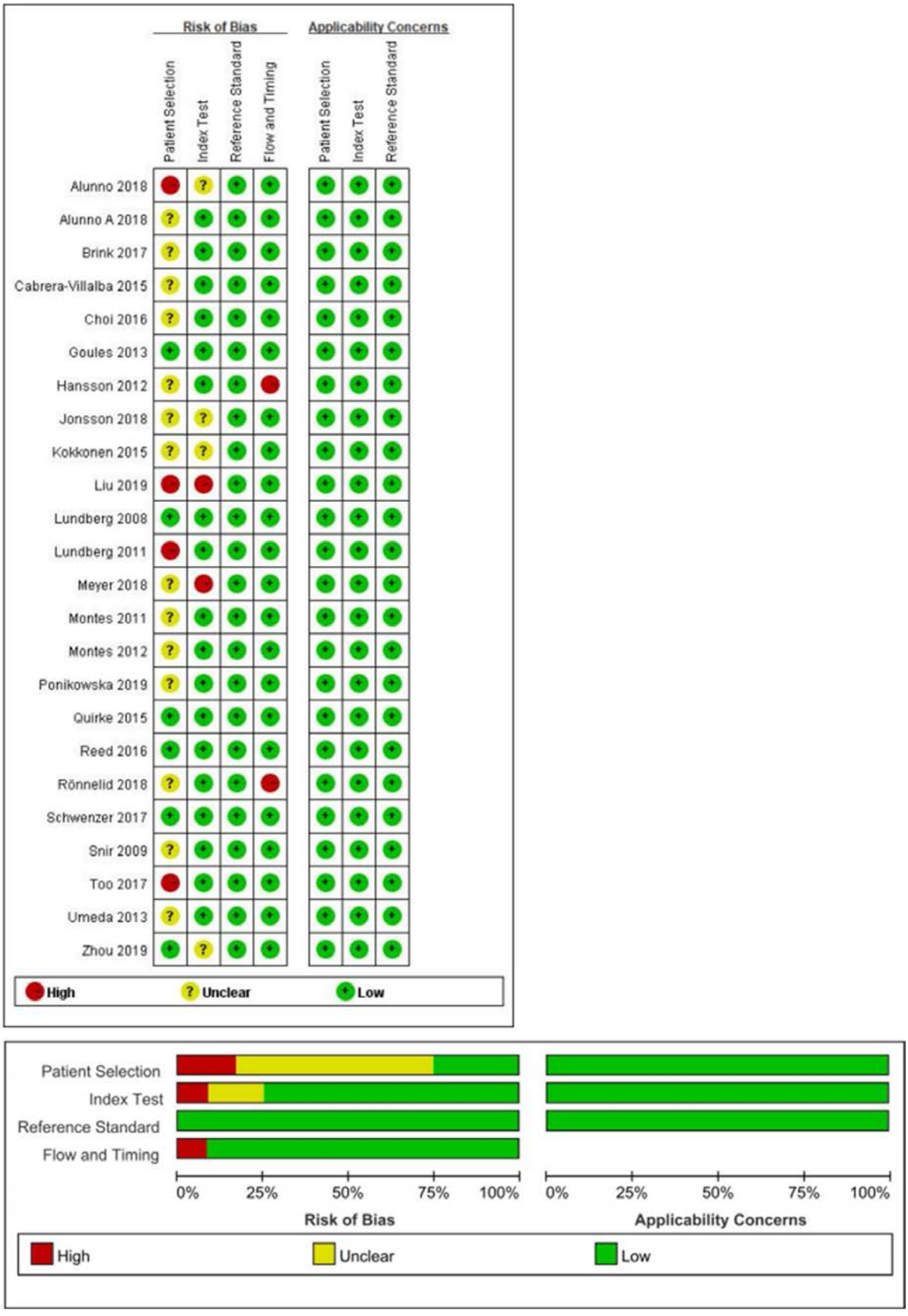

FIGURE 2 Literature quality assessment by using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool for eligible studies

The results of the included literature quality assessment are shown in Figure 2. We found a relatively high risk of bias or unclear risk of bias for patient selection because most of the articles did not specifically explain the sampling method of the included patients and it was difficult to achieve a consecutive or random sample of patients. A few studies indicated a high risk bias in flow and timing, because not all of the included patients in these studies underwent the anti-CEP 1 antibody test. However, regarding applicability, all studies had a low risk of bias.

3.3 | Diagnostic value of anti-CEP 1 antibody to RA

Among the 24 studies included in the meta-analysis, the sensitivity of the anti-CEP 1 antibody to RA ranged 17.4%-72.0%, and the specificity of the anti-CEP 1 antibody to RA ranged 76%-100%. Eleven studies out of 24 included studies showed the prevalence of the anti-CEP 1 antibody in the anti-CCP antibody negative RA patients or anti-CCP antibody-positive RA patients,[15-17,20-22,31,34,35,37,38] and the prevalences of the anti-CEP 1 antibody in the 2 groups of RA patients were 0%-41.2% and 32.6%-91.0%, respectively. The pooled sensitivity and specificity were 44% (95% CI: 38%-51%, $P < .0001$, $I^2 = 97.02\%$) and 97% (95% CI: 96%-98%, $P < .0001$, $I^2 = 92.77\%$), respectively (Figure 3A,B). Therefore, there was a significant heterogeneity among the studies. The pooled PLR was 14.81 (95% CI: 10.66-20.57, $P < .0001$, $I^2 = 81.29\%$), and the pooled NLR was 0.57 (95% CI: 0.52-0.64, $P < .0001$, $I^2 = 97.15\%$). The pooled PPV and NPV of the anti-CEP 1 antibody were 0.96 (95% CI: 0.95-0.97, $P < .0001$, $I^2 = 88.70\%$) and 0.53 (95% CI: 0.43-0.63, $P < .0001$, $I^2 = 99.60\%$), respectively. The pooled DOR was 25.83 (95% CI: 18.43-36.20), and the SROC showed that the AUC was 0.86 (95% CI: 0.82-0.88) (Figure 3C).

3.4 | Meta-regression and subgroup analysis

First, we evaluated the included studies to determine if there was an existing threshold effect. The Spearman's correlation coefficient for the anti-CEP 1 antibody was 0.342 ($P = .094$), indicating that there was no threshold effect.

However, there was substantial heterogeneity among the included studies, so we performed a meta-regression to explore the sources of heterogeneity. The following covariates were tested: the control group (healthy control and included disease control), diagnostic criteria for RA (the 1987 ACR criteria and 2010 ACR/EULAR criteria), and detection method for anti-CEP 1 antibody (commercial ELISA kit and non-commercial ELISA kit). Consequently, the control group and diagnostic criteria for RA were identified as significant contributors to the heterogeneity for specificity (both $P < .001$), whereas the detection method for RA contributed to the heterogeneity for sensitivity and specificity ($P < .05$ and $P < .001$, respectively) (Figure 4).

Furthermore, all covariates were subjected to subgroup analysis. In the controls subgroup, the group with healthy controls had a sensitivity of 42% (95% CI: 34%-50%, $P < .0001$, $I^2 = 98.12\%$), a specificity of 98% (95% CI: 97%-98%, $P < .0001$, $I^2 = 88.57\%$), and an AUC of 0.92 (95% CI: 0.89-0.94); the group including the disease controls had a sensitivity of 48% (95% CI: 40%-56%, $P < .0001$, $I^2 = 87.15\%$), a specificity of 95% (95% CI: 91%-98%, $P < .0001$, $I^2 = 93.24\%$), and an AUC of 0.78 (95% CI: 0.74-0.82). In the diagnostic criteria for the RA subgroup, the group diagnosed with RA according to the 1987 ACR criteria had a sensitivity of 41% (95% CI: 35%-48%, $P < .0001$, $I^2 = 97.51\%$), a specificity of 97% (95% CI: 96%-98%, $P < .0001$, $I^2 = 92.80\%$), and an AUC of 0.84 (95% CI: 0.80-0.87); and the group diagnosed with RA according to the 2010 ACR/EULAR criteria had a sensitivity of 57% (95% CI: 47%-67%, $P < .0001$, $I^2 = 90.55\%$), a specificity of 96% (95% CI: 91%-98%, $P < .0001$, $I^2 = 87.35\%$), and an AUC of 0.86 (95% CI: 0.83-0.89). In the detection method for RA subgroup, the group that detected the anti-CEP 1 antibody by using a commercial ELISA kit had a sensitivity of 59% (95% CI: 50%-68%, $P < .0001$, $I^2 = 88.04\%$), a specificity of 93% (95% CI: 85%-97%, $P < .0001$, $I^2 = 86.42\%$), and an AUC of 0.79 (95% CI: 0.76-0.83); the group that detected the anti-CEP 1 antibody by using a home-made ELISA kit had a sensitivity of 33% (95% CI: 28%-39%, $P < .0001$, $I^2 = 90.35\%$), a specificity of 98% (95% CI: 96%-99%, $P < .0001$, $I^2 = 87.07\%$), and an AUC of 0.70 (95% CI: 0.66-0.74); and the group that detected the anti-CEP 1 antibody by using microarray had a sensitivity of 48% (95% CI: 38%-58%, $P < .0001$, $I^2 = 98.34\%$), a specificity was 97% (95% CI: 96%-98%, $P < .0001$, $I^2 = 89.46\%$), and an AUC of 0.95 (95% CI: 0.93-0.97). To explore the different antigen sources and to determine if they affected the diagnostic performance of the home-made ELISA kit, we performed an additional subgroup analysis. The group using a CEP-1 sequence with C-terminal and N-terminal cysteines had a sensitivity of 35% (95% CI: 28%-42%, $P < .0001$, $I^2 = 91.04\%$), a specificity of 98% (95% CI: 95%-100%, $P < .0001$, $I^2 = 93.73\%$), and an AUC of 0.62 (95% CI: 0.57-0.66). The group using a CEP-1 sequence without cysteines had a sensitivity of 31% (95% CI: 22%-42%, $P < .0001$, $I^2 = 90.57\%$), a specificity of 97% (95% CI: 95%-100%, $P = .48$, $I^2 = 0\%$), and an AUC of 0.96 (95% CI: 0.94-0.98). Other details of the summary diagnostic index are shown in the supplementary files (see Table S2. [Appendix S2D]).

3.5 | Publication bias and sensitivity analysis

The Deeks' funnel plot asymmetry test showed that no publication bias was observed ($P = .65$) (see Figure S1 [Appendix S2E]). The sensitivity analysis, excluding each article to perform the meta-analysis again, indicated that the meta-analysis results were stable (See Figure S2 [Appendix S2F]).

4 | DISCUSSION

Patients with RA who receive early successful treatment can achieve effective remission and possibly prevent onset of extra-articular

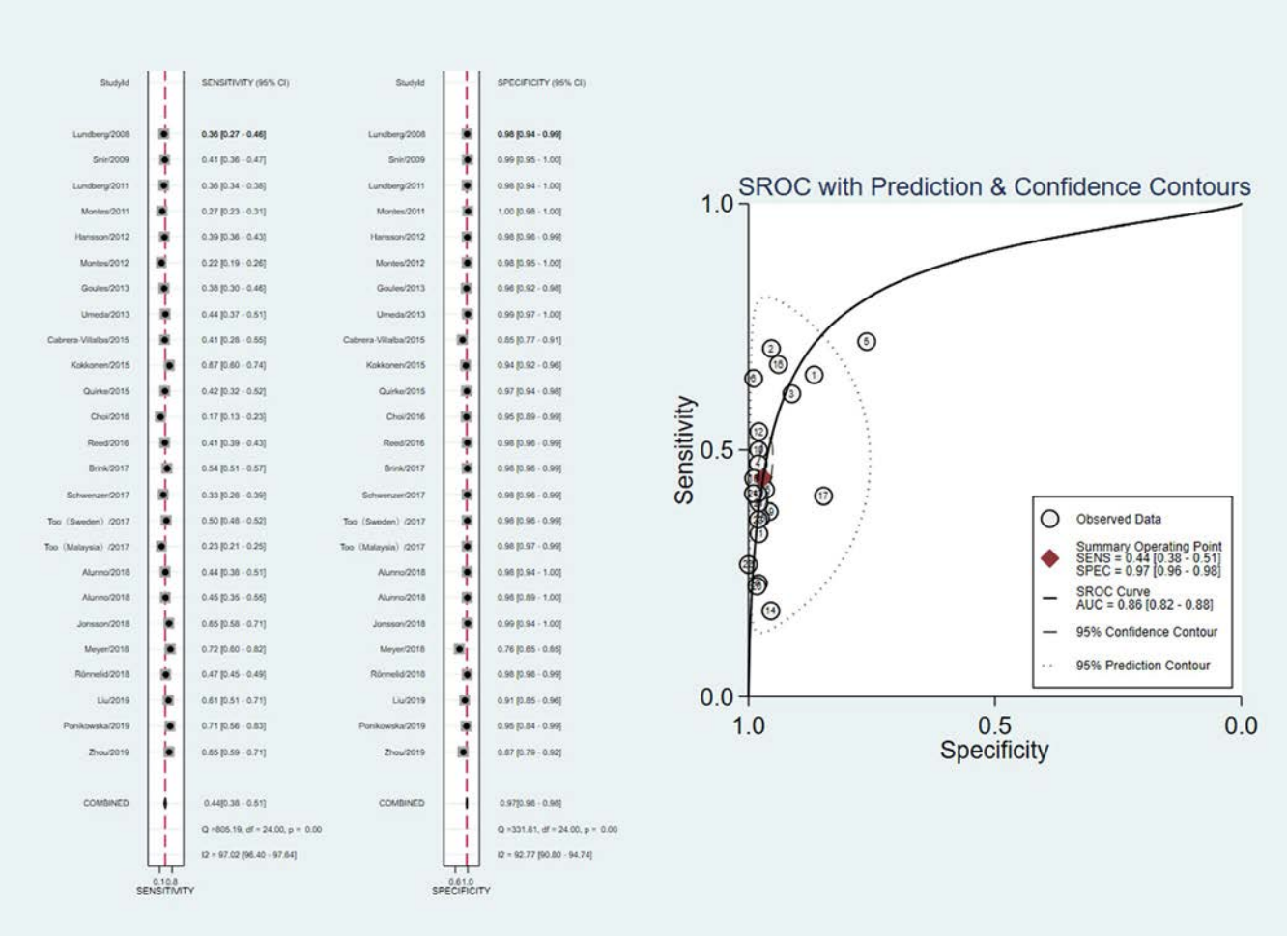

FIGURE 3 Forest plots and summary receiver operating characteristic curves of the anti-citrullinated α-enolase peptide 1 (anti-CEP 1) for rheumatoid arthritis (RA). (A) Sensitivity forest plot; (B) specificity forest plot; (C) summary receiver operating characteristic curve

manifestations. Therefore, it is necessary to identify RA as soon as possible. Many ACPAs have shown good performance for early diagnosis of RA.[39] The anti-CEP 1 antibody is one of the ACPAs that can be detected even before the onset of RA and may be involved at the beginning of RA.[40] Thus, detection of the anti-CEP 1 antibody may contribute to recognition of patients in early phase RA.

To our knowledge, this meta-analysis is the first to investigate the diagnostic value of anti-CEP 1 antibody for RA, with a total of 24 studies included. The potential diagnostic value of the anti-CEP 1 antibody for RA was mainly reflected by its predominant specificity (97%) and PLR (14.81), indicating that the subjects who tested positive for the anti-CEP 1 antibody had a 14.81-fold chance of developing RA relative to the chance for the subjects who tested negative. Therefore, the high PLR of the anti-CEP 1 antibody shows that a positive anti-CEP 1 antibody result has good accuracy for identifying subjects who have RA. However, the diagnostic value of the anti-CEP 1 antibody for RA was limited by its lower sensitivity (44%) and insufficiently low NLR (0.57), which suggests that the subjects who were suspected to have RA but tested negative for the anti-CEP 1 antibody could not be excluded from having RA. The higher pooled PPV (0.96) and lower pooled NPV (0.53) also indicated that subjects with the anti-CEP 1 antibody had a higher probability of RA and those without the anti-CEP 1 antibody could not be ruled out as having RA and that the predicted values could be affected by disease prevalence.[41] Therefore, more studies are needed to confirm this result by adjusting for the prevalence of RA. The DOR (25.83) revealed that the anti-CEP 1 antibody was very helpful for diagnosing RA, and the AUC of 0.86 indicated moderate performance of the anti-CEP 1 antibody for diagnosing RA.

We observed high heterogeneity among the included studies. Therefore, we tested several factors that may influence heterogeneity by performing meta-regression and subgroup analysis. The meta-regression analysis indicated that the control group, diagnostic criteria for RA, and detection method for the anti-CEP 1 antibody were significant contributors to the heterogeneity for specificity, whereas the subgroup analysis suggested that the heterogeneity values for each of the specificity of 3 factors were not significantly different (range of I^2: 86.42%-93.24%) and the specificity was higher than 93%. Additionally, the subgroup analysis indicated that the group diagnosed with RA according to the 2010 ACR/EULAR criteria had a sensitivity (57%) that was about 16% higher than that of the group diagnosed with RA according to the

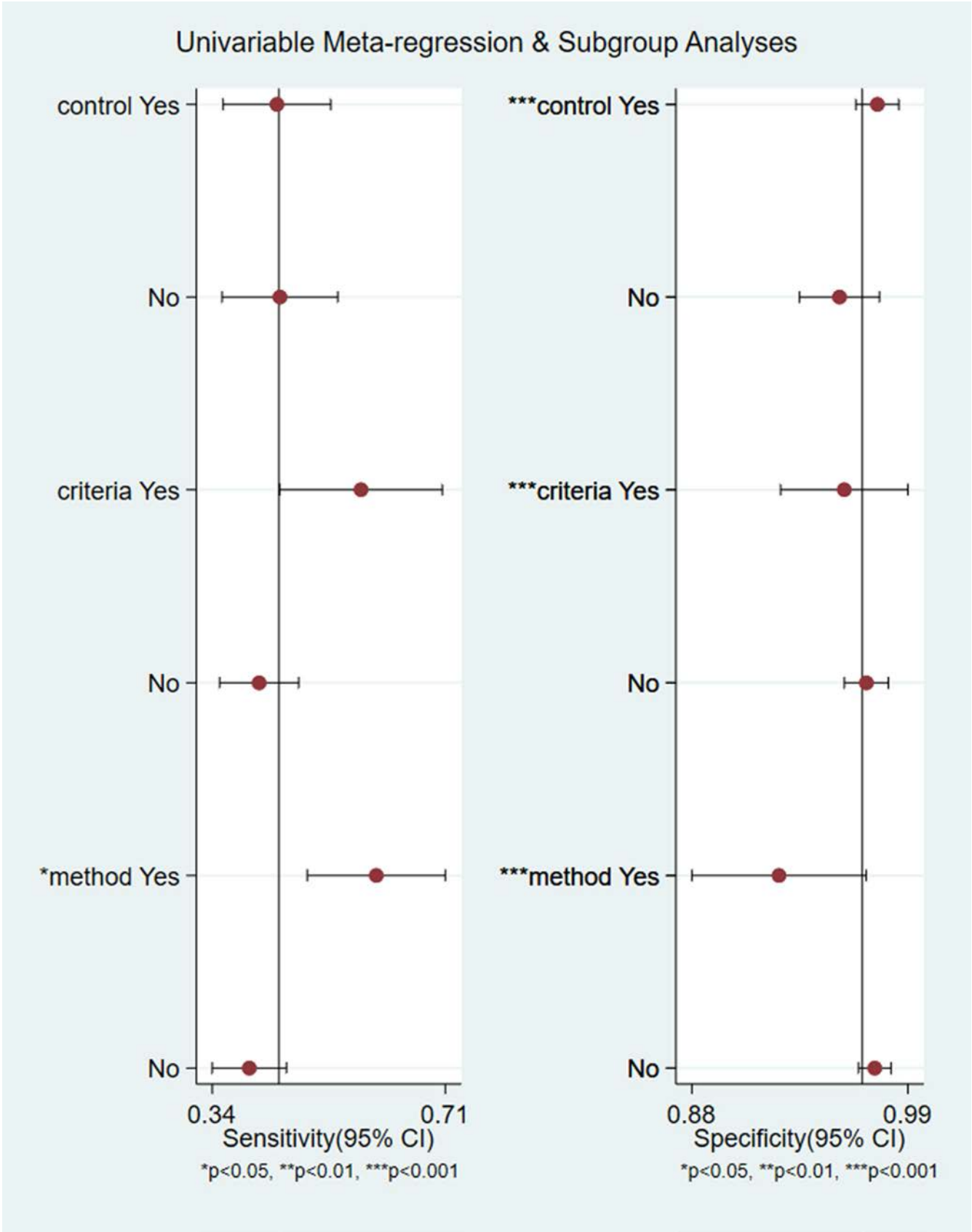

FIGURE 4 Univariable meta-regression and subgroup analysis of 3 covariates (control group, diagnostic criteria for rheumatoid arthritis (RA), method for detection of the anti-citrullinated α-enolase peptide 1 [anti-CEP 1] antibody)

1987 ACR criteria (41%). Clinically, there are 2 sets of criteria for diagnosing RA, and some studies have reported that the 2010 ACR/EULAR criteria have a higher sensitivity for identifying patients with RA but a lower specificity than that of the 1987 ACR criteria.[42-44] The results of the subgroup analysis were consistent with those of these published studies. The method for detecting the anti-CEP 1 antibody is a potential source of heterogeneity for sensitivity and specificity, which was tested by meta-regression and subgroup analysis. The group in which the anti-CEP 1 antibody was detected by using a commercial ELISA kit had the highest sensitivity (59%) of all subgroups, whereas the group in which the anti-CEP 1 antibody was detected by using a home-made ELISA kit had the lowest sensitivity (33%) among all subgroups, which indicated that the standardized commercial ELISA kit may improve the sensitivity for diagnosis of RA relative to that of the home-made ELISA kits that use a variety of materials and testing procedures. Additionally, the AUC of the commercial ELISA kit (0.79) was higher than that of the home-made ELISA kit (0.70), which indicated that detecting the anti-CEP 1 antibody by using the commercial ELISA kit had moderate diagnostic performance and was superior to that of the home-made ELISA kit. The group in which the anti-CEP 1 antibody was detected by using microarray had the highest AUC (0.95), which may be because only healthy donors were included in the control group. Therefore, the value of the microarray for detecting the anti-CEP 1 antibody should be investigated by additional studies that include a diseased-patient control. For the home-made ELISA kit, our data show that the sensitivity and specificity in the group using the CEP-1 peptide with C-terminal and N-terminal cysteines as coated antigens were slightly higher than the CEP-1 peptide without cysteines, but the AUC was lower. Therefore, an inconsistent antigen source may adversely affect the capability for detection of the anti-CEP 1 antibody and the diagnostic performance of home-made ELISA kits. Of note, although the meta-regression showed that 3 factors may contribute to the heterogeneity, the subgroup analysis did not explore the notable decrease in the I^2 value ($I^2 < 50\%$), which demonstrated that the combined effect of multiple factors may influence heterogeneity. However, some of our included studies had missing data, which prevented analysis of this issue.

To date, RA remains a clinical diagnosis and autoantibody tests only serve as an aid to clinical assessment because of the existence of patients with seronegative RA who are negative for both anti-CCP antibody and RF,[45] although anti-CCP antibody and RF are routinely detected as indicators for diagnosing RA. However, our meta-analysis indicated that due to the lower sensitivity of diagnosing RA and similar specificity relative to that of the anti-CCP antibody,[46] this demonstrated that anti-CEP 1 antibody detection is not superior to anti-CCP antibody detection for diagnosing RA. Nevertheless, some studies have indicated that detection of anti-CEP 1 antibody could identify patients with RA who had negative anti-CCP antibody tests. The available evidence shows that detection of the anti-CEP 1 antibody ranges 0%-41.2% in patients with RA who have negative anti-CCP antibody tests and from 32.6%-91.0% in patients with RA who have positive anti-CCP antibody tests.[15-17,20-22,31,34,35,37,38] The results were significantly different due to variations in sample size, study design or patient ethnicity, so further study is needed to investigate the reasons for these variations. Additionally, the first presentation of the anti-CEP antibody can be earlier than the increase in the anti-CCP antibody.[47] Thus, the anti-CEP 1 antibody may have supplementary diagnostic value in patients with RA by combining analysis of the anti-CCP and anti-CEP 1 antibodies. Presumably, they may benefit from early and aggressive interventions. Some studies have indicated that patients with anti-CEP 1 antibody-positive RA are more likely to develop bone erosions or interstitial lung disease (ILD) than patients with anti-CCP antibody-positive RA,[17,22,35] although the anti-CCP antibody is also associated with an increased risk of developing bone erosion or ILD,[22] which suggests that the anti-CEP 1 antibody is a better ACPA than the anti-CCP antibody to predict the prognosis of RA. The anti-CEP 1 antibody, one of the ACPA that targets a true physiological protein, may participate in the pathogen of RA-associated clinical manifestations.[48] High levels of ACPAs are associated with the development of bone erosion in patients with RA.[7,49] ACPAs may contribute to activating osteoclasts through their Fc glycan interactions with Fc receptors on osteoclasts to promote osteoclast activation and subsequent development of bone erosion, which is dependent on antibody-mediated effect.[50] RA-associated ILD frequently appears in patients with positive ACPA and cigarette smoking, but the specific mechanism of the underlying association between lung injury and ACPA generation remains unclear.

There were several limitations in our meta-analysis. First, some published articles in other databases were not evaluated. Second, we used the QUADAS-2 tool to assess the quality of the included studies, and a high risk of bias and an unclear risk of bias for patient selection were observed because most of the included studies did not explain the sampling method used to select the included patients. Third, ACPAs are related to genetic factors and may be one of the sources of heterogeneity, but the included studies were missing a lot of data, so we did not consider this relationship when we performed the meta-regression and subgroup analysis. Fourth, the stage of RA in patients may affect the overall diagnostic value of the anti-CEP 1 antibody for RA, so more studies are needed to investigate the diagnostic value of the anti-CEP 1 antibody for RA in different disease stages. Fifth, some studies have detected the anti-CEP 1 antibody by performing in-house assays, which vary in performance because of varying factors, such as antigen source, plates, conditions of coating, and detection reagents, so the diagnostic value of the anti-CEP 1 antibody detected by in-house assays should be validated.

5 | CONCLUSIONS

In summary, this review shows that the anti-CEP 1 antibody has moderate RA diagnostic value with relatively low sensitivity and high specificity. Moreover, the use of commercial ELISA kits for detecting the anti-CEP 1 antibody increases the RA diagnostic sensitivity.

ACKNOWLEDGEMENTS

The authors thank enago (http://www.enago.cn) for editing this manuscript.

CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

HL and YZ conceived and designed the study. HL and LB searched the literature, screened, and selected the eligible studies. HL and LB extracted data acquisition and conducted quality assessment. HL, CX, LL, SX, and HZ analyzed the data and made an interpretation. HL made a draft. All other authors reviewed it critically. All authors agree to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved. All authors take full responsibility for the integrity of the study and approved the final manuscript as submitted.

DATA AVAILABILITY STATEMENT

All data relevant to the study are included in the article or uploaded as Supplementary Information.

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SUPPORTING INFORMATION

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

How to cite this article: Li H, Li L, Liu C, et al. Diagnostic value of anti-citrullinated α-enolase peptide 1 antibody in patients with rheumatoid arthritis: A systematic review and meta-analysis. *Int J Rheum Dis*. 2021;24:633–646. https://doi.org/10.1111/1756-185X.14093

Received: 11 December 2020 | Revised: 26 January 2021 | Accepted: 11 March 2021

DOI: 10.1111/1756-185X.14108

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases

WILEY

Performance of a pre-administration infection screening questionnaire in patients with rheumatoid arthritis administered biological disease-modifying antirheumatic drugs

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Abstract

Aim: Pre-administration screening of active infections is imperative for the safe use of biological disease-modifying antirheumatic drugs (bDMARDs) in patients with rheumatoid arthritis (RA). However, a standardized screening method is lacking. We therefore implemented a novel systematic screening method with a simple predetermined questionnaire on infections and assessed its effectiveness.

Methods: We retrospectively reviewed medical records of individuals for whom intravenous bDMARDs were administered for RA from January 2016 to April 2019. We evaluated the performance of the new screening method based on physicians' assessments. In addition, a survey was administered to nurses, regarding their assessment of the usefulness of this new screening. The incidence of infections was also assessed.

Results: A total of 1636 cases underwent this new screening. The new screening method showed high sensitivity (0.97) and specificity (0.89) with a negative predictive value of 99.9%, as determined based on the physician's decision. Administration of bDMARDs was postponed in 37 (2.5%) patients, and there was only one case in which the screening failed to note an active infection. The nurses' survey demonstrated high agreement (87.5%) about the usefulness of this screening on the grounds of clarity, simplicity, ease, and time-saving effects. There was no significant increase in infections after implementation of this method.

Conclusions: Systematic screening with a predetermined simple questionnaire is effective as an infection screening method, with a high negative predictive value. This approach contributes to high satisfaction of nurses and a time-efficient practice by focusing on screen-positive cases without increasing infections.

KEYWORDS

bacterial infections, diagnostic screening programs, disease-modifying antirheumatic drugs, rheumatoid arthritis

1 | INTRODUCTION

Biological disease-modifying antirheumatic drugs (bDMARDs) are widely used to treat rheumatoid arthritis (RA); they often engender dramatic improvements in disease outcome.[1] Although the overall safety of bDMARDs has been established for long-term use, previous studies have demonstrated an increased incidence of infections when using bDMARDs.[2-4] Infections in patients with RA are related to increased mortality[5,6] and the interruption of immunosuppressive treatments with subsequent joint destruction and decreased quality of life.[7] Moreover, the immunosuppressive effects of bDMARDs, particularly the interleukin-6 blockade, can modify the presentation of an infection, such as fever, tiredness, and elevated C-reactive protein.[8,9] This renders it difficult to detect an active infection in patients treated with bDMARDs.[9] Therefore, a continuous and careful pre-administration screening for active infections is imperative for the safe use of bDMARDs.

Guidelines and previous studies have recommended screening, continuous monitoring, and preventive intervention for infections such as tuberculosis and viral hepatitis B and C before initiating bDMARDs.[10-12] Additionally, to decrease adverse infectious events, vaccinations and a reduced use of steroids have also been recommended.[10,11] Moreover, risk scores to assess the risk of infections are made and used in clinical practice.[13] However, a systematic preventive approach after initiating bDMARDs, such as a standardized way of detecting infection, is lacking.

In our hospital, patient conditions and vital signs were conventionally assessed with history-taking and physical examinations, on the day of intravenous bDMARD administration. However, there was no standardized, rigorously evaluated method for screening active infections. Therefore, in September 2017, we implemented a new screening method, in which infections were systematically screened according to a simple predetermined questionnaire before the patient was examined. It was expected that this new simple screening would detect infections successfully and would result in a more time-efficient practice.

The aim of this study was to evaluate the effectiveness and usefulness of the new screening method by comparing its results with the subsequent assessment regarding infections and by evaluating practitioners' assessments about the usability of the questionnaire. Additionally, we compared the incidence of infections before and after the implementation of this method.

2 | MATERIALS AND METHODS

2.1 | Study participants and data collection

We retrospectively reviewed cases of intravenous bDMARD administration for RA at St. Luke's International Hospital, a tertiary center in Tokyo, Japan from January 2016 to April 2019. Diagnosis of RA was based on the physician's assessment. Individuals given intravenous bDMARDs from January 2016 to August 2017 were classified into the conventional screening group, and those who underwent the new screening method from September 2017 to April 2019 were classified into the new screening group. We collected data regarding basic demographics, types of bDMARDs and other treatments, underlying diseases, the result of the screening with a predetermined questionnaire, the subsequent physician's decision, and infections.

2.2 | Predetermined questionnaire for infection screening

The predetermined questionnaire sheet is shown in Figure 1. The questionnaire items were selected based on a previous study regarding infections in patients treated with bDMARDS,[14] and the consensus of the physicians and nurses in our department. We selected three major questions on respiratory, urological, and skin symptoms. These reflect common sites of infections in patients treated with bDMARDs.[14] Abnormal vital signs were also included. Nurses were requested to report other symptoms or conditions not included in the questionnaire that they considered to be important. To increase the sensitivity of the screening tool, there were no limitations on the duration or timing of the reported symptoms. Furthermore, in November 2018, we updated the skin and soft-tissue section by adding "blisters" for specific detection of varicella zoster and oral herpes infections, because we experienced a screen-negative case with varicella zoster that resulted in the postponement of bDMARD

Predetermined questionnaire on infections

1: Does the patient have respiratory symptoms below?

(Cough, Sputum, Rhinorrhea, Sore throat, Shortness of breath)

2: Does the patient have urinary symptoms below?

(Pain on micturition, Frequent urination, Feeling of residual urine)

3: Does the patient have skin and soft tissue symptoms below?

(Injury, Skin redness and rash, Blisters)

4: Does the patient have abnormal vital signs?

- Body temperature ≥ 37.5°C
- Systemic blood pressure ≥180 or <90 mmHg
- Heart rate ≥100 /min or ≤50/min
- Respiratory rate ≥24/min
- SpO2 ≤94%

FIGURE 1 Predetermined questionnaire on infections. SpO2, pulse oximetry

administration after the physician's assessment. We regarded the result of this screening as positive if the patient had at least one symptom or condition.

2.3 | Definition of infections

Serious bacterial infection was defined as a bacterial infection requiring intravenous antibiotics or hospitalization, or resulting in death. Additionally, bDMARD-related and non-bDMARD-related infections were defined as infections that occurred within 2 weeks of or more than 2 weeks after the administration of bDMARDs, respectively. Data on viral infections that included varicella zoster virus and cytomegalovirus infections, as well as fungal infections, such as *Pneumocystis jirovecii* pneumonia and candidiasis, were also collected. The incidence of infection was presented as the number of cases per 100 person-years.

2.4 | Feedback on the screening questionnaire from nurses

Because this questionnaire was administered by nurses, and nurses conventionally reported the symptoms of infection before the implementation of this new screening, 16 nurses in our department were asked to complete a survey regarding their satisfaction with this new screening method. The survey questionnaire included a statement regarding the usefulness of the new screening method (strongly agree, agree, neutral, disagree, strongly disagree) and the reasons for their response.

2.5 | Statistical analyses

Descriptive statistics of the baseline characteristics of the conventional and new screening groups were calculated. Categorical data are presented as counts with percentages. Continuous data are presented as the mean (standard deviation [SD]) and median (interquartile range [IQR]) for continuous variables with normal and non-normal distributions, respectively. Each variable was compared between the groups in univariate analyses using the *t* test, nonparametric test, or χ^2 test, as appropriate.

In the new screening group, the number of scheduled administrations of intravenous bDMARDs was determined. The results of the screening with the questionnaire were compared with the subsequent physician's infection assessment of whether to administer bDMARDs or postpone bDMARDs. The sensitivity and specificity of the screening method for the physician's decision were calculated. The incidence of serious and opportunistic infections was compared between the conventional and new screening groups using Poisson regression analysis.

For all analyses, a *P* value less than 0.05 was considered significant. All analyses were performed using R version 3.3.2 (Vienna, Austria) and EZR, which is a graphical user interface for R.[15]

2.6 | Ethical approval

This study was approved by the institutional review board at St. Luke's International Hospital in Tokyo, Japan (Number: 19-R185). The need to obtain informed consent was waived owing to the retrospective design of the study. The study protocol complies with the guidelines of the 1964 Declaration of Helsinki and its later amendments.

3 | RESULTS

3.1 | Baseline characteristics

Among 3120 cases of bDMARD administration, 1636 cases underwent conventional assessment (total follow-up duration, 55 258 days) and 1484 underwent assessment with the novel screening method (total follow-up duration, 52 239 days). Although the conventional and new screening groups had significant differences in age (mean ± SD: 65.9 ± 12.9 vs 67.4 ± 12.5 years; $P = 0.001$) and use of infliximab as the bDMARD (301 ± 18.4 vs 205 ± 13.8; $P = 0.001$), there were no statistical differences between the groups with respect to other patient demographics, including sex, positive rheumatoid factor, and positive anti-cyclic citrullinated peptide antibody. In addition, there was significantly more concomitant use of tacrolimus, mizoribine, and salazosulfapyridine, and significantly less concomitant use of methotrexate and glucocorticoids in the new screening group than in the conventional screening group, as shown in Table 1.

3.2 | Evaluation of the new screening method in terms of the physician's assessment

Among the 1484 cases in the new screening group, 203 screened positive and 1281 screened negative. The reasons for a positive result were as follows: respiratory symptoms, 114 cases; skin and soft-tissue symptoms, 54 cases; urinary symptoms, 13 cases; other symptoms, 24 cases; and abnormal vital signs, eight cases. Among the screen-positive cases, physicians decided to administer bDMARDs in 167 cases and to postpone administration in 36 cases. Among the 1281 screen-negative cases, only one case actually had an infection (varicella zoster virus), as determined by the physician's assessment. The screening method using the predetermined questionnaire had high sensitivity (0.97, 95% confidence interval: 95% CI 0.86-1.00) and specificity (0.89, 95% CI 0.87-0.90) to the subsequent physician's decision of whether to administer bDMARDs or not (Figure 2). Physicians postponed bDMARDs in 37 (2.5%) cases in total, and positive and negative predictive values after this screening were 17.8% and 99.9%, respectively.

Nurses also reported other symptoms or conditions (beyond the screening questionnaire items) that they considered important in 24 cases. This included four cases without premedication; three

TABLE 1 Baseline characteristics

	Conventional screening cases (n = 1636)	New screening cases (n = 1484)	*P* value
Total follow up (days)	55 258	52 239	—
Age (y), mean ± SD	65.9 ± 12.9	67.4 ± 12.5	0.001
Female, n (%)	1389 (84.9)	1221 (82.3)	0.052
RF positive, n (%)	1459 (89.2)	1332 (89.8)	0.641
CCP positive, n (%)	1159 (70.8)	1073 (72.3)	0.382
Intravenous bDMARDs			
Infliximab, n (%)	301 (18.4)	205 (13.8)	0.001
Abatacept, n (%)	795 (48.6)	735 (49.5)	0.616
Tocilizumab, n (%)	631 (38.6)	611 (41.2)	0.143
csDMARDs			
Methotrexate, n (%)	835 (51.0)	694 (46.8)	0.018
Salazosulfapyridine, n (%)	524 (32.0)	541 (36.5)	0.010
Tacrolimus, n (%)	97 (5.9)	153 (10.3)	<0.001
Iguratimod, n (%)	243 (14.9)	202 (13.6)	0.330
Bucillamine, n (%)	321 (19.6)	264 (17.8)	0.199
Mizoribine, n (%)	53 (3.2)	166 (11.2)	<0.001
Glucocorticoid, n (%)	699 (42.7)	566 (38.1)	0.010

Abbreviations: bDMARDs, biological disease-modifying antirheumatic drugs; CCP, cyclic citrullinated peptide; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; RF, rheumatoid factor.

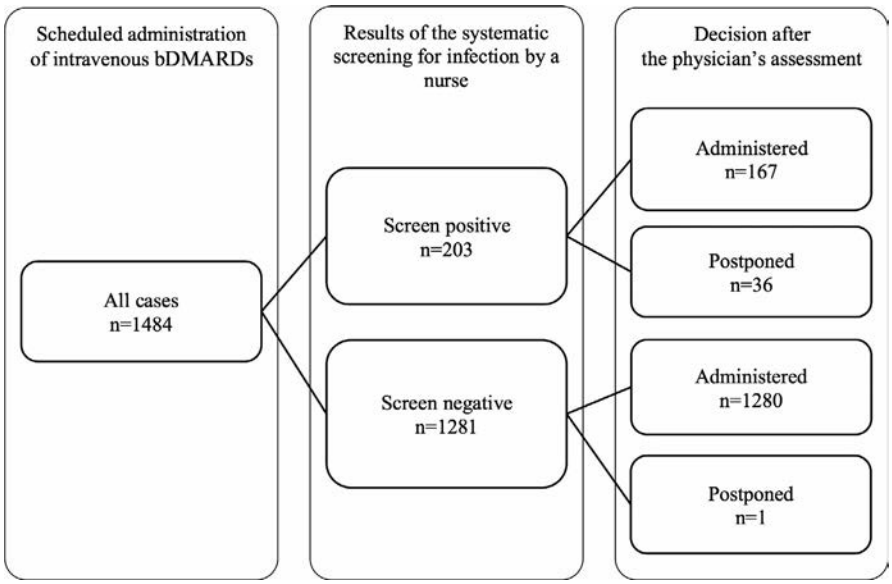

FIGURE 2 Results of screening by the nurse and subsequent decision of the physician. The sensitivity and specificity for the process of screening to the subsequent physician's decision are 0.97 [95% confidence interval 0.86-1.00] and 0.89 [95% confidence interval 0.87-0.90], respectively. bDMARDs, biological disease-modifying antirheumatic drugs

cases of oral ulcers and stomatitis; three cases of blisters (before November 2018, at which time "blisters" was added to the questionnaire); two cases of diarrhea; two cases of abnormal sensation in the oral mucosa; two cases of eye infection and hordeolum; one case of ear pain, black stool, history of allergy after last bDMARDs; and one case each for patient preference, hand tremor, gingival swelling, and dizziness. Of these cases, the physician decided to postpone bDMARD administration in three because of blisters, black stool, and hordeolum. Even when these cases were included as screen negatives, the screening tool still showed high sensitivity (0.89, 95% confidence interval 0.75-0.97) and specificity (0.90, 95% confidence interval 0.88-0.91).

3.3 | Results of the survey questionnaire completed by nurses

All 16 nurses in our department completed the survey questionnaire. A total of 14 (87.5%) nurses indicated that the predetermined screening questionnaire was useful. The distribution of responses regarding

TABLE 2 Comparison of the incidence of infection between the conventional and new screening groups

Incidence (/100 person-years)	Conventional screening cases (n = 1636)	New screening cases (n = 1484)	*P* value
Serious bacterial infection	3.96 (0.50-7.42)	5.59 (1.53-9.65)	0.599
bDMARD-related	0.66 (0.0-2.80)	0.70 (0.0-2.96)	>0.99
Non-bDMARD-related	3.30 (0.06-6.55)	4.89 (1.03-8.75)	0.572
Varicella zoster virus infection	1.98 (0.0-4.74)	3.49 (0.07-6.92)	0.497
bDMARD-related	0.66 (0.0-2.80)	0.70 (0.0-2.96)	>0.99
Non-bDMARD-related	1.32 (0.0-3.79)	2.79 (0.0-5.97)	0.441
Requiring hospitalization or IV antivirals	0	0.70 (0.0-2.96)	0.486
Cytomegalovirus infection	0.66 (0.0-2.80)	0.70 (0.0-2.96)	>0.99
Pneumocystis pneumonia	0	0	>0.99
Superficial candidiasis	6.61 (2.42-10.79)	7.69 (3.11-12.26)	0.828
Invasive candidiasis	0	0	>0.99

Note: All incidences are presented as the number (95% confidence interval) per 100 person-years.
Abbreviations: bDMARD, biological disease-modifying antirheumatic drugs; IV, intravenous.

the usefulness of this new screening method were as follows: strongly agree, nine (57%); agree, five (31%); neutral, one (6%); and strongly disagree, one (6%). The reasons for the positive responses were as follows: it made the assessment simple, clear, and easy, ten (63%); it was time-saving, eight (50%); it eliminated the necessity of reporting to the physician for negative cases, five (31%); and it was easy to write the patient charts, three (18%). The neutral and negative responses were due to confusion regarding whether to report cases with very mild symptoms or symptoms that had almost subsided.

3.4 | Differences in the incidence of infections between the conventional and new screening groups

There was no significant difference in the incidence of serious bacterial infection between the conventional and new screening groups on Poisson regression analysis (3.96, 95% CI 0.50-7.42 vs 5.59, 95% CI 1.53-9.65; $P = 0.599$). This result was maintained after further classification of serious bacterial infection into bDMARD-related and non-bDMARD-related serious bacterial infection, with just one case of bDMARD-related infection in each group. The incidence of varicella zoster virus infection did not significantly differ between the two groups (1.98, 95% CI 0.0-4.74 vs 3.49, 95% CI 0.07-6.92; $P = 0.497$), with just one case occurring within 2 weeks after bDMARD administration. There were no cases of active tuberculosis or *Pneumocystis* pneumonia. Several cases of candidiasis were observed in both groups, which were all superficial and not invasive (Table 2).

4 | DISCUSSION

The present study revealed that our predetermined questionnaire along with vital sign check was effective at screening for infection, with high sensitivity and specificity. Hence, infections can be screened appropriately based on this questionnaire and abnormal vital signs. Previously, there was no standardized, rigorously evaluated method for screening infections in patients treated with bDMARDs. To the best of our knowledge, this is the first report that evaluated a standardized pre-administration assessment of infections in patients with RA after the initiation of bDMARDs.

Our novel screening tool comprised ordinary questions regarding just three systems: (a) respiratory, (b) urological, and (c) skin or soft-tissue, along with abnormal vital signs. This is because a previous study showed that respiratory, urinary tract, cutaneous, and bloodstream infections accounted for approximately 90% of serious infections in patients with RA treated with bDMARDs.[14] It was also reported that vital signs are useful in detecting bloodstream infections.[16] The screening tool showed high sensitivity and specificity (0.97, IQR 0.86-1.00 and 0.89, IQR 0.87-0.90, respectively) with a negative predictive value of 99%. These results are favorable, considering that infection screening requires a higher sensitivity than specificity for the safe use of bDMARDs.

This screening can be generally effective to detect the patient who should be carefully checked for infections in low-risk patients, including young patients without comorbidities. Moreover, these specific questions might be potentially more useful in those in whom it is less easy to recognize symptoms of infections, such as elderly patients. Although there was one screen-negative case in which the physician subsequently detected varicella zoster, after adding blisters to the skin section of the questionnaire, there were no cases in which screening failed to detect infection resulting in postponement of bDMARDs.

We believe that these results support a time-efficient practice. It should be emphasized that history taking and physical examination are imperative for the diagnosis of infection. Moreover,

practitioners can examine patients more specifically and carefully if their screenings are positive. On the other hand, screen-negative patients could be examined in a time-efficient manner by confirming that the patients correctly understood and answered the questionnaire. Our screening approach is simple, and patients may be able to easily complete the screening questionnaire themselves. We may further adapt this method to allow self-screening before receiving intravenous or subcutaneous bDMARDs, which may improve the safety of bDMARD use in a time-efficient manner. We received favorable feedback from the nurses regarding the usefulness of the screening questionnaire, which we believe was due to its simplicity.

The present study also demonstrated that the incidence of serious infections did not significantly decrease or increase with the adoption of this new screening method in comparison to conventional examination. bDMARD-related infections (defined as infections occurring within 2 weeks after bDMARD administration) were notably very rare in both groups.

We designed and conducted this study in cooperation with nurses specializing in rheumatic diseases. The European League Against Rheumatism recommendations for the role of the nurse include providing encouragement to undertake extended roles after specialized training.[17] Clinical research might be included in this role, and it may be of great importance to conduct research with nurses in terms of advancing their careers. Moreover, we believe this kind of academic activity can contribute to advancing team medicine and improving the care of patients with rheumatic diseases through more active involvement of nurses in the practice.

The present study had several limitations. This was a retrospective single-center study, and only patients treated with intravenous bDMARDs were included. Second, we did not collect data regarding the actual time used to complete the screening and assessment by physicians, although the nurses agreed on the usefulness of this questionnaire as a time-efficient practice.

In conclusion, we demonstrated excellent sensitivity and specificity of a novel infection screening method with a simple predetermined questionnaire before the administration of bDMARDs in patients with RA. This method can support a time-efficient practice because screen-positive patients can be more carefully examined. Moreover, nurses are generally satisfied with the usefulness and simplicity of the questionnaire. Hence, this screening method may improve the safe use of bDMARDs and outcomes in RA by preventing infections. Furthermore, it may be possible to adapt this questionnaire to allow patients who receive subcutaneous bDMARDs to self-screen for infections.

ACKNOWLEDGMENTS

We thank all of the physicians, nurses, and other staff caring for the enrolled patients. We wish to thank Mr. Nakashima, Ms. Horikawa, and the Information System Unit at St. Luke's International Hospital for data collection support.

CONFLICT OF INTEREST

HT reports personal fees from Chugai Pharmaceutical, AbbVie, Ono Pharmaceutical, Kyowa-Kirin, Takeda Pharmaceutical, Astellas Pharma, Tanabe-Mitsubishi, and Ayumi, outside the submitted work; MK received an advisory fee/honoraria from AbbVie, Eisai, Tanabe-Mitsubishi, Novartis, Eli Lilly, Janssen, Ono, BMS, Ayumi, and Pfizer; MO received speaking fees and/or honoraria from Eli Lilly, Santen Pharmaceutical, Tanabe-Mitsubishi Pharma, Pfizer, and Abbott Japan.

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How to cite this article: Fukui S, Suda M, Furukawa S, et al. Performance of a pre-administration infection screening questionnaire in patients with rheumatoid arthritis administered biological disease-modifying antirheumatic drugs. *Int J Rheum Dis*. 2021;24:647–653. https://doi.org/10.1111/1756-185X.14108

Received: 27 September 2020 | Revised: 12 February 2021 | Accepted: 6 March 2021

DOI: 10.1111/1756-185X.14100

International Journal of Rheumatic Diseases

WILEY

ORIGINAL ARTICLE

Polymorphism of genes involved in methotrexate pathway: Predictors of response to methotrexate therapy in Indian rheumatoid arthritis patients

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Funding information
The work was supported by an intramural grant from the Institute to AA. AS was supported by Senior Research Fellowship of the Indian Council of Medical Research.

Abstract

Introduction: The adenosine pathway is one of the ways through which methotrexate (MTX) ameliorates inflammation. We therefore explored an association of polymorphism of genes involved in adenosine and MTX metabolic pathways with response to MTX.

Methods: Association of polymorphism in 7 genes (rs2236225 [*MTHFD1* 1958G>A], rs17602729 [*AMPD1* G>A], rs1127354 [*ITPA* C>A], rs1431131 [*TGFBR2* A>T], rs2372536 [*ATIC* C>G], rs11188513 [*ENTPD1* C>T] and rs5751876 [*ADORA2A* T>C]) with efficacy of MTX was studied in Indian rheumatoid arthritis (RA) patients. The patients, classified by European League Against Rheumatology (EULAR)/American College of Rheumatology (ACR) 2010 criteria, were DMARD (disease-modifying antirheumatic drug)-naïve, with Disease Activity Score (DAS28) >3.2. After 4 months of MTX monotherapy, patients were classified as responders (R) or non-responders (NR) based on EULAR response criteria. Genotyping was done by TaqMan 5' nuclease assay and association of gene polymorphisms with response to MTX was determined by Chi-squared test.

Results: Two hundred and twenty-six patients (86% female, median age 40 [interquartile range, IQR = 17.25] years), with disease duration of 24 (IQR = 38.25) months and DAS28-C-reactive protein score of 4.61 (IQR = 1.34) were enrolled. After therapy, 186 patients were classified as R and 40 as NR. GG genotype of *ATIC* ($P = .01$, odds ratio [OR] 2.56, 95% CI, 1.04-6.30) and CC genotype of *ITPA* ($P = .009$, OR 1.34, 95% CI 1.02-1.76) genes were found to be associated with the response. On binary logistic regression analysis, GG genotype of *ATIC* and CC of *ITPA* genes were independent predictors of the response.

Conclusion: Polymorphisms of *ATIC* and *ITPA* genes alone or with clinical variables were associated with response to MTX therapy in Indian RA patients.

KEYWORDS

biomarker, gene polymorphism, methotrexate, rheumatoid arthritis

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a chronic disease which, if not treated early, leads to joint and cartilage damage.[1] Achieving good clinical response during early disease is the key to prevention of tissue-damage and morbidity which appear within 2 years of disease onset.[2] A significant variation exists in clinical course as well as response to treatment in RA patients.[3] Methotrexate (MTX) is the recommended first-line disease-modifying antirheumatic drug (DMARD) for newly diagnosed RA patients.[4] However, about 40% of patients do not respond to MTX.[5] Thus, markers that can identify patients who are likely to respond may be useful in clinical practice.

Genetic biomarkers are most robust as they do not change with disease activity, they are not influenced by drugs and with progress in genomics the cost has also considerably reduced. One of the major mechanisms of action of MTX is release of adenosine. MTX efficacy has been associated with the release of adenosine.[6,7] Hence, polymorphism of genes involved in adenosine production and signaling could be associated with response to MTX.

These genes include *ATIC*, *ITPA*, *AMPD1*, *ADORA2A*, *ENTPD1*, and *TGFBR2*. The enzyme encoded by *ATIC* is inhibited by MTX, resulting in accumulation of 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside and adenosine in an extracellular milieu.[8] *ITPA* encodes for an enzyme which hydrolyzes inosine triphosphate (ITP) to inosine monophosphate (IMP). IMP is also formed by deamination of adenosine monophosphate (AMP). Hence, deficiency of *ITPA* will affect AMP and adenosine balance. Polymorphism in *ITPA* (rs1127354) 94C>A gene causes ITPA deficiency.[9] *AMPD1* encodes an enzyme which deaminates AMP to IMP. Polymorphism of *AMPD1* (rs17602729) 34C>T gene in exon 2 results in production of truncated AMPD peptide[10] thereby increasing adenosine levels. The *ENTPD1* gene encodes for CD39 protein which hydrolyzes ATP to AMP, thereby facilitating adenosine production. The C allele of *ENTPD1* (rs11188513) C>T gene polymorphism is associated with low CD39 expression.[11] Signaling by transforming growth factor (TGF)-β via TGFβR2 receptor increases the expression of CD39. Defect in TGF-β signaling is caused by presence of A allele which eventually reduces CD39 expression.[12] Most of the anti-inflammatory response of adenosine is mediated via *ADORA2A*. The *ADORA2A* (rs5751876) 1976T>C gene polymorphism in exon 2 results in variable expression of the receptor.[13]

Another enzyme associated with purine pathway is encoded by *MTHFD1* gene. It catalyzes the interconversion of 1-carbon derivatives of tetrahydrofolate which are substrates for methionine, thymidylate, and de novo purine syntheses. Minor allele (A) of *MTHFD1* (rs2236225) 1958G>A gene is associated with reduced de novo purine synthesis.[14]

In the light of above observations, in this study, we have explored the association of polymorphisms in 7 genes associated mainly with adenosine pathway, *ATIC*, *MTHFD1*, *ITPA*, *AMPD1*, *ENTPD1*, *TGFBR2* and *ADORA2A* with response to MTX therapy in Indian RA patients. Single nucleotide polymorphisms (SNPs) were chosen based on the review of literature and those that have been studied earlier were chosen.

2 | MATERIALS AND METHODS

2.1 | Patients

Two hundred and twenty-six RA patients fulfilling the American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) 2010 classification criteria for RA[15] were enrolled. The Disease Activity Score at 28 joints (DAS28) in all patients was >3.2.[16] All patients were DMARD (MTX or any other DMARD)-naïve at inclusion. Exclusion criteria comprised any contraindications to MTX therapy, pregnancy, lactation or refusal to provide consent for participation in the study. The Institutional Ethics Committee approved the study and a written informed consent was provided by all patients. All patients were not examined by the same clinician.

Baseline clinical details, physician and patient global assessment, Health Assessment Questionnaire (HAQ), and DAS28 were collected from all patients. A blood sample was collected for genotyping (n = 226). Initially the patients were treated with 10 mg/wk MTX and then the dose was escalated by 2.5 mg after every 2 weeks until DAS28 < 2.6 or a maximum tolerated dose of 25 mg/wk was achieved. After 4 months of MTX therapy, clinical parameters were assessed again and based on EULAR response criteria, patients were classified into responder (R) and non-responder (NR) groups.[17] A priori sample size calculation was not done in the study.

2.2 | Isolation of DNA from blood

Whole blood DNA was isolated using the 'salting out' method.[18] In brief, 300 μL lysis buffer was mixed with 1 mL ethylenediaminetetraacetic acid blood for 15 minutes and centrifuged (5800 *g*, 10 minutes, 4°C). After discarding supernatant, the pellet was re-suspended (by intense tapping) in 300 μL lysis buffer and centrifuged as above. The 'tapping and washing' step was repeated once and 200 μL deionized water was added to the pellet. Further, 100 μL proteinase K buffer and 20 μL 10% sodium dodecyl sulfate was added and mixed. Later, 100 μL deionized water and 100 μL of 5 mol/L NaCl was added and mixed. Finally, 5:1 ratio of phenol/chloroform (400 μL) was added and mixed by swirling the tube for 10 minutes. The mixture was centrifuged (15500 *g*, 30 minutes, 4°C), aqueous layer was separated and mixed with 1 mL chilled absolute alcohol, and centrifuged again (15500 *g*, 20 minutes, 4°C). The pellet was washed with 70% alcohol (500 μL) by centrifuging the tube. Later, the pellet was dried in a dry bath (56°C, 5 minutes). Deionized water (100 μL) was used to resuspend the pellet. Concentration and purity of DNA was determined by using Nano-Drop spectrophotometer (Nanodrop Inc).

2.3 | Genotyping assay

Seven SNPs were genotyped using dual color TaqMan hydrolysis probe (Thermo Fischer Scientific; Table 1). Real-time polymerase chain reaction was setup in a LC480 Real-Time system (Roche) and

TABLE 1 Details of polymorphisms analyzed in the study

Gene	SNP ID	Context sequence [VIC/FAM]	MAF (1000 genomes)
ATIC	rs2372536	TGTAA**[C/G]**TGTTGAGGAGG CTGTGGAGCAAATT	G = 0.28
AMPD1	rs17602729	GCAGCAAAAGTAATGCAATACTCAC**[T/C]** TTTCTCTTCAGCTGTATGAAGTAAA	T = 0.04
ITPA	rs1127354	CGTTCAGATTCTAGGAGATAAGTTT**[A/C]**CATGCACTTTGGTGGCACAGAAAAT	A = 0.09
TGFBR2	rs1431131	TAAAAAGCACATCTTCATTTAGACC**[A/T]** TCCTTATTTCCAAAGCTCTTTTGAT	T = 0.38
ENTPD1 (CD39)	rs11188513	TCCACTACCTGACTACTGTCATTCA**[C/T]** AGGCATTCTGTTCCACAGCAGGCCA	C = 0.42
ADORA2A	rs5751876	ACCCTGAGCGGAGGCCCAATGGCTA**[T/C]**GCCCTGGGGCTGGTGAGTGGAGGGA	C = 0.44
MTHFD1	rs2236225	GGCAATTCCTCCATCATTGCAGACC**[A/G]**GATCGCACTCAAGCTTGTTGGCCCA	A = 0.34

Abbreviations: *ADORA2A*, adenosine receptor 2A; *AMPD1*, adenosine monophosphate deaminase 1; *ATIC*, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase; *ENTPD1*, ectonucleoside triphosphate diphosphohydrolase 1; *ITPA*, inosine triphosphatase; MAF, minor allele frequency; *MTHFD1*, methylenetetrahydrofolate dehydrogenase 1; SNP, single nucleotide polymorphism; *TGFBR2*, transforming growth factor beta receptor 2; VIC & FAM, fluorescent dyes.
Bold value indicates statistically significant results.

TABLE 2 Comparative baseline data of rheumatoid arthritis patients (N = 226)

Characteristics	Values	Responders (n = 186)	Non-responders (n = 40)	*P* values
Female/male, number (%)	194 (86)/32 (14)	160 (86)/26 (14)	34 (85)/6 (15)	.87
Age, y	40 (17.25)	40 (17)	43 (21.75)	.43
Duration of disease, mo	24 (38.25)	24 (37.25)	24 (60.75)	.39
Tender joint count	10 (9)	10 (9)	7 (5.75)	**.02**
Swollen joint count	7 (8)	8 (7.25)	5 (6)	**.01**
HAQ score	1.75 (1.5)	1.75 (1.13)	1.88 (5.16)	.34
CRP, mg/dL	1.85 (2.89)	1.9 (2.92)	1.56 (2.61)	.41
Disease Activity Score of 28 joints (DAS28-CRP)	4.61 (1.34)	4.65 (1.26)	4.16 (1.62)	**.048**
DAS28-ESR	6 (1.31)	6.08 (1.29)	5.65 (1.4)	**.004**
Number of patients positive for IgM-RF (%)	189 (84)	154 (83)	35 (87.5)	.47
Number of patients positive for ACPA (%)	211 (93)	174 (93.5)	37 (92.5)	.81

Note: All values are given as median (interquartile range). Gender, RF and ACPA positivity are given as numbers (%).
Abbreviations: ACPA, anti-citrullinated protein antibody; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; RF, rheumatoid factor.
Bold value indicates statistically significant results.

data were analyzed using Light Cycler 480 software. Two color hydrolysis allele specific probes were labeled with fluorescent reporter dyes VIC (allele 1, 2-chloro-7-phenyl-1, 4-dichloro-6-carboxyfluorescein) and FAM (allele 2, carboxyfluorescein). The reaction mixture (10 μL) comprised genotyping master mix (Thermo Fischer Scientific), TaqMan probe (1×) and 20 ng of DNA. As negative control, a reaction without any genomic DNA was setup with every assay. Amplification protocol was as follows: 10 minutes at 95°C, 40 cycles of 15 seconds at 95°C for denaturation, and 1 minute at 60°C for annealing and extension.

TABLE 3 Allelic association of 7 SNPs with response to methotrexate

Gene	SNP	Allele frequency (R = 186)	Allele frequency (NR = 40)	HWE, *P* value	Allele association, *P* value* OR (95% CI)
ATIC	rs2372536 C>G	C = 0.50 G = 0.50	C = 0.65 G = 0.35	.27	**.01;** 1.90 (1.15-3.14) for allele G
AMPD1	rs17602729 C>T	C = 0.95 T = 0.05	C = 0.98 T = 0.02	.49	.40
ITPA	rs1127354 C>A	C = 0.89 A = 0.11	C = 0.78 A = 0.22	.19	**.006;** 2.34 (1.36-4.34) for allele C
TGFBR2	rs1431131 A>T	A = 0.64 T = 0.36	A = 0.58 T = 0.42	.80	.30
ENTPD1	rs11188513 C>T	C = 0.38 T = 0.62	C = 0.30 T = 0.70	.95	.20
ADORA2A	rs5751876 T>C	T = 0.71 C = 0.29	T = 0.7 C = 0.3	.20	.82
MTHFD1	rs2236225 G>A	G = 0.46 A = 0.54	G = 0.5 A = 0.5	.56	.51

Abbreviations: *ADORA2A*, adenosine 2a receptor; *AMPD1*, adenosine monophosphate dehydrogenase 1;*ITPA*, inosine triphosphate pyrophosphatase; *ATIC*, 5-aminoimidazole-4-carboxamide ribonucleotide transformylase; *ENTPD1*, ectonucleoside triphosphate diphosphohydrolase 1; *MTHFD1*, methylenetetrahydrofolate dehydrogenase, cyclohydrolase and formyltetrahydrofolate synthetase 1; NR, non-responder; R, responder; SNPs, single nucleotide polymorphisms; *TGFBR2*, transforming growth factor beta receptor 2.

Bold value indicates statistically significant results.

2.4 | Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences 16.0 (SPSS Inc). Comparisons of age, disease duration, tender joint count (TJC), swollen joint count (SJC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), HAQ score and baseline DAS28 were analyzed using Mann-Whitney *U* test. Comparison of baseline mean DAS28 in R and NR between allelic and genotypic groups of 7 genes polymorphism was done by *t* test and one-way analysis of variance.

Associations of 7 SNPs, gender, rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) with response to MTX were analyzed using Chi-squared test. Multiple logistic regression analysis of age, DAS28-ESR, TJC, SJC and, *ATIC* and *ITPA* gene polymorphisms were done to identify an independent marker to predict response to MTX therapy. Both clinical and genetic variables with *P* value ≤.3 were taken as independent variables and response status was selected as dependent variable. For additive model, genotypic groups were assigned as follows: non-associated allele, 0; heterozygous, 1 and associated allele homozygous variant, 2. For dominant model, associated allele homozygous and heterozygous were assigned as 1; and non-associated allele homozygous variant as 0. In recessive model, associated allele homozygous was assigned as 1; and heterozygous + non-associated allele homozygous variant as 0. *P* value <.05 was considered statistically significant. All data are represented as median and interquartile range (IQR).

3 | RESULTS

3.1 | Patient characteristics

Two hundred and twenty-six patients (86% female), with median age of 40 (IQR, 17.25) years, disease duration of 24 (IQR, 38.25) months and DAS28 of 4.61 (IQR 1.34) were enrolled (Table 2).

Based on EULAR response criteria, 186 patients were classified as responders (R) and 40 as non-responders (NR) to MTX therapy. Significant difference was observed in the baseline DAS28-ESR (*P* = .004), DAS28-CRP (*P* = .048), TJC (*P* = .02) and SJC (*P* = .01) between R and NR groups of patients. No difference was observed between R and NR patients in other clinical characteristics such as gender, age, disease duration, HAQ score, and positivity for CRP, RF and ACPA (Table 2).

3.2 | Association of genes polymorphism with response to MTX

Allelic distribution of all gene polymorphisms was in Hardy-Weinberg equilibrium (HWE; Table 3). In allelic associations, G allele of *ATIC* gene (rs2372536) 347 C>G was associated with response to MTX (odds ratio [OR] 1.90; 95% CI 1.15-3.14; *P* = .01). In addition, C allele of *ITPA* gene (rs1127354) 94C>A was also associated with the response (OR 2.34; 95% CI 1.36-4.34; *P* = .006; Table 3).

TABLE 4 Genotypic association of 7 SNPs with response to methotrexate

Gene	SNP	Genotype frequency (R = 186)	Genotype frequency (NR = 40)	Genotypic association *P* value* OR (95% CI)
ATIC	rs2372536 C>G	CC = 41 CG = 102 GG = 43	CC = 16 CG = 20 GG = 4	**.01;** 2.56 (1.04-6.30)
AMPD1	rs17602729 C>T	CC = 169 CT = 17 TT = 0	CC = 38 CT = 2 TT = 0	.39
ITPA	rs1127354 C>A	CC = 146 CA = 39 AA = 1	CC = 23 CA = 16 AA = 1	**.009;** 1.34 (1.02-1.76)
TGFBR2	rs1431131 A>T	AA = 77 AT = 83 TT = 26	AA = 13 AT = 20 TT = 7	.57
ENTPD1	rs11188513 C>T	CC = 26 CT = 88 TT = 72	CC = 3 CT = 18 TT = 19	.42
ADORA2A	rs5751876 T>C	TT = 98 TC = 69 CC = 19	TT = 20 TC = 16 CC = 4	.94
MTHFD1	rs2236225 G>A	GG = 43 GA = 85 AA = 58	GG = 9 GA = 22 AA = 9	.49

Abbreviations: *ADORA2A*, adenosine 2a receptor; *AMPD1*, adenosine monophosphate dehydrogenase 1;*ITPA*, inosine triphosphate pyrophosphatase; *ATIC*, 5-aminoimidazole-4-carboxamide ribonucleotide transformylase; *ENTPD1*, ectonucleoside triphosphate diphosphohydrolase 1; *MTHFD1*, methylenetetrahydrofolate dehydrogenase, cyclohydrolase and formyltetrahydrofolate synthetase 1; NR, non-responder; R, responder; SNPs, single nucleotide polymorphisms; *TGFBR2*, transforming growth factor beta receptor 2.

Bold value indicates statistically significant results.

Among genotypic associations, GG genotype of *ATIC* gene (OR 2.56; 95% CI 1.04-6.30; *P* =.01) was associated with response to MTX. Also, CC genotype of *ITPA* gene (OR 1.34; 95% CI 1.02-1.76; *P* =.009) was associated with the response (Table 4). In R and NR, no significant difference was observed in baseline mean DAS28 within allele and genotypes of 7 genes (Table 5).

Binary logistic regression analysis of 3 genetic models to predict response to MTX therapy included 6 parameters: DAS28-ESR, TJC, SJC, age; and *ATIC* and *ITPA* gene polymorphisms. The GG genotype of *ATIC* (rs2372536; OR 6.09; 95% CI 1.65-22.46; *P* = .007) and CA genotype of *ITPA* (rs1127354; OR 3.35; 95% CI 1.48-7.58; *P* = .004) genes were identified as independent predictors of response in the 'additive' model. The association with response was significant even after Bonferroni correction (GG genotype of *ATIC* gene [*P* =.04] and CC genotype of *ITPA* gene [*P* =.02]).

The GG genotype of *ATIC* gene (OR 2.21; 95% CI 1.02-4.76; *P* = .04) predicted response in the 'dominant' model; however, this was not significant after Bonferroni correction. GG genotype of *ATIC* (OR 3.41; 95% CI 1.08-10.82; *P* = .04) and CC genotype of *ITPA* (OR 2.99; 95% CI 1.38-6.44; *P* = .005) genes served as independent markers in the 'recessive' model (Table 6). However, only CC genotype of *ITPA* gene remained significant (*P* =.03) after Bonferroni correction.

4 | DISCUSSION

Nearly one-third of RA patients do not respond to MTX monotherapy. Hence, attempts are underway to identify the biomarker(s) which could predict the response. With respect to baseline clinical parameters, we observed significantly higher SJC and TJC and higher DAS28-ESR and DAS28-CRP in R as compared to NR patients, suggesting an association of enhanced inflammatory burden with response to MTX. Corroborative findings have been reported in a study where low disease activity (measured as DAS28) was associated with poor response to MTX.[19] However, contradictory results were reported in another study demonstrating high baseline DAS28 as a strong predictor of unresponsiveness to MTX.[20] Also, the association of high TJC and SJC with MTX response in our study is contrary to some other studies reporting an association of high TJC and SJC with unresponsiveness to MTX.[19,20]

Of the 7 genes studied, only *ATIC* and *ITPA* polymorphisms were found associated with the response to MTX. We observed an association of G allele and GG genotype in *ATIC* (rs2372536) 347C>G gene polymorphism with response to MTX therapy. This polymorphism reflects change of threonine to serine in exon 5, although it is not yet known whether this change affects the function of enzyme. Some other studies have also analyzed an association of *ATIC* (rs2372536)

TABLE 5 Baseline DAS28 score in rheumatoid arthritis patients in responders and non-responders to methotrexate therapy by genotype

Gene	Single nucleotide polymorphism	R (n)	Mean ± SD	NR (n)	Mean ± SD
ATIC	rs2372536 C>G	CC = 41 GC = 102 GG = 43	4.57 ± 1.07 4.86 ± 0.98 4.62 ± 0.98	CC = 16 GC = 20 GG = 4	4.22 ± 1.16 4.63 ± 1.32 4.50 ± 1.74
AMPD1	rs17602729 C>T	GG = 169 AG = 17	4.73 ± 0.99 4.88 ± 1.21	GG = 38 AG = 2	4.44 ± 1.27 4.69 ± 2.07
ITPA	rs1127354 C>A	CC = 146 CA = 39 AA = 1	4.76 ± 0.98 4.67 ± 1.11 4.85	CC = 23 CA = 16 AA = 1	4.09 ± 1.04 4.93 ± 1.48 5.22
TGFBR2	rs1431131 A>T	TT = 25 AT = 83 AA = 78	4.86 ± 1.27 4.64 ± 0.95 4.81 ± 0.98	TT = 7 AT = 20 AA = 13	4.95 ± 1.17 4.53 ± 1.29 4.07 ± 1.28
ENTPD1	rs11188513 C>T	TT = 72 CT = 88 CC = 26	4.71 ± 0.99 4.78 ± 1.09 4.71 ± 0.79	TT = 19 CT = 18 CC = 3	4.45 ± 1.13 4.55 ± 1.52 3.95 ± 0.5
ADORA2A	rs5751876 T>C	TT = 98 TC = 69 CC = 19	4.81 ± 0.99 4.69 ± 0.99 4.62 ± 1.18	TT = 19 TC = 17 CC = 4	4.29 ± 1.09 4.67 ± 1.56 4.27 ± 0.85
MTHFD1	rs2236225 G>A	AA = 58 AG = 85 GG = 43	4.85 ± 0.99 4.72 ± 1.02 4.64 ± 1.02	AA = 9 AG = 22 GG = 9	4.37 ± 0.64 4.25 ± 1.33 5.03 ± 1.56

Note: None of the comparisons showed any significant difference.

Abbreviations: *ADORA2A*, adenosine receptor 2A; *AMPD1*, adenosine monophosphate deaminase 1; *ATIC*, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase; DAS28, Disease Activity Score of 28 joints; *ENTPD1*, ectonucleoside triphosphate diphosphohydrolase 1; *ITPA*, inosine triphosphatase; *MTHFD1*, methylenetetrahydrofolate dehydrogenase 1; NR, non-responder; R, responder. $P < .05$ *SD, standard deviation; *TGFBR2*, transforming growth factor beta receptor 2.

347C>G gene polymorphism with efficacy of MTX. Similar findings were reported in a Caucasian cohort where GG genotype was associated with response to MTX[21] whereas in a Dutch cohort, CC genotype was associated with good response.[22] Discrepant results were recorded in a meta-analysis comprising of 5 studies with 1056 RA patients, which reported an association of GG + GC genotype with unresponsiveness to MTX. However, upon ethnicity-based stratification of patients, the association was evident in Caucasians but not in Asian populations.[23] Among 3 studies available on Indian populations, no association was seen between response to MTX and *ATIC* gene polymorphism.[24-26]

The polymorphism in *ITPA* (rs1127354) 94C>A gene results in *ITPA* deficiency.[27] We observed an association of C allele and CC genotype in *ITPA* gene polymorphism with response to MTX. A study in a Dutch population has reported similar association,[22] whereas no association was observed in American [28] and British [29] cohorts. Differences in association can be attributed to variation in study design and population. For instance, the outcome was measured as disease activity (American cohort) rather than response at the end of 6 months (British cohort) or at 4 months in our study. The present study comprised an early RA patient population as compared to the other 2 studies.

We did not observe any association of *MTHFD1*, *AMPD1*, *ENTPD1* and *TGFBR2* gene polymorphisms with response to MTX. With respect to *MTHFD1*, 3 other studies, in British, New Zealander and Serbian populations, have reported similar findings.[29-31] Two studies in Indian and British populations have also reported no association between *AMPD1* and MTX response.[26,29] However, a study in a Dutch population observed an association of T allele of *AMPD1* (rs17602729) 34C>T gene with good clinical response to MTX.[22] No prior study is available on association of *ENTPD1* gene polymorphism with MTX efficacy. An allele of *TGFBR2* (rs1431131) A>T gene was associated with poor response to MTX therapy in a Brazilian population.[12] No other study has examined this association. We also did not find an association of *ADORA2A* gene polymorphism with response to MTX. Similar findings were reported in a British population.[32] However, in a north Indian population, T allele of *ADORA2A* (rs5751876) gene polymorphism was associated with poor response to therapy.[26] Contradictory results between various studies can be related to variability in MTX dosage, sample size, duration of disease, response criteria and ethnicity.

In binary logistic regression analysis, we observed an association of good response to MTX in a model that included age, DAS28-ESR, TJC, SJC and polymorphism of *ATIC* and *ITPA* genes. Another prediction model comprising of *ATIC* and *ITPA* genes polymorphism along with gender, RF status, smoking status, disease activity, *AMPD1* (rs17602729) C>T, and *MTHFD1* (rs17850560) A>G gene polymorphisms has been proposed.[33] However, a prediction model developed in one ethnic group may not be applicable to other groups. For instance, a prediction

TABLE 6 Binary logistic regression analysis of 6 variables in different models

Model	Variables	B (SE)	*P* value	OR (95% CI)	*P* value (Bonferroni correction)
Additive	Age	0.02 (0.02)	.25	1.02 (0.99-1.05)	1.50
	BL-DAS28-ESR	−0.67 (0.37)	.06	0.51 (0.25-1.05)	0.36
	TJC	0.01 (0.06)	.90	1.01 (0.89-1.14)	5.46
	SJC	0.03 (0.06)	.7	1.03 (0.90-1.16)	3.84
	ATIC (rs2372536)	1.81 (0.67)	**.007**	6.09 (1.65-22.46)	**0.04**
	ITPA (rs1127354)	1.21 (0.42)	**.004**	3.35 (1.48-7.58)	**0.02**
	Constant	−0.10 (1.86)	.96	0.90	5.76
Dominant	Age	0.02 (0.02)	.26	1.02 (0.99-1.05)	1.56
	BL-DAS28-ESR	−0.80 (0.35)	**.02**	0.45 (0.23-0.9)	0.12
	TJC	0.02 (0.06)	.73	1.02 (0.91-1.14)	4.38
	SJC	0.03 (0.06)	.59	1.03 (0.92-1.17)	3.54
	ATIC	0.79 (0.39)	**.04**	2.21 (1.02-4.76)	0.24
	ITPA	1.24 (1.47)	.40	3.45 (0.19-61.65)	2.4
	Constant	1.68 (1.66)	.31	5.34	1.86
Recessive	Age	0.02 (0.02)	.25	1.02 (0.99-1.05)	1.5
	BL-DAS28-ESR	−0.66 (0.36)	.06	0.52 (0.26-1.04)	0.36
	TJC	0.02 (0.06)	.79	1.02 (0.90-1.15)	4.74
	SJC	0.01 (0.06)	.93	1.01 (0.89-1.14)	5.58
	ATIC	1.23 (0.59)	**.04**	3.14 (1.08-10.82)	0.24
	ITPA	1.10 (0.39)	**.005**	3 (1.38-6.44)	**0.03**
	Constant	−0.04 (1.8)	.98	0.96	5.88

Note: Odds ratio (OR) and 95% CI (confidence interval) by logistic regression analysis of 7 variables with (*P* value ≤.3) to predict response to methotrexate therapy.

Abbreviations: *ATIC*, 5-aminoimidazole-4-carboxamide ribonucleotide transformylase; BL, baseline; DAS, Disease Activity Score of 28 joints; ESR, erythrocyte sedimentation rate; *ITPA*, inosine triphosphate pyrophosphatase; SJC, swollen joint count; TJC, tender joint count.

Bold value indicates statistically significant results.

model developed in an American population, comprising 3 gene polymorphisms (*ATIC*, SLC191A1 and thymidylate synthase [TSER*2/*3]) could not predict MTX efficacy when tested on a British population.[34,35] Similarly, a model developed in a Slovenian population comprising MTX dose, presence of erosions, baseline DAS28 and 4 gene (*AMPD1*, TYMS, SLC19A1 and SLCO1B1) polymorphisms did not perform well in a Serbian population of RA patients.[36] Therefore, all polymorphism data available on Indian populations need to be collated and analyzed to generate a robust model to predict the response to MTX in Indian RA patients.

The strength of our study is inclusion of RA patients newly started on MTX monotherapy. Patients were followed up at 4 months to predict the disease outcome, which shall help in tailoring the therapy within a short period of disease onset. One of the major limitations of this study is the skewness in the sample, with fewer numbers of NRs compared to responders. Also, more composite score models are presently being analyzed for predicting the association of gene polymorphisms with drug response. Since multiple factors affect pharmacokinetics and pharmacodynamics of a drug, sensitivity and specificity of a single gene or marker is low. Hence, composite score analysis comprising clinical and genetic parameters should be considered in search for a robust marker for drug efficacy.

In summary, we have explored an association of polymorphism in 7 genes with MTX response, which previously was not investigated extensively. Our study suggests an association of G allele and GG genotype in *ATIC* and C allele and CC genotype in *ITPA* gene polymorphisms with MTX response. However, these findings must be replicated in larger and independent cohorts of RA patients.

ACKNOWLEDGEMENT

We thank Dr Sudhir Sinha for the critical review of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this paper.

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How to cite this article: Singh A, Gangadharan H, Gupta V, Patro PS, Misra R, Aggarwal A. Polymorphism of genes involved in methotrexate pathway: Predictors of response to methotrexate therapy in Indian rheumatoid arthritis patients. *Int J Rheum Dis*. 2021;24:654–662. https://doi.org/10.1111/1756-185X.14100

Received: 3 November 2020 | Revised: 2 March 2021 | Accepted: 6 March 2021

DOI: 10.1111/1756-185X.14105

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases

WILEY

Clinical characteristics of Vietnamese patients with idiopathic inflammatory myopathies and autoantibodies to aminoacyl-transfer RNA synthetases

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Funding information
The Swedish Research council; Grants provided by Region Stockholm (ALF project); Asia Pacific League of Associations for Rheumatology

Abstract

Objective: To assess clinical phenotypes of anti-aminoacyl-transfer RNA synthetases (aaRS) autoantibodies in Vietnamese patients of Kinh ethnicity with idiopathic inflammatory myopathies (IIM).

Methods: In a cross-sectional study 23 patients with anti-aaRS autoantibodies were compared to 36 patients with other myositis-specific antibodies and to 69 seronegative patients with IIM. Assessments included muscle performance, extra-muscular involvement, and disease activity according to the International Myositis Assessment and Clinical Studies (IMACS). Sera were tested by a line immunoassay (Euroline Myositis Profile 4).

Results: The frequency of anti-Jo-1 antibodies was 56.5%, anti-EJ antibodies 26.1%, and anti-PL-7 antibodies 17.4%, while anti-PL-12 and anti-OJ antibodies were not present in any case. All patients with anti-aaRS autoantibodies had signs of myositis. At time of investigation 22/23 patients had muscle weakness, 52.2% arthritis, 34.8% Raynaud's phenomenon, 73.9% fever, 14.3% mechanic's hands and 56.5% dysphagia. Interstitial lung disease was present in 52.2%, and pulmonary hypertension in 56.5%. The anti-aaRS autoantibody positive group had higher disease activity in the domains of skin and pulmonary disease compared to the seronegative group and had lower disease activity in skeletal disease compared to the anti-melanoma differentiation-associated protein 5-positive patients. The clinical presentation of antisynthetase syndrome was similar between the aaRS autoantibody specificities with the exception of more frequent pulmonary hypertension in anti-Jo-1 positive patients.

Conclusions: Different aaRS autoantibody specificities may vary between different ethnic populations for reasons that still need to be clarified. Furthermore, the high frequency of pulmonary hypertension is noteworthy but otherwise clinical manifestations associated with aaRS autoantibodies did not differ from other ethnic populations.

KEYWORDS

antisynthetase autoantibodies, dermatomyositis, Kinh ethnicity, myositis, polymyositis

1 | INTRODUCTION

Idiopathic inflammatory myopathies (IIM) collectively named myositis, represent a group of rare disorders characterized by dysfunction of skeletal muscle and inflammatory infiltrates in muscle tissue and frequent involvement of extra-muscular organs such as skin, joints, lung, heart and the gastrointestinal tract. Based on different clinical and histopathologic manifestations, patients with myositis are often subclassified into dermatomyositis (DM), polymyositis (PM), inclusion body myositis (IBM) and more recently also into the subset named immune-mediated necrotizing myopathy (IMNM).[1-4]

Autoantibodies are common in IIM, present in up to 80% of patients with PM and DM, and less frequently in IBM.[5,6] Recently a number of new autoantibody specificities have been identified in patients with IIM. They can be classified as myositis associated autoantibodies (MAAs) including anti-Ro52, anti-La, anti-Ku, anti-PM-Scl and anti-U1RNP that can also be found in other autoimmune diseases, and in myositis-specific autoantibodies (MSAs).[7] The MSAs are not only specific for myositis but are also strongly associated with distinct clinical phenotypes. The most frequent MSA, the anti-Jo1 autoantibody, present in up to 30% of Caucasian patients with IIM, is associated with a clinical entity known as antisynthetase syndrome (myositis, interstitial lung disease [ILD], non-erosive arthropathy, mechanic´s hands, fever, and Raynaud´s phenomenon).[8] ILD is especially prevalent in antisynthetase syndrome, occurring in about 75% of patients with anti-Jo1 autoantibodies compared to about 30% of patients with IIM in the absence of antisynthetase autoantibodies.[8] There are 7 other less frequently occurring aminoacyl-transfer RNA synthetase (aaRS) autoantibodies (anti-EJ, anti-OJ, anti-PL-12, anti-PL-7, anti-Ha, anti-Zo and anti-KS) all associated with features of antisynthetase syndrome.[9]

The different anti-aaRS autoantibodies have been reported with different clinical phenotypes in patients with different ethnicities. Thus in Caucasian patients myositis is more frequent in anti-Jo1 positive compared to anti-PL-7 or anti-PL-12 positive patients who have a higher frequency of ILD, whereas in Japanese patients anti-PL-7 was more often associated with myositis compared to anti-PL-12. Patient survival was also conditioned by the anti-aminoacyl-transfer RNA synthetases (anti-ARS) specificity, and was significantly lower in patients with anti-PL-7/12 autoantibodies than in anti-Jo1 positive patients.[10-12] Thus different anti-aaRS autoantibodies may be associated with different clinical phenotypes in different ethnic populations. In this study we aimed to assess the clinical phenotype of anti-aaRS autoantibodies in Vietnamese patients of Kinh ethnicity with IIM.

2 | PATIENTS AND METHODS

2.1 | Patients

This is a descriptive study including all patients who were seen at the Rheumatology Department at Bach Mai Hospital, Hanoi, Vietnam, between March 2011 and December 2013. From a cohort of 151 patients of Kinh ethnicity with IIM who were subject to a cross-sectional study previously published, we selected the aaRS autoantibody positive (n = 23) patients for a thorough review regarding clinical and laboratory data.[13] The aaRS antibody positive patients were compared to 69 patients seronegative for MSAs and MAAs and to patients from the 3 largest groups with other MSAs namely anti-signal recognition particle (anti-SRP) (n = 17), anti-melanoma differentiation-associated protein 5 (anti-MDA5) (n = 11) or anti-Mi-2 (n = 8) autoantibodies. IIM was defined by experienced rheumatologists as probable or definite PM/DM according to the Bohan and Peter criteria.[14,15] In the cohort of 151 patients with IIM, 74 patients fulfilled the criteria for definite and 14 for probable PM and 55 for definite DM and 8 probable DM; information for the respective subgroups is presented in Table 1. The patients who did not have classical skin manifestations of DM according to the Bohan and Peter criteria were excluded from the analysis. Patients with clinical overlap syndromes were also excluded. Information on age, gender, disease duration, initial symptoms, accumulated clinical manifestations and treatment was recorded. At time of study all patients had detailed clinical and laboratory examination including computed tomography (CT) scan of thorax, abdomen and pelvis, and echocardiogram. Gastroscopy was performed when clinically indicated. Women had a gynecological examination. The assessment also included disease activity using the myositis disease activity assessment tool (MDAAT) proposed by the International Myositis Assessment and Clinical Studies group (IMACS).[16]

Patients were treated with immunosuppressive agents according to the treating physician´s choice. All patients were treated with glucocorticoids. Methotrexate and azathioprine were the most often used immunosuppressive drugs. Patients with steroid refractory disease received intravenous pulse cyclophosphamide. All patients with ILD received combination therapy including methylprednisolone pulse therapy, plus methotrexate, azathioprine or cyclophosphamide.

Antisynthetase syndrome (ASS) was defined as a positive aaRS autoantibody together with at least 1 of the following clinical manifestations: myositis, ILD, fever, Raynaud's phenomenon, arthritis, or mechanic's hands.[17] *Pulmonary involvement* was systematically investigated at time of study and in some cases at time of diagnosis. Pulmonary function was tested according to the American Thoracic Society guidelines, using standard equipment.[18] High resolution computed tomography (HRCT) of the lungs without intravenous contrast during end inspiration was performed in all patients. A radiologist who is a specialist of respiratory disease evaluated the HRCT findings in a blinded fashion. ILD was defined as presence of either: (a) chest radiograph abnormalities indicative of fibrosis, and pulmonary function tests; forced expiratory volume in 1 second (FEV_1) < 80%, and forced vital capacity (FVC) < 80% (predicted), and total lung capacity (TLC) < 80% and/or >80% predicted FEV_1/FVC; or (b) abnormal findings on HRCT scan, showing at least 1 of the following features: reticulation and fibrosis, traction bronchiectasis, honeycombing, ground-glass opacification.[19]

TABLE 1 Clinical features and laboratory characteristics at time of investigation of 23 patients with anti-aminoacyl-transfer RNA synthetase (aaRS) autoantibodies compared to patients with anti-SRP, anti-Mi2 or anti-MDA5 autoantibodies and to autoantibody negative patients with idiopathic inflammatory myopathy (IIM)

Feature	Antisynthetase n = 23	Anti-SRP n = 17	Anti-Mi-2 n = 8	Anti-MDA5 n = 11	No antibody n = 69
Age, mean ± SD, y	45.8 ± 13.9	42.2 ± 13.8	48 ± 16.5	39.1 ± 10.8	43.3 ± 16.6
Disease duration, mean, mo ± SD	16.0 ± 22.3	31.7 ± 37.2	19 ± 28.7	18.1 ± 9.9	21.9 ± 30.3
Female, n (%)	16 (70)	15 (88)	7 (88)	9 (82)	49 (71)
Diagnosis, PM/DM	**9/14**	9/8	5/3	5/6	**44/25**
Definite PM/DM	7/12	8/8	5/2	3/4	36/22
Probable PM/DM	2/2	1/0	0/1	2/2	8/3
Constitutional manifestations, n (%)					
Fever	**17 (74)**[§]	9 (53)	6 (75)	11 (100)	36 (52)
Cutaneous involvement, n (%)					
Heliotrope rash	10 (71)	7 (87.5)	3 (100)	5 (83)	17 (68)
Gottron´s papules	9 (64.3)	4 (50)	3 (100)	5 (83)	15 (60)
Mechanic´s hands	2 (14.3)	1 (5.9)	1 (12.5)	1 (9.1)	2 (8.0)
Skeletal manifestations, n (%)					
Myositis	23 (100)	17 (100)	8 (100)	11 (100)	69 (100)
Severe proximal muscle weakness, n (%)	7 (30)	5 (29)	4 (50)	4 (36)	18 (26)
Arthritis, n (%)	**12 (52)**[§]	**2 (12)**[‡]	3 (38)	**9 (82)**[δ]	21 (30)
Cardiovascular manifestations, n (%)					
Raynaud's phenomenon	8 (35)	3 (18)	4 (50)	6 (55)	22 (32)
Pericarditis	**7 (30)**[§]	3 (17.6)	2 (25)	2 (18.2)	7 (10)
Pulmonary involvement, n (%)					
Interstitial lung disease	**12 (52)**[§]	4 (24)	**1 (13)**[*]	2 (18)	22 (32)
Pulmonary hypertension	13 (56.5)	7 (41.2)	4 (50)	4 (36.4)	28 (40.6)
Gastrointestinal involvement, n (%)					
Dysphagia	13 (56.5)	11 (64.7)	3 (37.5)	7 (63.6)	34 (49.3)
Laboratory investigations					
Raised CRP, n (%)	**17 (74)**[†]	10 (59)	6 (75)	5 (46)	29 (42)
CK, IU/L, mean ± SD	3019 ± 5716.2	**5205.8 ± 9812.1**[δ]	**5889.4 ± 7527.9**[§]	926.8 ± 1791.6	1565 ± 2890.1
Treatment					
Prednisolone mean daily dosage, mg ± SD	27.2 ± 12.2 (min = 5, max = 40)	26.5 ± 14.6 (min = 0, max = 40)	37.5 ± 7.1 (min = 20, max = 40)	26.8 ± 15.6 (min = 5, max = 40)	27.1 ± 15.5 (min = 0, max = 40)
Number of patients with methotrexate/azathioprine/cyclophosphamide/mycophenolate mofetil	4/4/9/0	6/1/6/1	5/2/1/0	4/1/4/0	16/13/14/0

Abbreviations: CK, creatine kinase; CRP, C-reactive protein; DM, dermatomyositis; IIM, idiopathic inflammatory myopathy; NS, not significant; Reference values for CK: 26-140 IU/L; PM, polymyositis; SD, standard deviation.

[§]$P < .05$ compared to antibody negative group (negative against all investigated antibodies). [†]$P < .01$ compared to antibody negative group. [‡]$P < .01$ compared to anti-aaRS antibody positive group. [*]$P < .05$ compared to anti-aaRS antibody positive group. [δ]$P < .01$ compared to antibody negative group. [#]$P < .0005$ compared to antibody negative group.

In patients with symptoms indicating an infection, bronchoscopy and bronchoalveolar lavage was performed to rule out tuberculosis or other infections. In addition, skin test of tuberculosis and extended history for exposure to tuberculosis was performed.

Cardiac involvement was defined by abnormalities on electrocardiogram or echocardiogram, performed in all patients at time of this study.

Pulmonary hypertension was defined as a systolic pulmonary arterial pressure >30 mm Hg as estimated by Doppler echocardiography.

Muscle involvement was defined as abnormalities on electromyography, or in muscle biopsy. Muscle strength was tested by manual muscle test (MMT)-8, with a maximal score of 80.[20] Muscle weakness was defined as MMT-8 score below 80. Active muscle inflammation was defined as creatine kinase (CK) level above at least twice the upper limit of normal level (>280 IU/L) and muscle disease activity on visual analog scale (VAS) ≥ 5 in the MDAAT.

Laboratory tests at the time of clinical examination included blood tests for erythrocyte sedimentation rate (ESR), hemoglobin, leukocytes, platelets, C-reactive protein (CRP), CK, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine, protein and albumin.

Sera from all individuals who gave consent were stored at −80°C until analyses were performed.

2.2 | Autoantibodies

Sera were tested by a myositis antibody line immunoassay (LIA) in the Department of Clinical Immunology, Uppsala University Hospital, Sweden, according to the manufacturer's instructions (Myositis Profile 4 Euroimmun) including: anti-Jo1 (histidyl), anti-PL-7 (threonyl), anti-PL-12 (alanyl), anti-EJ (glycyl), anti-OJ (isoleucyl-tRNA synthetase), anti-SRP, anti-Mi-2 (alpha and beta chains investigated separately), anti-MDA5, anti-transcriptional intermediary factor-1 gamma, anti-nuclear matrix protein 2 (anti-NXP2), anti-small activating enzyme, anti-PM-Scl (100 kD and 75 kD units tested separately) and anti-Ku. Scanned densitometry data were calculated using the Euroline Scan software (Euroimmun). The autoantibody profile of this group has previously been reported.[13] A validation of the used LIA was recently published.[21] The cut off for each autoantibody was ≥11 densitometry units for all autoantibodies. When investigating 60 healthy Swedish blood donors with the myositis LIA, 2 individuals showed weak reactivity for anti-NXP2 and anti-Mi-2 alpha, respectively, whereas all other reactions were negative.

2.3 | Human leukocyte antigen cell surface receptor (HLA-DR) typing

HLA-DR typing was performed by sequence-specific primer polymerase chain reaction assay (SSP-PCR; DR low-resolution kit; Olerup SSP AB).

2.4 | Ethics

This study complies with the Declaration of Helsinki, (as revised in Brazil 2013), and was approved by the local ethics committee at Hanoi Medical University (1720/IRB-HMU) and informed consent has been obtained from the subjects in this study.

2.5 | Statistical analyses

Data analyses were performed using Statistical Package for the Social Sciences (SPSS) version 10.0. Student's *t* test and the Mann-Whitney *U* test were used for comparison of continuous variables (age). We used either the Chi-square test (for sample sizes of >5) or Fisher's exact test (for sample sizes of ≤5) for discrete variables (gender, prevalence of laboratory tests and symptoms). *P* values <.05 were considered to be statistically significant. The antisynthetase group was compared to the anti-Mi-2, the anti-SRP, the anti-MDA5 autoantibody positive groups and the seronegative groups respectively. Although correction for multiple testing would be appropriate for our study, we present raw *P* values, but consider possible type I errors when making the conclusions.

3 | RESULTS

Twenty-two of the 23 aaRS autoantibody positive patients had 1 single MSA and one patient had 2 MSAs, anti-EJ and anti-Mi-2. The most commonly detected anti-aaRS antibody was anti-Jo-1 (n = 13), followed by anti-EJ (n = 6) and anti-PL-7 (n = 4). Anti-PL-12 and anti-OJ were not present in any case. Demographic and clinical data are summarized in Table 1. A higher prevalence of anti-aaRS autoantibodies was observed in patients with DM (n = 14) than in PM (n = 9) whereas in the seronegative group PM (n = 44) was more common than DM (n = 25). Clinical features and laboratory characteristics in patients with anti-aaRS autoantibodies (Jo1, PL-7 and EJ) are presented in Table 1. All 23 anti-aaRS autoantibody positive patients fulfilled the criteria for ASS.

The anti-aaRS positive group did not differ from the seronegative patients with IIM or from the patients with 1 of the other myositis-specific antibodies concerning age, gender or mean disease duration at time of investigation. At time of investigation anti-aaRS-positive patients had higher frequencies of fever, arthritis, ILD, pericarditis and raised CRP compared to the seronegative patients. The anti-aaRS-positive patients also had higher frequency of arthritis compared to the anti-SRP-positive patients and higher frequency of ILD compared to the anti-Mi-2 positive patients (Table 1). A higher frequency of arthritis compared to the seronegative group was also recorded in the anti-MDA5 positive patients (Table 1).

Next, we compared disease activity applying the MDAAT score of patients with anti-aaRS autoantibodies to the seronegative comparator group and to the patients with each of the other myositis-specific antibodies. The anti-aaRS autoantibody positive group had a higher disease activity in the domains skin and pulmonary disease compared to the seronegative group but had lower disease activity in skeletal disease compared to the anti-MDA5-positive patients (Table 2). The anti-MDA5-positive patients also had a higher disease activity in the cutaneous disease compared to the seronegative group (Table 2). At time of investigation the mean CK level of patients with anti-aaRS autoantibodies was 3019 U/L and for the seronegative group 1565 U/L, but the difference was not statistically significant. Additionally the patients with anti-SRP autoantibodies

TABLE 2 Disease activity assessment at time of investigation of 23 patients with anti-aminoacyl-transfer RNA synthetase (aaRS) autoantibodies compared to patients with anti-SRP, anti-Mi2 or anti-MDA5 autoantibodies and autoantibody negative patients with idiopathic inflammatory myopathies (IIM)

Feature, mean ± SD	Antisynthetase n = 23	Anti-SRP n = 17	Anti-Mi-2 n = 8	Anti-MDA5 n = 11	No antibody n = 69
Constitutional disease activity, VAS (max score = 10)	5.6 ± 2.6	5 ± 2.4	5.9 ± 2.9	5.6 ± 2.5	4.8 ± 2.8
Cutaneous disease activity, VAS, mean ± SD (max score = 10)	**4.0 ± 3.5**[†]	2.9 ± 3.2	4 ± 4.0	**5.8 ± 3.2**[#]	2.3 ± 2.9
Muscle disease activity, VAS, mean ± SD (max score = 10)	5.5 ± 2.6	5.5 ± 2.5	6.9 ± 3.0	5.6 ± 2.5	5.1 ± 2.6
Skeletal disease activity, VAS (max score = 10)	1.9 ± 2.9	1 ± 2.2	1.9 ± 2.3	**4.4 ± 3.9***	1.8 ± 3.1
Gastrointestinal disease activity, VAS (max score = 10)	2.6 ± 3.0	2.3 ± 2.7	2.9 ± 3.9	2.9 ± 2.4	2.3 ± 2.8
Pulmonary disease activity, VAS, mean ± SD (max score = 10)	**3.7 ± 3.5**[§]	2.0 ± 1.8	3.5 ± 3.2	2.5 ± 1.8	2.4 ± 2.7
Cardiovascular disease activity, VAS (max score = 10)	1.3 ± 2.2	0.5 ± 1.1	1.4 ± 1.6	0.9 ± 1.6	0.48 ± 1.2

Abbreviations: IIM, idiopathic inflammatory myopathy; NS, not significant; SD, standard deviation; VAS, visual analog scale.
[§]$P < .05$ compared to antibody negative group (negative against all investigated antibodies). [†]$P < .01$ compared to antibody negative group. *$P < .05$ compared to anti-aaRS antibody positive group. [#]$P < .0005$ compared to antibody negative group.

or anti-Mi-2 had significantly higher serum levels of muscle enzymes compared to the seronegative patients (Table 1). Seventy-four percent of patients with anti-aaRS autoantibodies had raised CRP compared to 42% in the seronegative group.

Finally, we compared the cumulative presence of clinical manifestations between the 3 aaRS autoantibody positive groups, anti-Jo1, anti-EJ and anti-PL7 (Table 3). Pulmonary hypertension was present in 69%, 25% and 50% of the anti-Jo1, anti-PL7 and anti-EJ positive patients respectively and ILD in 46%. 75%, and 50% of the anti-Jo1, anti-PL7, and anti-EJ positive patients respectively. These differences were not statistically significant.

No association between anti-aaRS-positivity or the other autoantibody defined subgroups and HLA-DRB1 haplotypes was detected in our study. The frequencies of HLA-DRB1 haplotypes are presented in Table 4.

Two patients with anti-aaRS autoantibodies (anti-Jo1 and anti-EJ antibodies), 1 anti-MDA5 positive patient, 1 anti-Mi-2 positive patient and 3 seronegative patients died during the follow-up. Cause of death was rapidly progressive ILD (RPILD) in the 2 anti-aaRS positive patients and in the seronegative group RPILD in 1, infectious pneumonia in 1 and vasculitis and infection in a 3rd case. The patient with anti-MDA5 autoantibodies died due to infectious pneumonia and the patient with anti-Mi-2 autoantibodies died due to myocarditis, pulmonary hypertension and RPILD.

4 | DISCUSSION

In our cohort of Vietnamese patients of Kinh ethnicity with anti-aaRS autoantibodies, anti-Jo1 was the most frequent autoantibody, followed by anti-EJ and anti-PL7 autoantibodies. Furthermore, aaRS autoantibodies were more common in patients within the subgroup DM compared to the PM subgroup. The aaRS autoantibody positive patients had a high frequency of extra-muscular manifestations and all fulfilled the proposed criteria for ASS.

That anti-Jo1 autoantibodies were the most common of anti-aaRS autoantibodies in our cohort is similar to what has been reported from other cohorts with different ethnicities. However, anti-EJ antibodies being the second most common anti-aaRS autoantibodies is different from reports in Caucasian and Japanese cohorts, although our data need to be interpreted with caution due to the low number of patients. Antisynthetase autoantibodies constitute an important risk factor for ILD in patients with IIM. ILD frequently predominates at presentation and contributes to the high morbidity and mortality in patients with ASS and was one of the most common extra-muscular manifestations in our patients with ASS found in 51%, a frequency comparable to what has previously been reported in other ethnic groups.[22-24] Similar to other ethnic groups, the frequency of ILD was in particular high in patients with anti-PL7 autoantibodies where 3 of our 4 cases had ILD.[12,25] All of our patients with anti-aaRS autoantibodies had signs of myositis, thus there was no difference between anti-aaRS antibody specificities, which was unexpected and different from previous reports.[26,27] Our data on clinical associations with different antisynthetase autoantibodies in Asian patients of Kinh ethnicity differ from a previous report from Japan with 165 patients having anti-aaRS autoantibodies.[28] Myositis was found in less than 60% of Japanese patients during follow-up and the frequency of myositis varied among anti-aaRS subgroups. The patients with anti-Jo1, anti-EJ and anti-PL7 autoantibodies had a higher frequency

TABLE 3 Clinical manifestations at time of investigation in patients subgrouped by the 3 different aminoacyl-transfer RNA synthetase autoantibodies

Characteristic	Anti-Jo-1 (n = 13) n (%)	Anti-PL-7 (n = 4) n (%)	Anti-EJ (n = 6) n (%)	*P* value
Age, mean ± SD y	42.7 ± 14.6	39 ± 8.3	57 ± 9.4	NS
Diagnosis, PM/DM	7/6	1/3	1/5	NS
Constitutional manifestations n (%)				
Fever	8 (61.5)	4 (100)	5 (83.3)	NS
Skin lesions n (%)				
Heliotrope rash	4 (66.7)	3 (100)	3 (60)	NS
Gottron's papules	5 (83.3)	1 (33.3)	3 (60)	NS
Mechanic´s hands	1 (16.7)	0 (0)	1 (20)	NS
Skeletal manifestations n (%)				
Arthritis	7 (53.8)	2 (50)	3 (50)	NS
Myositis	13 (100)	4 (100)	6 (100)	NS
Muscle weakness	12 (92.3)	4 (100)	6 (100)	NS
Manual muscle test-8, mean ± SD	61 ± 16	52 ± 20	54 ± 27	NS
Dysphagia	7 (53.8)	2 (50)	4 (66.7)	NS
Raised SGOT	7 (53.8)	3 (75)	2 (33.3)	NS
Raised SGPT	6 (46.2)	3 (75)	3 (50)	NS
Raised lactic dehydrogenase	5 (38.5)	2 (50)	1 (16.7)	NS
CK, IU/L, mean ± SD, (ref; 26-140 IU/L)	3364 ± 6947.1	4966 ± 5230.7	972 ± 1777.1	NS
Cardiovascular manifestations n (%)				
Raynaud's phenomenon	5 (38.5)	2 (50)	1 (16.7)	NS
Pericarditis	4 (30.8)	2 (50)	1 (16.7)	NS
Pulmonary involvement n (%)				
Interstitial lung disease, PM/DM	6 (46) (4/2)	3 (75)	3 (50)	NS
Pulmonary hypertension	9 (69)	1 (25)	3 (50)	NS
Laboratory investigations				
Raised CRP, n (%)	10 (76.9)	3 (75)	4 (66.7)	NS
Disease activity assessment, mean ± SD				
Cutaneous disease activity, VAS (max score = 10)	3.2 ± 3.4	4.3 ± 3.1	5.7 ± 4.1	NS
Muscle disease activity, VAS (max score = 10)	5.1 ± 2.4	6.8 ± 2.2	5.5 ± 3.5	NS
Joint disease activity, VAS (max score = 10)	2.9 ± 3.1	0	1.2 ± 2.9	NS
Pulmonary disease activity, VAS (max score = 10)	3.4 ± 3.5	5.3 ± 2.5	3.2 ± 4.0	NS

Abbreviations: CK, creatine kinase; CRP, C-reactive protein; DM, dermatomyositis; SGOT, serum glutamic oxalo-acetic transaminase; SGPT, serum glutamate pyruvate transaminase; NS, not significant, reference values for CK = 26-140 IU/L; PM, polymyositis; SD, standard deviation; VAS, visual analog scale.

of myositis compared to anti-PL12, anti-KS and anti-OJ. In addition, ILD was found in nearly all Japanese patients with anti-aaRS autoantibodies, a strikingly higher frequency in comparison with our cohort.

Notably, pulmonary arterial hypertension (PAH) was equally frequent as ILD in our patients with anti-aaRS autoantibodies and was more often recorded in patients with anti-Jo-1 autoantibodies compared to patients with other aaRS autoantibodies although the difference was not statistically significant. The frequency of PAH in our cohort is higher compared to other populations where PAH was reported in 7.9%-14.8% of patients with anti-aaRS autoantibodies.[29,30] An explanation for the high frequency observed in our cohort may be because all patients were screened with echocardiography. Moreover, both anti-aaRS-positive and anti-MDA5-positive patients had a high prevalence of arthritis and in the anti-aaRS-positive patients the frequency of arthritis was higher compared to the anti-SRP-positive patients (52% vs 12%).

The high frequency of DM skin rash in our patients with anti-aaRS autoantibodies is in agreement with previous reports from Japan,[28,31] but different compared to a Chinese population in which the frequency of DM was lower.[32] This difference may indicate that other factors than the autoantibodies may influence the clinical

TABLE 4 Human leukocyte antigen cell surface receptor (HLA-DR)B1 alleles in Vietnamese patients with anti-aaRS autoantibodies, patients with anti-SRP, anti-Mi2 or anti-MDA5 autoantibodies and seronegative IIM

	Antisynthetase (n = 22)	Anti-SRP (n = 17)	Anti-Mi-2 (n = 8)	Anti-MDA5 (n = 11)	Seronegative IIM (n = 68)
HLA-DRB1*	n (%)	n (%)	n (%)	n (%)	n (%)
*01	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.5)
*03	5 (22.7)	2 (11.8)	0 (0)	0 (0)	6 (8.8)
*04	5 (22.7)	1 (5.9)	2 (25)	3 (27.3)	11 (16.2)
*07	1 (4.5)	0 (0)	1 (12.5)	0 (0)	4 (5.9)
*08	4 (18.2)	2 (11.8)	2 (25)	1 (9.1)	9 (13.2)
*09	2 (9.1)	4 (23.5)	0 (0)	2 (18.2)	14 (20.6)
*10	3 (13.6)	0 (0)	2 (25)	0 (0)	12 (17.6)
*11	0 (0)	2 (11.8)	0 (0)	0 (0)	4 (5.9)
*12	13 (59.1)	3 (17.6)	3 (37.5)	9 (81.8)	30 (44.1)
*13	2 (9.1)	2 (11.8)	2 (25)	1 (9.1)	7 (10.3)
*14	2 (9.1)	6 (35.3)	2 (25)	1 (9.1)	1 (1.5)
*15	4 (18.2)	7 (41.2)	1 (12.5)	0 (0)	20 (29.4)
*16	1 (4.5)	0 (0)	0 (0)	0 (0)	5 (7.4)

Abbreviations: IIM, idiopathic inflammatory myopathy.

*No significant differences were found between the groups.

phenotypes in anti-aaRS positive patients, where both environmental and genetic factors may contribute.[22] However, this question could not be addressed in our study with only 1 ethnic group.

The disease activity score in the pulmonary domain of the MDAAT score was higher at time of investigation in patients with ASS compared to patients without autoantibodies in our study. The patients with anti-PL-7 antibodies had the highest pulmonary activity score of the 3 anti-aaRS autoantibody positive subgroups. When we compared disease activity at time of investigation, the DM patients with anti-aaRS autoantibodies had more active disease in the skin domain compared to DM patients without autoantibodies. The patients with anti-aaRS autoantibodies have been reported to have higher disease activity compared to patients without autoantibodies.[33] In addition, patients with aaRS autoantibodies have poor prognosis with a high mortality rate due to ILD and severe myositis. However, patients with anti-Jo-1 antibodies often have a more favorable prognosis and lower mortality rate than patients with other aaRS autoantibodies.[12] In our cohort the occurrence of fever and CRP levels was also significantly higher in patients with anti-aaRS antibodies compared to the seronegative patients with IIM at time of investigation despite ongoing immunosuppressive treatment. The active disease at time of investigation was in some cases explained by a disease flare due to patient´s decision to stop treatment. This could possibly also explain the persisting muscle weakness at time of evaluation in some cases. However, another explanation for the in general severe manifestations observed in our cohort may be a selection of severe cases in this referral center but also that the patients had a delay before myositis diagnosis and thereby a delay in start of immunosuppressive treatment. Alternatively, patients in this ethnic group have a more severe disease compared to Caucasian patients. To address these questions comparative studies are needed. A selection bias may also affect the autoantibody pattern that we found. A clear limitation of our study is the low number of patients, making comparisons of the small subgroups with different aaRS autoantibodies unreliable.

In conclusion, Vietnamese myositis patients of Kinh ethnicity with anti-aaRS autoantibodies had a high frequency of extramuscular manifestations. Some differences in clinical phenotypes associated with anti-aaRS autoantibodies compared to previous reports were observed such as a high frequency of pulmonary hypertension supporting the need for careful surveillance of extramuscular organ manifestations including pulmonary hypertension in patients with anti-aaRS autoantibody positive IIM, at least in patients of the Kinh ethnicity.

ACKNOWLEDGEMENTS

Dr Ingrid Lundberg has received grants from The Swedish Research Council and grants provided by Region Stockholm (ALF project). Dr Thuy was supported by a research grant from APLAR in 2013 for doctors under the age of 40 years.

CONFLICT OF INTEREST

Dr Ingrid Lundberg has received research grants from Astra-Zeneca and Bristol-Myers Squibb and has served on the advisory board of Corbus Pharmaceuticals, Inc. The other authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Authors' contributions to the paper: Dr Thuy Nguyen Thi Phuong designed the study, collected data, wrote the first draft of the manuscript, performed 50% of the work; Dr Lan Nguyen Thi Ngoc was involved in study design 5%-10%; Dr Johan Rönnelid performed the autoantibody assay, 5%-10%; Dr Leonid Padyukov performed the

genetic analyses, 5%-10%; Ingrid E. Lundberg was involved in study design, discussing the results and writing the manuscript, 10%-20%.

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How to cite this article: Phuong TNT, Ngoc LNT, Rönnelid J, Padyukov L, Lundberg IE. Clinical characteristics of Vietnamese patients with idiopathic inflammatory myopathies and autoantibodies to aminoacyl-transfer RNA synthetases. *Int J Rheum Dis*. 2021;24:663–670. https://doi.org/10.1111/1756-185X.14105

Received: 21 September 2020 | Revised: 13 January 2021 | Accepted: 20 February 2021

DOI: 10.1111/1756-185X.14094

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases | WILEY

High cost of illness in fibromyalgia patients in Iran, irrespective of disease severity: A prospective cost study

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Funding information
The funding source of this study was provided by Guilan University of Medical Sciences.

Abstract

Aims: This study aimed to estimate the economic burden of fibromyalgia (FM) in 6 months, using a cost-diary, and to evaluate its relationship with the disease severity.

Methods: This is a prospective cost-of-illness study on 62 participants with an FM diagnosis within a 6 month period. Patients completed the questionnaires, including FIQR (Revised Fibromyalgia Impact Questionnaire) and SF-12 (12-item short-form survey). The cost-diary method was used to track the cost of the disease. The participants received six cost-diary booklets during the study period to report their FM-related costs, hours, and days of productivity loss. The final costs are reported in US dollars.

Results: Most of the participants were women (90.3%) with a mean (±SD) age of 40.80 (±5.50) years and a mean (±SD) FIQR score of 54.38 (±14.13). Moreover, 45.2% of patients fulfilled all six booklets, whereas 24.2% returned only one booklet. The participants showed a mean (±SD) direct healthcare, non-healthcare, and indirect cost of $ 2817.08 (±$ 1860.04), $ 1497.98(±$ 1358.21), and $ 1449.05(±$ 3637.41) per patient for 6 months, respectively.

Conclusion: Fibromyalgia is associated with high health-related and non-health-related costs in our country, irrespective of its severity. This study warrants urgent consideration in managing the disease burden on both patients and society.

KEYWORDS

cost diary, economic, fibromyalgia, Revised Fibromyalgia Impact Questionnaire, Short-form 12-item survey

1 | INTRODUCTION

Fibromyalgia (FM) with a global incidence of 2.7%-4.7%, is a poorly understood condition of widespread pain, fatigue, multiple somatic symptoms, and associated comorbidities.[1,2] Accumulating data suggest that FM incurs a high clinical and economic burden on both patients and societies, comparable with other chronic diseases such as diabetes and hypertension. The substantial burden of this condition may be caused by several factors, such as the exhausting challenges in diagnosis and management of patients, presence of various comorbidities, low quality of life, and marked social invalidation.[3,4]

Patients with FM often have a long journey to correct diagnosis and management. They undergo multiple visits, numerous investigations, and treatments, even after being diagnosed with FM, mainly because of the patient's and physician's dissatisfaction with their consultations. There is no reference standard of diagnosis or

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[Correction added on 03 April 2021, after first online publication: In the authors list, Banafshesh Gavidel-Parsa name has corrected to Banafshesh Ghavidel-Parsa].

effective targeted treatment for FM, mainly because of the heterogeneous clinical and biological properties of the condition.[5,6] In the absence of a definite route for diagnosis and treatment, the presence of multiple disabling symptoms despite normal appearance and clinical findings could lead to misunderstanding and rejection of patients by their social environment, including medical caregivers. The presence of concomitant comorbidities could add more complexity to judgment by observers. This scenario results in a tremendous clinical, social, and economic burden. As expected, the benign appearance of FM could place governments and societies into a deep trap of incurred clinical and social burden because of the "iceberg" nature of FM, which is representative of hidden burden of this illness [4,7,8]

Notably, FM patients report far greater use of direct healthcare and non-healthcare resources, regardless of the initial FM diagnosis. Direct healthcare costs are defined as the medical supplies and services used by the patients.[9] Outpatient physician visits, prescribed medications, physiotherapy, and complementary medicine are among the most common examples of direct healthcare costs used by FM patients.[4] Direct non-healthcare costs comprise non-medical goods, and resources expended by the patients and their families somewhere out of the healthcare services, such as the costs due to transportations for reaching the clinics, over-the-counter medications, and seeking help for housekeeping or caregivers.[9] In addition to direct costs, indirect cost is the hidden aspect of the high economic burden of chronic disorders such as FM. It is related to the transient or permanent loss of productivity and the need for sick leave. The indirect cost could be estimated by the acquisition of hours/days of absence in the workplace for employed patients as well as the inability to fulfill household chores or other unpaid works for unemployed patients.[4]

It has been estimated that the mean annual measurable cost per FM patient ranges from $ 2274 to $ 9573 (US dollars) or even more than approximately $ 13 000 in various studies depending on the severity of symptoms and the route of cost calculation. However, the vast majority of these studies were conducted retrospectively using questionnaires,[10,11] insurance databases,[12,13] and medical files.[11,14-16] Although retrospective studies are safe from patient recall errors and result in larger study populations, they may lack a complete collection of direct costs and probably have no coverage of indirect costs.[17-19]

It is believed that a self-report or interview-based method provides more reliable and detailed results in cost-of-illness studies.[17,19] Questionnaires and diaries are among the proposed forms of the self-report-based method, both with their pros and cons.[17] While questionnaires are dependent on the patients' memory, diaries can precisely record events and costs.[20]

Minimal efforts have been made until now to study the diary method and its feasibility in the cost studies of chronic diseases. On the other hand, prospective data, addressing the relation of direct and indirect costs to FM severity, are scarce and conflicting. The present study aimed to prospectively evaluate the direct healthcare and non-healthcare costs and productivity losses among Iranian FM patients in a diary-based method. We also aimed to study the feasibility of the cost-diary method among our patients. Furthermore, we sought to analyze the relation of direct and indirect costs by FM severity level.

1.1 | Study design and patient selection

This is a 6 month prospective study on 95 patients with FM syndrome referred to the FM outpatient clinic of Razi Hospital, Rasht between July 2018 and August 2019. Patients who were included in this study were older than 18 years of age and fulfilled the 2016 American College of Rheumatology classification criteria for FM. The diagnosis of all participants was confirmed by one rheumatologist (BGH), an expert in the diagnosis and management of FM. Patients were excluded if they were under 18 years of age or had a systemic inflammatory rheumatic disease, cultural or educational barriers to cooperate, known confounding medical illness (such as malignancy and disabling medical condition) at the time of enrollment, and any non-rheumatic cause of pain.

Demographic data (including age, sex, occupation, income, hours of paid work, and educational level) were obtained from all participants. A contact telephone number was also obtained to remind patients of their appointments. All the patients were asked to fill out the Revised Fibromyalgia Impact Questionnaire (FIQR), Short-Form 12-item Health Survey (SF-12) at the initial visit. An experienced medical assistant offered help if patients did not understand the meaning of the questions.

To calculate the sample size, we used the formula of $N = (Z_{\alpha/2})^2(SD)^2/d^2$,[21] with 95% confidence intervals and a level of significance of 5% and $Z_{\alpha/2}$ of 1.96. The standard deviation (SD) and error of margin (d), were obtained from the study by Lacasse et al,[19] which had 57 participants. The final sample size was 95 patients with 15% dropout.

Written informed consent was obtained from all participants. The study complied with the Declaration of Helsinki and was approved by the local ethics committee (IR.GUMS.REC.1398.366).

2 | INSTRUMENTS

2.1 | Revised Fibromyalgia Impact Questionnaire (FIQR)

The validated Persian version of the FIQR was used as an instrument assessing the disease severity of FM.[22] It contains 21 individual questions. All questions are based on an 11-point numeric rating scale of 0-10 with "10" denoting the “worst”. This is divided into three sets of domains: function, overall impact, and symptoms. Then, the total FIQR scores would be the sum of the three modified domain scores.

2.2 | Short-Form Health Survey (SF-12)

Health status was assessed by the validated Persian version of SF-12, including eight dimensions: physical functioning, physical role, social role, emotional role, bodily pain, general health, vitality, and mental health. The scores range from 0 to 100, and lower scores indicate worse possible conditions.[23]

2.3 | Cost diary

Cost diary is an instrument, developed and partially validated by Goosens et al[17] and used for the cost estimation of a condition. Goosens et al[17] found that there is no significant difference in the patients' reports and medical records. Therefore, the cost diary could be used without the need for checking medical reports. To decrease recall bias, we chose a 1 month period between the visits.

All participants were instructed in both oral and written form to fill out the six booklets, each covering 1 month. Patients were supposed to write down the number of physician visits, their chief complaint, purchased medication (whether prescription drugs or over the counter), and all other expenses related to the condition.

Each patient attended seven face-to-face interviews—one initial visit to obtain demographic data and baseline questionnaires, and six visits to deliver the completed booklets and receive the next one. The response rate was calculated as the proportion of completed diaries to the total number of diaries (six for each patient). To minimize the partial responses and missing information, we also discussed the completed diaries at each visit. Furthermore, patients were asked to bring their purchased medicine and disease-related bills. Before each visit, telephone contacts were made to remind patients of their upcoming appointment. If patients decided to quit the study, the interviewers kindly asked them to bring the booklet as soon as possible. For those who returned at least one but less than six booklets, the mean value over the available booklets was calculated and extrapolated to 6 months.

2.4 | Estimates of cost

Resource use and associated cost were estimated by diary records. Medical resources and para-clinical tests (laboratory and imaging tests) were included only if the physician (BGH) approved their relevance to FM or its comorbidities. Direct healthcare costs (including visits to general practice, specialist care, physiotherapy, hospitalization, prescribed medications, and para-clinical tests) were estimated by the reported number of each healthcare utilization, multiplied by the contemporary tariff of the Mministry of Health. The contemporary tariff is determined for each healthcare service annually by the Ministry of Health and the tariff is different for public and private services. In this study we used the tariff of private services. If the patients had paid more than the tariff, the recorded costs in the diary were calculated. Direct non-healthcare costs (including over-the-counter drugs, alternative medicines, transportation, hours of paid and unpaid household help, and health activities) were calculated based on the real costs reported by the patient in the dairy. The cost of unpaid household help was assessed by the number of reported visit days multiplied by the lowest daily wage of the Ministry of Labor. No transportation cost was calculated if the patients had used personal cars for transportation. Indirect costs (defined as the value of productivity loss due to disease-related absence) were calculated by the friction cost method, which is defined as the number of days or hours that the patient did not attend or left work due to illness unless their position was replaced by another employee.[24] The daily wage of the Ministry of Labor was used for the households and those patients who avoided revealing their income. The costs were recorded in Rial and converted to US dollars at the 2017 gross domestic product-based purchasing power parity of 9035.96 : 1 (Rial : US$).

2.5 | Statistical analysis

Descriptive statistics were used to calculate the mean and standard deviation for continuous variables, and the frequency and percentage for categorical variables.

Linear regression analysis was performed to test whether or not the patient characteristic (including age, level of education, disease duration, and marital status) and severity of the condition were associated with the response rate and the costs. To compare the means of costs between different severity groups of FIQR, the one-way analysis of variance test, and Brown-Forsythe test were used for homogeneous and non-homogeneous data, respectively. To measure the effect size, η-squared was used.[25]

Levene's test was also applied to evaluate the homogeneity of variance between the groups.

Statistical calculations were performed using the SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, NY, USA), and statistical significance was evaluated at the level of 0.05.

3 | RESULT

3.1 | Patient demographics and clinical characteristics

A total of 95 patients with a diagnosis of FM were enrolled during the study period. From these, 62 patients fulfilled at least one cost-diary booklet and joined the statistical analysis. In total, 42.5% of diaries were returned. Thirty-three (34.7%) patients did not return the first cost-diary booklet so were excluded from the study. Among those who returned at least one booklet, 45.2% returned the full set of booklets. The response rate among the participants was calculated based on the number of returned completed booklets.

Patients had a mean (±SD) age of 40.80 (±5.5) years and they were predominantly from the 31-44 years age group (64.5%). The vast majority of patients were female (90.3%). The demographics and clinical characteristics of eligible participants were described in Table 1. There was no particular patient characteristic that predisposed patients to higher cost except for marital status. It was shown that married patients tended to have a lower cost compared with single patients ($P = 0.03$).

Antidepressant drugs were the most prescribed drugs, and 95.1% of patients used them to alleviate their symptoms. There was no breakdown of antidepressants as the majority of patients were

prescribed Duloxetine. Most (82.2%) patients also used supplementary drugs. Only 3.2% of patients used one class of drugs whereas the majority (56.5%) used four or more classes of drugs (Table 2).

3.2 | Direct and indirect costs estimation during 6 months follow up

The costs related to the use of healthcare services, non-healthcare services, and loss of productivity are shown in Table 3. Direct healthcare costs during the 6 months were estimated at $ 174 658.7 in total and a mean (±SD) of $ 2817 (±$ 1860) for each patient. The para-clinical tests (laboratory and imaging tests) with a total cost of $ 53 105.74 were the predominant compartment of direct healthcare costs (30.40%). No hospitalization costs were reported during the study, so the total costs of physician visits were attributed to outpatient visits including a total cost of $ 44 617.27 for specialist visits versus $ 2371 for general physician visits, implying the tendency of patients with FM to visit physicians with different specialties. The prescribed drugs appropriated 11.47% ($ 20 045.54) of total direct healthcare-related costs with the predominance of antidepressant drugs, and supplements with a mean (±SD) of $ 109.63 (±$ 187.61) and $ 99.83 (±$ 223.55), respectively (Table 3).

Direct non-healthcare costs were estimated at a mean of $ 1497.98 (±$ 1358.22) for each patient and $ 84 209.87 in total, which was almost two times lower than total direct healthcare costs. The nursing cost was the main compartment of the direct non-healthcare cost (72.18%). Costs related to exercise and activities (gyms and pool fees) were estimated at $ 6398.38 in total. Alternative/complementary medicine costs were mostly related to herbs.

The indirect costs accounted for almost 25% of total costs, which were comparable to the direct non-healthcare costs, which was 24% of total costs.

3.3 | Components of indirect costs

Unemployed patients had a mean (±SD) missed hours of 83.04 (±107.94) hours, whereas missed hours in employees were 59.88 (±307.58) hours. Total days of sick leave were 138 days during the 6-month period for all the employees (Table 3).

3.3.1 | Analysis of costs by the disease severity

There was a trend for higher direct and indirect costs in the mild severity group with a mean of $ 3126.75 (±$ 2264.97), and $ 4833.76 (±$ 3903.93) per patient, for indirect and direct costs, respectively. However, the differences among the severity groups were not statistically significant with *P* values of 0.413, 0.058, 0.549, and 0.202 for direct healthcare, direct non-healthcare, total direct, and indirect costs, respectively. The η-squared of severity was just 0.03 for direct healthcare, 0.09 for direct non-healthcare, 0.05 for indirect costs,

TABLE 1 Patients' characteristics

Characteristic (n = 62)	Value
Age, mean (SD), years	40.80 (5.50)
Age groups, n [%], years	
≤30	4 [6.5%]
31-44	40 [64.5%]
≥45	17 [27.4%]
Sex, n [%]	
Female	56 [90.3%]
Male	6 [9.7%]
Education, mean (SD), years	12.39 (14.64)
Marital status, n [%]	
Married	51 [82.3]
Non-married	11 [17.7]
Employment status, n [%]	
Non-employed	45 [72.6%]
Employed	17 [27.4%]
FIQR score, mean (SD)	54.38 (14.13)
Disease severity (FIQR), n [%]	
Mild	11 [17.7%]
Moderate	30 [48.4%]
Severe	21 [33.9%]
SF-12 score, mean (SD)	
Mental component	43.18 (9.73)
Physical component	34.88 (8.36)
Time to diagnosis, mean (SD), month	53.58 (64.27)
Disease duration, n [%]	
≤1 month	4 [6.5%]
2-12 month	9 [14.5%]
>12 month	49 [79%]
Interval between the diagnosis and study entry	
≤1 month	35 [56.5%]
2-12 month	14 [22.6%]
>12 month	13 [21%]
Number of physicians visit per patient, mean (SD) [% of patients]	
General physician	1.21 (2.42) [34%]
(Sub)Specialist	
Internist	0.64 (0.30) [4%]
Rheumatologist	11.12 (4.1) [100%]
Endocrinologist	0.2 (0.66) [13%]
Neurologist	0.35 (1.1) [13%]
Gastroenterologist	0.06 (0.31) [4%]
Gynecologist	0.06 (0.30) [4%]
Urologist	0.01 (0.12) [1%]
General surgeon	0.01 (0.12) [1%]
Ophthalmologist	0.80 (0.32) [4%]
Otolaryngologist	0.03 (0.25) [1%]

(Continues)

TABLE 1 (Continued)

Characteristic (n = 62)	Value
Orthopedics	0.04 (0.38) [1%]
Pain medicine	0.01 (0.12) [1%']
Response rate, n (% of patients)	
1/6	15 [24.2%]
2/6	6 [9.7%]
3/6	8 [12.9%]
4/6	3 [4.8%]
5/6	2 [3.2%]
6/6	28 [45.2%]

Note: The sum of Age groups percentage might not equal 100% due to missing data (n = 1).

Abbreviations: FIQR, Revised Fibromyalgia Impact Questionnaire; SD, standard deviation; SF-12, 12-item short-form survey.

TABLE 2 Frequency of prescribed medications

Medication type	Number (%) of patients
Anti-depressant	19 063 (95.1%)
Supplementary	5079 (82.2%)
Anti-epileptic	5071 (46.7%)
Analgesic	4383 (17.7%)
Sedative/Hypnotic	980 (17.7%)
Gastrointestinal	1359 (19.62%)
Corticosteroid	86 (9.6%)
Anti-migraine	300 (3.2%)
Miscellaneous	4513 (54.7%)
One of the above	2 (3.2%)
Two of the above	8 (12.9%)
Three of the above	17 (27.4%)
Four or more of the above	35 (56.5%)

and 0.02 for total direct costs. The calculated effect size was small according to Tatsuoka et al,[26] showing that despite the sample size, severity may not be a significant indicator of costs in our population (Table 4).

3.4 | Analysis of costs by the interval between the diagnosis and the study entry

The diagnosis of FM was confirmed in more than half of our included patients within less than 1 month before the study enrollment (56.5%). These patients, who were newly diagnosed, had more direct healthcare ($ 3248.92 ± $ 2271.46), direct non-health related ($ 1403.33 ± $ 1614.11), and indirect costs ($ 1825.42 ± $ 4724.88). However, this difference was not statistically significant ($P > 0.05$; Table 5).

3.5 | Response rate determinants

No single factor was significantly associated with a higher response rate, except for the function domain of FIQR ($P = 0.04$). It was shown that the response rate rose with the increase of age, disease duration, FIQR total score, but this relationship was not statistically significant. After using dummy coding for marital status, the response rate decreased when the patients were single, divorced, or widowed (Table 6).

3.6 | Excluded patients

Of 95 patients at the beginning of the study, 33 patients did not return any booklet and were excluded from the study. The dropout group of patients had a mean age of 47.3 ± 8 years, and mean years of education of 10.4 ± 3.4 years. 81.8% of them were married, and 72.8% were not employees. The mean FIQR in this group was 55.0 ± 14.9, and the mean mental and physical components of SF-12 were 44.5 ± 9.5 and 34.0 ± 7.8, respectively. There was no significant difference in baseline characteristics between this dropout group and the patients that completed the study protocol.

4 | DISCUSSION

In our prospective study, we used a cost-diary method to estimate the costs and pattern of healthcare utilization among patients with FM in the north of Iran. Our results demonstrated the substantial direct and indirect costs associated with FM in our population, which were not related to the severity of this condition. We also found that the cost-diary method was acceptable, and feasible for use in our population compared with the original study.[17]

Consistent with other studies, our study revealed that FM patients are among high users of healthcare resources, non-healthcare resources, and patients with poor physical and mental health status, which could lead to absenteeism and disability (Figure 1). Our FM patients had $ 4175.29 ± $ 2383.99 in total direct costs per patient for 6 months. This cost was high compared with another study in Iran, which estimated the total direct costs of $ 170.7 ± $ 107.9 annually. This discrepancy can be related to the method of currency exchange, because we used purchasing power parity instead of the exchange rate used by the other study. Our result was also more comparable with the annual cost of the condition in the USA, which was around $ 7973 ± $ 7341 and higher than Germany and France ($ 2234 ± $ 2641, $ 924 ± $ 862), respectively.[27] The most expensive components of the direct healthcare-related costs in our population were para-clinic costs ($ 856.54 ± $ 813.40 for 6 months per patient) and specialist visit costs ($ 719.63 ± $ 263.76 for 6 months per patient). The dissatisfaction of FM patients and their physicians with the FM diagnosis and management alongside the direct and feasible access to specialists in our country are thought to be responsible for multiple visits, extensive investigations, and consequently

TABLE 3 Direct and indirect costs

	Mean (SD) costs per patient, US $	Total costs, US $
Direct healthcare costs	2817.08 (1860.04)	174 658.7
Physician visit costs		
General physician	38.24 (73.15)	2371.08 [1.35%]
(Sub)Specialist	719.63 (263.76)	44 617.27 [25.54]
Physiotherapy	37.93 (209.83)	2351.71 [1.34%]
Para-clinic costs		
Laboratory	444.62 (456.80)	27 566.65 [15.78%]
Imaging	411.92 (740.75)	25 539.09 [14.62%]
Total prescribed drugs		
Anti-depressant	109.63 (187.61)	6796.84 [3.89%]
Supplementary	99.83 (223.55)	6189.51 [3.54%]
Anti-epileptic	40.05 (72.94)	2482.98 [1.42%]
Analgesic (NSAID)	24.38 (34.94)	1511.36 [0.86%]
Analgesic (others)	3.09 (11.29)	191.49 [0.10%]
Analgesic total	27.47 (35.57)	1702.85 [0.97%]
Sedative/Hypnotic	5.10 (22.84)	316.32[0.18%]
Gastrointestinal	0.87 (2.73)	54.50 [0.03%]
Corticosteroid	0.78 (3.03)	48.41 [0.02%]
Anti-migraine	2.75 (15.46)	170.65 [0.09%]
Miscellaneous[a]	25.19 (47.15)	1561.92[0.89%]
Direct non-healthcare costs	1497.98 (1358.22)	84 209.87
OTCs	28.68 (95.03)	1778.45 [1.91%]
Assistive devices	20.87 (91.47)	1293.72 [1.39%]
Alternative medicines	24.55 (73.31)	1521.88 [1.63%]
Exercise	103.20 (229.95)	6398.38 [6.88%]
Unpaid household help	902.07 (1389.15)	55 928.43[60.21%]
Paid household help	179.39 (507.61)	11 122.22 [11.97%]
Transportation	99.46 (125.86)	6166.78 [6.63%]
Indirect cost	1449.05 (3637.41)	89 841.00
Missed, h		
Nonemployee(housewives)	83.04 (107.94)	5148.75
Employee	6.36 (24.38)	394.50
Employee absenteeism, d	2.23 (11.80)	138.00

Note: Data are presented as mean (SD) [% of total cost].

Abbreviation: NSAIDs, non-steroidal anti-inflammatory drugs; OTC, over-the-counter drugs; SD, standard deviation; US$, US dollars.

[a]Miscellaneous drug groups include anti-Parkinsonism drugs, drugs for joint and bone health, gastrointestinally associated drugs, and others.

TABLE 4 Comparison of costs between different Revised Fibromyalgia Impact Questionnaire severity groups

Type of cost	Mild	Moderate	Severe	*P* value	η^2
Direct healthcare cost	3126.75 ± 2264.97	3011.09 ± 2127.92	2377.68 ± 1053.35	.410	0.03
Direct non-healthcare cost	1707.01 ± 2098.23	894.91 ± 966.36	1837.39 ± 1630.53	.058	0.09
Total direct cost	4833.76 ± 3903.93	3906.01 ± 2138.91	4175.29 ± 1652.72	.540	0.02
Indirect cost	3131.95 ± 8123.34	832.53 ± 1507.32	1448.26 ± 1391.79	.202	0.05

Note: Data are presented in US dollar and as mean ± standard deviation. η^2 represents eta-squared. *P* value <0.05 is considered statistically significant.

TABLE 5 Comparison between costs in different time intervals after the diagnosis

Type of cost	≤1 month	2-12 months	>12 months	*P* value	η^2
Direct healthcare cost	3248.92 ± 2271.46	2501.23 ± 959.79	1994.53 ± 759.660	.088	0.07
Direct non-healthcare cost	1403.33 ± 1614.11	1278.03 ± 1290.32	1323.13 ± 1485.38	.962	0.00
Total direct cost	4652.25 ± 2778.74	3779.27 ± 1495.66	3317.66 ± 1716.56	.178	0.05
Indirect cost	1825.42 ± 4724.88	1079.77 ± 1389.55	833.40 ± 963.76	.648	0.01

Note: Data are presented in US dollars and as mean ± standard deviation. η^2 represents eta-squared. *P* value <0.05 is considered statistically significant.

TABLE 6 Relationship between response rate and patients' characteristics

	P value	*B* coefficient[a]
Age, year	.596	0.016
Level of education	.462	−0.026
Disease duration, year	.217	−0.068
Marital status[b]	.083	−1.245
FIQR (total score)	.320	0.019
FIQR (function domain)	.044*	0.029
FIQR (overall domain)	NS	0.002
FIQR (symptom domain)	NS	0.002
SF-12 (physical health)	NS	0.005
SF-12(mental health)	NS	0.007

Abbreviations: NS, not significant; RFIQ, Revised Fibromyalgia Impact Questionnaire; SF-12, 12-item short-form health survey.

*P value <0.05 is considered statistically significant.

[a]B stands for regression coefficiency and its positive values represent direct correlation with response rate and vice versa for negative values.

[b]For marital status, dummy coding was used and higher values represent married status.

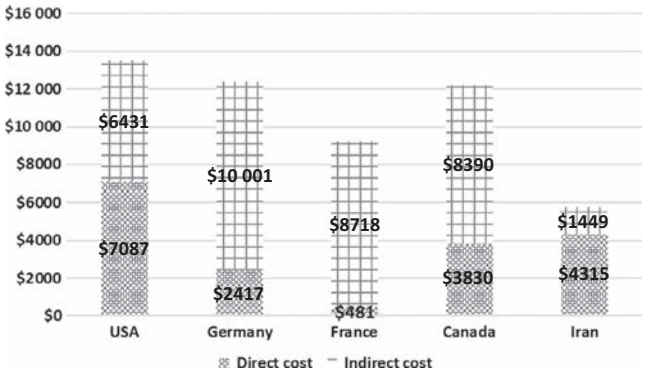

FIGURE 1 Direct and indirect costs associated with fibromyalgia in different countries. Values for USA, Germany, and France are in 2009 US dollars and values for Canada and Iran are in 2001 and 2017 US dollars, respectively

high costs. In contrast, Europe and Canada benefit from the referral system, which may lower the unnecessary costs ($ 564 ± $ 262, $ 297 ± $ 200, and $ 390.28 ± $ 465.6 in Germany, France, and Canada, respectively, for annual physician visit costs) (Figure 1).[19,27] The most frequent visits were made to rheumatologists and endocrinologists, which is understandable due to pain being the main symptom, and the presence of concomitant thyroid disease in a considerable percentage of our patients. The prevalence of thyroid diseases in FM patients might be related to similar symptoms and frequent checking of thyroid function tests in FM patients, which results in higher detection of thyroid disease in these patients.

Interestingly, the total costs for prescribed drugs were lower than the costs for physician visits. Moreover, para-clinic test costs were higher than the physiotherapy costs. These results are probably due to the "doctor shopping behavior" of the FM patients who are looking for satisfaction and reassurance about disabling and invalidated multiple symptoms.

Although the total costs of prescribed drugs were lower than the other direct healthcare cost units, antidepressant medications were consumed in 95.1% by the patients. Such results may be related to the initial prescription of antidepressants by the rheumatologist at the time of enrollment for management of the illness in our population. Additionally, the lower costs of psychotherapy could be justified by the higher tariff and lack of insurance support in our healthcare system. Unsurprisingly, in our study, 56.5% of patients used four or more classes of drugs, and only 3.2% used a single medication. Although polypharmacy is common in FM, evaluating its efficacy is in question. It is believed that over time and due to the variation of symptoms in FM, multiple medications with the same or different mechanisms may be prescribed for patients.[28] Altogether, this pattern of health care, which leads to exhaustion of healthcare providers and invalidation of patients, seems worrisome and must be addressed when setting priorities for the future effectiveness of healthcare services.

Another crucial component of the direct costs in this study was non-healthcare resources utilization. Although it was lower than the direct healthcare costs in total, unpaid household help as the main component of non-healthcare costs incurred substantial costs ($ 902.07 ± $ 1389.15 for 6 months per patient), which was comparable to the visit or para-clinic units of direct healthcare costs. This cost was not directly paid in cash by the patients; however, its economic burden both on patients and society is undeniable. Paid and unpaid household help was found to be the most important resource use among all directly related costs. This is congruent with the study by Boonen et al,[29] which used the cost diary in the evaluation of FM patients' resource utilization. The high costs on this issue

indicate the marked impact of FM on the function and daily life of our patients.

Over-the-counter drugs were used by 37% of our patients, and incured relatively lower cost compared with the other components of direct costs ($ 28.68 ± $ 95.03 for 6 months per patient). Moreover, the acceptable costs for the exercise and activity unit in the non-healthcare costs could be attributed to the tertiary level of care; as after the confirmation of FM diagnosis by the rheumatologist, all patients were encouraged to do graduated exercise at least three times a week.

As shown in many studies, fibromyalgia has a considerable effect on loss of productivity. Most employed patients lose their job due to disability; others who attend their job have a notable decrease in productivity.[30] In a study conducted by Lacasse et al[19] in 2016 in Canada, employed and unemployed patients had a loss of productivity equivalent to 3.19 and 14.34 weeks per year, respectively. As a result, the indirect costs of FM are notably higher compared with many other diseases. Boonen et al[29] found that FM and chronic low back pain had indirect costs of $ 2573 and $ 2939 per year, respectively. These results were higher than $ 834 in ankylosing spondylitis. In our study, the means of the productivity loss for each patient were equivalent to 59.88 and 83.04 hours during 6 months for employed and unemployed patients, respectively. The Indirect costs consisted of 25% of the total cost in our study, which was lower than the previous studies. Knight et al[27] showed that indirect cost accounted for 57%, 91%, and 77% in the USA, France, and Germany, respectively. One reason for the lower costs in our study might be the high percentage of unemployment (72.6%) because we used the lowest daily wage of employment for calculating the indirect cost of unemployed patients. Although there are generally more unemployed women in our population than in western countries, the studies have shown that the magnitude of unemployment in FM is also significant compared with other chronic diseases.[31]

It is worth noting that the direct non-healthcare and productivity costs were equally comparable in our unemployment-dominant population. Although it could be representative of a similar impact of FM on the private and professional lives of these patients, caution must be exercised in the interpretation of lower indirect cost, which may be underestimated in the context of unemployment conditions.

Consistent with the previous studies, our findings suggest the substantially impaired quality of life of FM patients. The patients suffered from poor mental (43.18 ± 9.73) and physical (34.88 ± 8.36) health status, which were lower than in the general population.[32] Studies have shown that among a variety of pain disorders, only patients with end-stage renal failure have a lower quality of life compared with patients with FM.[32,33] Poor health, especially in the physical domain, could markedly influence the utilization of formal and non-formal resources.

We used the cost-diary method developed by Goosens et al.[17] This prospective method, unlike the routine retrospective method of collecting data, relies on the patient's self-report, which is more beneficial for collecting the direct costs and productivity-loss information in FM patients.[17,19] In our study, we could track the information of 42.5% of given diaries. This rate is lower compared with the study of Goosens et al,[17] in which 68% of total diaries were returned. However, the percentages of the patients who completed the whole set of diaries for the entire 6 months were comparable to the study conducted by Goosens et al (45.2% vs 50%, respectively).[17] Given the lower response rate in our patients, the success of this method seems to depend on the frequent reminding of patients about arranged appointments and the emphasis on the correct filling of their cost-diary booklet. There was a nonsignificant trend to higher response rate with older age, being single (whether being widowed, divorced, or separated), and more severe disease. Even so, only the functional domain score of FIQR was found to have a positive, statistically significant correlation with the response rate. This follows previous studies, which have shown that patients with poorer health status and worse distress have had a higher response rate.[17]

Although the existing retrospective studies tended to show that the FM costs increased when illness severity rose, the data are still controversial.[11,34] There were many barriers to the certainty of this relationship in these studies, such as not confirming the FM diagnosis based on the validated criteria, patients' enrollment biases, heterogeneous FM diagnosis by the various specialists, estimation of FM severity not at the same time of diagnosis or cost estimation, and finally retrospective method. To the best of our knowledge, there is no prospective research that has investigated the relationship between FM cost and disease severity. Our study did not show any significant relationships between increased cost and severity. Conversely, we found a trend for increased costs in FM patients with mild severity. As our cost drivers were the multiple testing and physician visits, we think the milder condition created more doubt toward the FM diagnosis and as a result warranted more investigation, even after the FM labeling.

Another factor that may affect disease cost is the delay in the diagnosis. Kim et al[35] compared the costs 3 months before and after diagnosis and showed a significant decline in costs after the diagnosis. When we compared the costs between the different periods after the diagnosis, there was a trend to higher costs in newly diagnosed patients. However, this difference was not statistically significant. The higher cost may be attributed to more imaging and para-clinical tests in newly diagnosed patients, as well as the patients' and physicians' dissatisfaction with their diagnosis.

This study had some limitations. First, we mostly enrolled patients with a new FM diagnosis and we did not include the costs related to the patients' pre-diagnostic period. Therefore, we could not compare the different components of direct and indirect costs in these two periods. Second, in our study, the economic burden of FM was not compared with inflammatory rheumatological diseases, other chronic pain conditions, or healthy individuals. The third was the inherent limitation of the cost-diary method, which was the dropout and compliance rate in this prospective acquisition method. Although the response rate was acceptable, around 30% dropout occurred, which was higher than the estimated dropout rate calculated for the sample size. To ensure that this rate of dropout does not affect our result, a comparison between

the enrolled and dropout participants was conducted. The result showed no difference between the two groups in terms of probable cofounding variables. As this study was set in a tertiary FM clinic, it was different from the routine or general level of care. As our data came from patients who were actively seeking care, our findings may not be generalizable to the total FM patient population. Finally, as the majority of our patients were unemployed women, these data are insufficient to generalize employment. Therefore, these outcomes warrant future investigation in epidemiological studies with larger sample sizes.

This study provides valuable insights into the economic burden of FM patients in Iran. The significant cost was related to direct medical costs, and its major components were physician office visits and para-clinic tests even after the FM diagnosis. This result shows the high prevalence of doctor shopping behavior in our population, which warrants a need for the prompt attention to shift the resource utilization of FM patients from an unnecessary and exhausting diagnostic process (probably with particular attention to the referral system) towards effective healthcare services. The high costs in the formal and non-formal household care are comparable with direct medical costs, as well as considerable productivity loss, which elucidated the hidden poor functioning of FM patients from the perspective of private and professional life. The high burden of FM irrespective of illness severity highlighted the importance of attention to the economic and clinical aspects of FM beyond the arbitrary scores.

ACKNOWLEDGMENT

The authors thank Guilan University of Medical Sciences for providing the financial source of the work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection, analysis, and the first draft of the article were performed by FG, EN, and BGP. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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How to cite this article: Gharibpoor F, Nasrollahzadeh E, Ghavidel-Parsa B, Ghaffari ME, Bidari A. High cost of illness in fibromyalgia patients in Iran, irrespective of disease severity: A prospective cost study. *Int J Rheum Dis*. 2021;24:671–680. https://doi.org/10.1111/1756-185X.14094

Received: 29 October 2020 | Revised: 8 February 2021 | Accepted: 18 February 2021

DOI: 10.1111/1756-185X.14095

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases | WILEY

Association of F11R polymorphisms and gene expression with primary Sjögren's syndrome patients

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Funding information
Kaohsiung Medical University Chung-Ho Memorial Hospital, Grant/Award Number: KMUH102-2M14

Abstract

Aims: *F11R* gene encodes junctional adhesion molecule-A protein (JAM-A), which is expressed in various types of cells and is involved in leukocyte extravasation during inflammation. Sjögren's syndrome (SS) is a chronic systemic inflammatory disease that involves lymphocytes invasion of exocrine glands. *F11R* has been studied in autoimmune diseases, but any association between *F11R* and SS has not yet been investigated. Therefore, experiments were undertaken to examine the relationships among *F11R* gene polymorphism, messenger RNA (mRNA) expression and SS patients.

Methods: Three hundred and twenty-nine patients with SS, and 223 healthy controls were enrolled in their recruitment from the Kaohsiung Medical University Hospital. Genomic DNA was extracted from peripheral blood mononuclear cells and gene polymorphisms were genotyped by TaqMan real-time polymerase chain reaction (PCR). F11R mRNA expression was quantitated by quantitative real-time PCR with TaqMan Gene Expression Assay.

Results: Our study showed the genotype -688A/C (rs6695707) was not found in relation to SS patients. The odds ratio of -436A/G (rs12567886) genotype was notably associated with less susceptibility of SS in human leukocyte antigen (*HLA*)-*DR2* negative and *HLA-DR3* negative individuals. F11R mRNA expression was lower in SS patients than in the cells of healthy controls.

Conclusion: The result indicated that G allele of -436A/G genotype has the potential protective effect against SS disease condition. F11R mRNA was expressed significantly lower in SS patients.

1 | INTRODUCTION

F11R gene encodes junctional adhesion molecule-A protein (JAM-A). F11R/JAM-A is a transmembrane glycoprotein which is expressed in various types of cells including epithelial cells, endothelial cells, lymphocytes, monocytes, polymorphonuclear (PMN) cells, dendritic cells and platelets.[1-3] The biological function of F11R protein is multifunctional, for instance, F11R is involved in recruiting intracellular signaling, regulating cellular permeability, stimulation of cell translocation during inflammatory processes.[4] F11R is one of the components for cell-cell tight junctions, which maintains the epithelial barrier membrane of the adjacent epithelial cells. F11R serves as an important protein in

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recruiting monocytes and lymphocytes to migrate across paracellular spaces.[5] The destruction of cellular barriers would cause the damage of the tissue by immunocompetent cells. Previous studies reported that deficiency of *F11R* mice showed the decrease of leukocyte extravasation in inflammatory meningitis and skin inflammation.[6,7] In addition, a previous report demonstrated that the recruitment of leukocytes by F11R was stimulus-specific.[8] F11R also influenced the production of cytokines.[9] Chemokines secretion was decreased and nuclear factor (NF)-κB activation was repressed in a *F11R* knockout mice model.[10]

Sjögren's syndrome (SS) is a complicated slow progressive systemic disease, but the precise etiology still remains unknown. SS is a chronic systemic inflammatory disease which involves lymphocyte invasion of exocrine glands, mainly lacrimal glands and salivary glands, and also other organs.[11] However, the pathogenesis of SS has not been elucidated. *F11R* has been studied in autoimmune diseases, but the association between *F11R* and SS is not known.[2] Therefore, it is important to elucidate the roles of *F11R* gene in the immunopathogenesis of SS. In this study, we have carried out experiments to examine the relationship of *F11R* gene polymorphisms and messenger RNA (mRNA) expression with SS patients.

2 | METHODS

2.1 | Clinical subjects

Three hundred and twenty-nine SS patients, and 223 healthy controls were enrolled in their recruitment from the Kaohsiung Medical University Hospital (KMUH). The patients met the 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for primary SS.[12] All patients were informed and gave their consent to participate in this study. This study was approved by the Institutional Review Board of KMUH.

2.2 | Genomic DNA and total RNA extraction

Peripheral blood mononuclear cells (PBMC) were collected by Ficoll-paque (GE Healthcare) purification and genomic DNA was extracted from PBMC of patients from KMUH. Total RNA were extracted by the method of QIAmp RNA Blood Mini Kit (Qiagen). RNA was reverse-transcribed to complementary DNA (cDNA) with random primers and high-capacity cDNA Archive kit (Applied Biosystems, Life Technologies). The experimental procedure was followed as the manufacture supplied.

2.3 | Single nucleotide polymorphism (SNP) genotyping and data analysis

Genotypes -688A/C (rs6695707) and -436A/G (rs12567886) of *F11R* were assessed. The 2 SNPs were chosen from the National Center for Biotechnology Information (NCBI) dbSNP for validation and higher heterozygosity compared with others. The *F11R* SNPs genotyping were performed by TaqMan SNP Genotyping Assays (Applied Biosystems, Life Technologies) by real-time polymerase chain reaction (PCR) with ABI 7500 Real-Time PCR systems. The assay was carried out as previously described. Odds ratio (OR) and 95% confidence interval (CI) were calculated to evaluate the risk of disease. *HLA-DR2* and *HLA-DR3* typing were determined by HLA-Ready Gene (inno-train, Germany) following the supplier's procedure. The *t* test, χ^2 test and Fisher's test were used to evaluate the genotype and allele frequencies and F11R mRNA expression, respectively. The statistical analysis was conducted by IBM SPSS Statistics version 19.

2.4 | F11R mRNA expression and autoantibodies detection

A total of 120 SS patients and 92 healthy control individuals were assayed for the F11R mRNA expression. The mRNA expression was performed by the TaqMan Gene Expression Assay (Applied Biosystems, cat. no. 4369016) and real-time PCR reaction mix (Applied Biosystems, cat. no. 4369016). The enzyme was first activated at 95°C for 10 minutes, and PCR condition was at 95°C for 10 seconds, 60°C for 1 minute (repeat 40 cycles) on ABI 7500 Real-Time PCR systems. RNA polymerase II is the input control as previously described.[13] Anti-SSA/Ro, anti-SSA/La were detected by fluorescence enzyme immunoassay (FEIA) (EliA, Phadia AB, Sweden). Anti-Ro/La titer greater than 10 IU/mL was positive, less than 7 IU/mL was negative. Antinuclear antibody (ANA) was detected by indirect fluorescence assay (IFA) (DiaSorin Inc, USA). Titers greater than 1:160 were positive, and less than 1:40 were negative.

3 | RESULTS

3.1 | G allele of -436A/G is negatively associated with SS

In this study, we have carried out experiments to determine 2 polymorphic sites on the promoter region of *F11R* gene in SS patients. Our result showed the genotype -688A/C (rs6695707) was not found in relation to SS patients (Table 1). The genotype -436A/G (rs12567886) was notably associated with susceptibility of SS. The OR of -436 A/G + G/G genotypes was significantly associated with decreased risk of SS (OR = 0.25, P <.001, 95% CI = 0.11-0.54) (Table 1). This result indicated that the G allele was a protective allele in SS disease with OR 0.24, P <.001, 95%CI = 0.11-0.53 (Table 1).

3.2 | G allele of -436 was less susceptible with SS in *HLA-DR2*(-) and *HLA-DR3*(-) individuals

The genotype of *F11R* promoter sequence -436A/G (rs12567886) was related to the susceptibility of SS in *HLA-DR2*/*HLA-DR3* positive

TABLE 1 Genotype and allele frequencies of *F11R* promoter in primary Sjögren's syndrome patients and healthy controls

Position	Sjögren's syndrome (n = 329)	Control (n = 223)	*P*	OR (95% CI)
-688				
Genotype				
A/A	217	147		1
A/C	93	67	NS	0.94 (0.65-1.37)
C/C	19	9	NS	1.43 (0.63-3.25)
Dominant model				
A/A	217	147		1
A/C + C/C	112	76	NS	1.00 (0.70-1.43)
Recessive model				
A/A + A/C	310	214		1
C/C	19	9	NS	1.46 (0.65-3.28)
Allele				
A	527	361		1
C	131	85	NS	1.06 (0.78-1.43)
-436				
Genotype				
A/A	320	200		1
A/G	9	22	<.001	0.26 (0.12-0.57)
G/G	0	1	NS	
Dominant model				
A/A	320	200		1
A/G + G/G	9	23	<.001	0.25 (0.11-0.54)
Recessive model				
A/A + A/G	329	222		
G/G	0	1	NS	
Allele				
A	649	422		1
G	9	24	<.001	0.24 (0.11-0.53)

Abbreviation: NS, not significant.

and *HLA-DR2*/*HLA-DR3* negative patients (Table 2). Notably, the OR of G allele of -436 A/G position in *HLA-DR2* negative and *HLA-DR3* negative SS patients was significantly low (OR = 0.16, 95% CI = 0.04-0.60; Table 2). *F11R* genotype -436 A/G was shown to be less susceptible to SS disease in *HLA-DR2* negative and *HLA-DR3* negative individuals. These results suggested that G allele of -436A/G has the ameliorative effect to the susceptibility of SS in *HLA-DR2* negative and *HLA-DR3* negative individuals (OR = 0.17, 95% CI = 0.05-0.62; Table 2).

3.3 | mRNA expression of *F11R* in SS patients was lower than those of healthy controls

We performed the TaqMan real-time PCR to evaluate the mRNA expression of *F11R* gene in SS patients. mRNA expression of *F11R* was 0.386 ± 0.253 in SS patients, and was 0.552 ± 0.346 in healthy controls, *P* value <0.001 (Table 3). F11R mRNA expression in SS patients was lower than those of healthy controls. We observed that F11R mRNA expression in SS patients was lower than healthy controls also in the alleles of genotype -688 and position -436 A allele (Table 3). The F11R mRNA level in SS patients was lower than healthy controls after stratifying with *HLA-DR2* or *HLA-DR3* (data not shown). The F11R mRNA expression of position -436 G allele was higher in SS patients as compared to the healthy controls, but without significant difference (Table 3).

3.4 | Autoantibodies were negatively associated with F11R mRNA level in SS patients

In clinical outcome of SS patients, we noticed that the autoantibodies were negatively associated with F11R mRNA level in SS patients. F11R mRNA expression was low in anti-Ro/anti-La positive individuals, although it was not statistically significant. In contrast, F11R mRNA was significantly less expressed in ANA positive SS patients.

TABLE 2 Genotype and allele frequencies of *F11R* promoter in primary Sjögren's syndrome patients stratified with *HLA-DR2* and *HLA-DR3*

	HLA-DR2(+) or *DR3*(+)				*HLA-DR2*(-) and *DR3*(–)			
	Sjögren's syndrome	Control			Sjögren's syndrome	Control		
Position	(n = 86)	(n = 67)	*P*	OR (95%CI)	(n = 128)	(n = 76)	*P*	OR (95%CI)
-688								
Genotype								
A/A	62	44		1	84	48		1
A/C	20	21	NS	0.68 (0.33-1.39)	37	22	NS	0.96 (0.51-1.82)
C/C	4	2	NS	1.42 (0.25-8.09)	7	6	NS	0.67 (0.21-2.10)
Allele								
A	144	109		1	205	118		1
C	28	25	NS	0.85 (0.47-1.54)	51	34	NS	0.86 (0.53-1.41)
-436								
Genotype								
A/A	83	61		1	125	66		1
A/G	3	6	NS	0.37 (0.09-1.53)	3	10	.005	0.16 (0.04-0.60)
G/G	0	0			0	0		
Allele								
A	169	128		1	253	142		1
G	3	6	NS	0.38 (0.09-1.54)	3	10	.006	0.17 (0.05-0.62)

Abbreviation: HLA, human leukocyte antigen; NS, not significant.

TABLE 3 The mRNA expression of *F11R* in primary Sjögren's syndrome patients and control

		F11R mRNA expression		
		Sjögren's syndrome	Control	*P*
		0.386 ± 0.253 (n = 120)	0.552 ± 0.346 (n = 92)	<.001
Position	Allele			
-688	A	0.380 ± 0.250 (n = 192)	0.551 ± 0.337 (n = 147)	<.001
	C	0.410 ± 0.262 (n = 48)	0.556 ± 0.379 (n = 37)	.039
-436	A	0.383 ± 0.251 (n = 236)	0.556 ± 0.350 (n = 176)	<.001
	G	0.554 ± 0.327 (n = 4)	0.468 ± 0.171 (n = 8)	NS

Abbreviation: mRNA, messenger RNA; NS, not significant.

These results indicated there was a trend of F11R mRNA levels in less amount in anti-Ro/anti-La positive individuals, and particularly in ANA positive SS patients.

4 | DISCUSSION

We have investigated 2 polymorphic sites on the promoter of *F11R* gene in SS patients. The 2 SNPs were chosen from NCBI dbSNP for good validation and higher heterozygosity compared with others. The results indicated that the promoter genotype -688A/C (rs6695707) of *F11R* gene was found to have no relation to the susceptibility with SS patients. However, we noticed that the genotype -436A/G (rs12567886) of *F11R* was associated with less susceptibility to SS, particularly in the *HLA-DR2* negative and *HLA-DR3* negative individuals. Taking the results together, our study shows that the G allele of -436A/G genotype has the potential to have ameliorative effect of SS disease condition in *HLA-DR2* negative and *HLA-DR3* negative individuals. The F11R is important in maintenance of the tight junction between the endothelial cells and epithelial cells, and F11R is also expressed in lymphocytes.[2] The binding of F11R with its ligands contributes to the interaction between endothelial cells and epithelial cells leading to stabilizing intercellular junctions. The interaction between lymphocyte integrin with F11R protein facilitates lymphocyte infiltration and diapedesis of lymphocytes during inflammation.[1,2,14,15]

mRNA expression of *F11R* was lower in SS patients as compared to the healthy controls (Table 4). We also observed that the F11R mRNA expression of position -436 G allele was higher in SS patients as compared to the healthy controls, although without significant difference (Table 4). *F11R* genotypes -436 A/G and -436

TABLE 4 Relationship between autoantibodies and F11R messenger RNA expression in primary Sjögren's syndrome (SS) patients

Clinical feature	F11R mRNA expression	*P*
Anti-SSA/Ro test		
Positive	0.349 ± 0.246	.053
Negative	0.450 ± 0.267	
Anti-SSB/La test		
Positive	0.306 ± 0.197	.062
Negative	0.412 ± 0.268	
Antinuclear antibody test		
Positive	0.277 ± 0.159	.010
Negative	0.428 ± 0.272	

G/G were shown to be less susceptible to SS disease in *HLA-DR2* negative and *HLA-DR3* negative individuals (Table 2). Therefore, these results demonstrated that G allele of *F11R* genotype -436 was a protective allele. In our study, we observed that the F11R mRNA expression of position -436 G allele in SS patients was not significantly higher than those of the healthy controls. This result was only from a small sample size because of insufficient amount of extracted RNA. This sample size might serve as a training cohort, and further validation study will be required. Autoantibodies anti-Ro, anti-La and ANA are associated with different autoimmune diseases, such as SS and systemic lupus erythematosus.[16] One of the main pathologies of SS is anti-Ro antibodies.[17] Our results indicated that the autoantibodies anti-Ro, anti-La and ANA were negatively associated with F11R levels in SS patients. There was a trend of F11R mRNA to be less in SS autoantibody positive individuals. The correlation between the level of F11R mRNA and autoantibodies or disease severity requires further study. In our previous study, we found that the mRNA expression level of *F11R* was higher in rheumatoid arthritis patients with secondary SS than those of healthy controls.[18] The high level expression of F11R was involved in the recruitment of lymphocytes and immune cells. The low expression of F11R was also reported in autoimmune thyroid diseases.[19] F11R was increasingly expressed in Graves' disease but F11R was decreased in Hashimoto thyroiditis; both are thyroid autoimmune diseases. Highly expressed F11R recruited monocytes transmigration to the epithelium of patients with Graves' disease. In Hashimoto disease, F11R was less expressed and resulted in loss of integrity tightness of cell junction leading to the transmigration of immunocompetent cells and eventually caused the destruction of thyroid glands.[19] Silencing of *F11R* promoted the migration and proliferation of human keratinocytes and epidermal cells.[20,21] Thus, F11R is not necessary for the migration of lymphocytes and immune cells. F11R is important to maintain the epithelial barrier membrane of the adjacent epithelial cells. F11R serves as important protein in recruiting monocytes and lymphocytes to migrate across paracellular spaces.[2] Cytokines influence the integrity of the epithelium by altering tight junction protein expressions and the change of cell-cell junction complex permeability.[22,23] The level of F11R could be decreased by proinflammatory cytokines, such as interleukin-8 and tumor necrosis factor-α in autoimmune diseases.[9] Previous studies have shown that loss of JAM-A negatively regulates polarized migration of PMN cells, while having a positive effect on dendritic cells.[10,24] Because cytokines are involved in the pathogenesis of autoimmune diseases, the physiological role of cytokines and F11R in the development of autoimmune diseases needs to be elucidated. Further experiments have to confirm if the presence of F11R contributes to disease severity. We cannot rule out the possibility of disease severity in SS can be partially explained by *F11R* polymorphisms and F11R mRNA expression. The regulatory role of F11R is complicated, and the pathogenesis of autoimmune diseases must be determined in further studies.

ACKNOWLEDGEMENT

This research was financially supported by Kaohsiung Medical University Hospital, grant number KMUH102-2M14.

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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How to cite this article: Fang T-J, Li R-N, Lin Y-Z, et al. Association of F11R polymorphisms and gene expression with primary Sjögren's syndrome patients. *Int J Rheum Dis*. 2021;24:681–686. https://doi.org/10.1111/1756-185X.14095

Received: 15 December 2020 | Revised: 22 February 2021 | Accepted: 26 February 2021

DOI: 10.1111/1756-185X.14096

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases

WILEY

Joint hypermobility and its association with self-reported knee health: A cross-sectional study of healthy Australian adults

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Funding information
National Health and Medical Research Council of Australia – Centre for Research Excellence and Neuromuscular Disorders, Grant/Award Number: 1031893; Australian Podiatry Education and Research Foundation

Abstract

Aim: The primary aim of this study was to determine the association between generalized joint hypermobility (GJH), knee-specific hypermobility (KSH) and self-reported knee health in an Australian population. Secondary aims included elucidating ethnic/gender differences in GJH/KSH prevalence and knee health, and identifying KSH using a novel knee extension range of motion cut-off method.

Method: Knee extension range, Beighton score, and 5 domains of the Knee Injury and Osteoarthritis Outcome Score (KOOS) were collected from adults aged 18-101 years self-identifying as healthy, and were grouped by ethnicity and gender. Two established Beighton score criteria and 1 novel knee extension range cut-off method were used to determine GJH and KSH respectively. Point-biserial correlation tested the associations between GJH/KSH and KOOS. Differences in GJH/KSH prevalence and knee health between ethnic/gender groups were determined with the Chi-squared test.

Results: Of 732 participants (50% male), 80.3% were Caucasian. No correlations were found between GJH and KOOS while a very weak correlation was found between KSH and 1 KOOS domain ($r > -.30$; $P = .04$). Prevalence of GJH was higher in non-Caucasians (17.4% vs 5.6%, $P < .001$) and females (4.4% vs 1.1%, $P = .007$). Prevalence of KSH between ethnic and gender groups was not significantly different ($P = .50$ and $P = .69$ respectively). Non-Caucasians scored higher (better) in all KOOS domains than Caucasians (all $P < .05$).

Conclusion: Those who met the age- and gender-specific criteria for GJH/KSH did not report worse knee health than their non-hypermobile counterparts. Clinicians can assure individuals who exhibit GJH/KSH that these are not associated with lower knee health and function.

KEYWORDS

Beighton score, cut-off, generalized joint hypermobility, Knee injury and Osteoarthritis Outcome Score, knee-specific hypermobility

1 | INTRODUCTION

Musculoskeletal disorders of the knee can be detrimental to an individual's long-term knee health, leading to joint pain, disability and reduced quality of life.[1-3] Knee osteoarthritis is an example of a musculoskeletal disorder that may lead to recurrent pain and disability, negatively affecting both knee and global health.[2] Australians living with osteoarthritis are up to 3 times more likely to experience severe pain and psychological distress.[4] While multiple mechanisms have been hypothesized to explain how poor knee health could arise from musculoskeletal disorders, biomechanical features are consistently recognized as key risk factors for ongoing knee pain and disability.[5,6]

Joint hypermobility is a biomechanical feature, defined as the capacity of a joint to exceed normal active and/or passive physiological mobility,[7] that is influenced by age, gender, and ethnicity.[8] When hypermobility is present in 5 or more joints, it is termed generalized joint hypermobility.[7,8] Both generalized joint hypermobility and knee-specific hypermobility have been suggested as indicators of poorer knee health. A seminal study found a greater prevalence of joint pain, particularly at the knee, in Canadians with generalized joint hypermobility who were otherwise healthy.[9] Subsequent studies reported similar findings in Caucasians,[10-12] African-Americans,[13] and Middle Eastern populations.[14,15] Conversely, other studies suggest generalized joint hypermobility as a factor protecting hypermobile Caucasians[16-18] and African Americans[17,18] from developing osteoarthritis. Such conflicting results regarding the association between generalized joint hypermobility or knee-specific hypermobility with knee health and pain may be explained by the diverse set of outcome measures and their suggested reference values/cut-offs used to quantify these factors. Additionally, the lack of standardized methodologies to classify people as demonstrating generalized joint or knee-specific hypermobility further challenges the validity of reported associations.

Currently, no study has investigated the association of knee health with generalized joint or knee-specific hypermobility in the ethnically diverse Australian population. Clarification of these relationships is essential to ascertain the impact and effects of joint hypermobility on knee health, and to determine the role of screening for hypermobility in routine medical examinations. Therefore, the primary aim of this study was to determine the association of generalized joint hypermobility and knee-specific hypermobility with knee health in a large healthy Australian adult population. Secondary aims of this study include identifying ethnic and gender differences in generalized joint hypermobility, knee-specific hypermobility prevalence and knee health, as well as establishing age- and gender-specific knee extension range of motion cut-offs to appropriately define knee-specific hypermobility across the lifespan.

2 | MATERIALS AND METHOD

2.1 | Design

This study involved 732 adults (aged 18-101 years) who participated in the 1000 Norms Project,[19] a cross-sectional observational study investigating outcome measures of self-reported health and physical function in 1000 healthy individuals aged 3 to 101 years. Participants were stratified for age and gender (Table 1).

2.2 | Population and participants

The data of eligible participants aged 18-101 years were used in the current study. Participants considered themselves healthy for their age, could participate in age-appropriate activities of daily living and did not report any significant health conditions affecting their physical performance. Potential participants were excluded if they were diagnosed with diabetes mellitus; malignant cancers; demyelinating, inflammatory, or degenerative neurological conditions; pregnancy; severe cardiac/pulmonary disease; class 3 obesity (body mass index ≥ 40 kg/m^2); joint replacement; infectious or inflammatory arthropathies; severe mobility impairment requiring mobility aids for ambulation. Ethics approval was granted by the institutional Human Research Ethics Committee (HREC 2013/640) and written informed consent was received from all participants prior to the study.

2.3 | Procedures

As the prevalence of generalized joint hypermobility differs between ethnicities and genders,[20] participants were separated into ethnic and gender cohorts in accordance with the standards of the Australian Bureau of Statistics.[21] Participants were classified as Caucasian if they self-identified as European, American, or Oceanic, and those who identified otherwise were categorized as non-Caucasian.

The Beighton scoring system was used to classify the presence of generalized joint hypermobility, and has been reported to have excellent inter-rater reliability,[22] and concurrent validity.[20] It assesses mobility of 4 joints bilaterally, as well as the lumbo-pelvic complex. One point out of 9 is allocated for each of the following tests: (1) passive apposition of left and/or right thumb to contact the flexor aspect of the forearm (2 points); (2) passive hyperextension of left and/or right 5th metacarpophalangeal joint beyond 90° (2 points); (3)

TABLE 1 Gender and ethnic characteristics of included participants, categorized into age-groups

Age category, y	Male, n (%)	Caucasian, n (%)	Subtotal, n
18-29	66 (50)	64 (48)	132
30-39	50 (50)	68 (68)	100
40-49	50 (50)	83 (83)	100
50-59	50 (50)	86 (86)	100
60-69	50 (50)	94 (94)	100
70-101	100 (50)	193 (97)	200
Total:	366 (50)	588 (80)	732

hyperextension of the left and/or right humero-ulnar joint beyond 10° (2 points); (4) hyperextension of the left and/or right tibiofemoral joint beyond 10° (2 points); and (5) placement of both palms on the floor directly in front of the feet with both knees extended in standing (1 point). The Beighton score was originally developed to be interpreted dichotomously;[8] that is, classifying a person as having generalized joint hypermobility or not. The commonly utilized Beighton score cut-off of ≥ 4/9 to dichotomize individuals with or without generalized joint hypermobility was employed to allow comparison of the results of this study to others. This cut-off methodology is described herein as the "≥4/9 Beighton criterion".

A novel Beighton score cut-off method was recently established to better define "hyper-" mobility, where generalized joint hypermobility prevalence should represent the 5% most mobile individuals for age and gender.[23] Utilizing this revised method, Beighton score cut-offs were recommended for females aged 18-39 years to be ≥ 5, aged 40-59 years to be ≥ 4, aged 60-69 years to be ≥ 3, and those aged 70 years and above to be ≥ 2, while Beighton score cut-offs were recommended for males aged 18-39 years to be ≥ 4, aged 40-59 years to be ≥ 2, and those aged 60 years and above to be ≥ 1.[23] This was employed as the 2nd generalized joint hypermobility classification method, described herein as the "age- and gender-specific Beighton criterion".

Knee extension range was measured using a universal goniometer (Baseline, Fabrication Enterprises Inc.). The greater the knee extension range, the more hypermobile the knee joint. Participants were instructed to lie supine on a plinth with their hip positioned in neutral extension, adduction, and abduction. The ankle of the dominant limb was rested on a rolled-up towel, permitting the knee to drop into hyperextension. The goniometry was undertaken twice by either 1 of 2 experienced physiotherapists and the mean of their 2 knee extension measurements was used for analysis. Inter-rater reliability between the 2 assessors was demonstrated to be excellent.[19]

Novel knee extension range cut-offs were used to determine if participants demonstrated knee-specific hypermobility. Similar to the age- and gender-specific Beighton score criterion, these cut-offs identified the 5% of individuals in each age- and gender-specific cohort with the greatest knee extension mobility, thereby determining the prevalence of knee-specific hypermobility.[24] These cut-offs were utilized as the knee-specific hypermobility classification method, described herein as the 'knee-specific hypermobility criterion'.

Participants completed the Knee injury and Osteoarthritis Outcome Score (KOOS), which is a valid and responsive knee-specific instrument that assesses knee-related quality of life.[25] It is widely used to determine the impact of various knee conditions that can result in osteoarthritis.[26] The KOOS quantifies an individual's self-perceived knee health in 5 domains: pain, other symptoms, activities of daily living (ADL), sports and recreation participation (sport/rec), and quality of life (QoL). A maximum score of 100 for each domain represents excellent self-reported knee health, with a score of 0 representing poorest knee health.[25] Scoring of KOOS domains does not permit aggregation.[27]

2.4 | Statistical analysis

Descriptive analyses of the Beighton score, knee extension range, and 5 domains of the KOOS for both groups (ethnicity and gender) were performed. To determine the association between generalized joint hypermobility and knee-specific hypermobility with self-reported knee health, point-biserial correlation tests were used. Correlations of $r < .30$ were deemed very weak, $.30 \le r \le .49$ as weak, $.50 \le r \le .69$ as moderate, $.70 \le r \le .89$ as strong, and $r \ge .90$ as very strong.[28] The secondary aim of identifying ethnic and gender differences in the prevalence of generalized joint hypermobility, knee-specific hypermobility and self-reported knee health was addressed with Chi-squared tests on Beighton Scores, knee extension range, and all KOOS domains between participants with and without generalized joint hypermobility/knee-specific hypermobility in their ethnic and gender cohorts. *P* values of <.05 were accepted *a priori* as statistically significant. All statistical analyses were undertaken using SPSS v. 22 (IBM SPSS Statistics for Windows).

3 | RESULTS

3.1 | Demographics

Of the original 1000 participants, 268 were excluded as they did not meet the 18 and over age inclusion criterion, leaving a remainder of 732 participants in this study (Table 1). Mean age, ethnic-specific mean Beighton Scores (out of 9), knee extension range, and scores for the 5 domains of knee health (KOOS) were generated. Mean Beighton Scores and KOOS domain scores were statistically significantly higher in non-Caucasians than Caucasians (all $P < .05$), except mean age where the non-Caucasians were significantly younger than the Caucasian cohort ($P < .001$; Table 2).

3.2 | Association between generalized joint hypermobility or knee-specific hypermobility and the KOOS domains

Point-biserial correlation coefficients indicating the extent of associations between dichotomized generalized joint and knee-specific hypermobility (participants either meeting or not meeting the 3 cut-off criteria) with continuous KOOS measures have been calculated (see Appendix S1). The only significant, yet very weak correlation, was found between knee-specific hypermobility and the KOOS QoL domain score in all male participants ($r = -.11$, $P = .04$).

3.3 | Age- and gender-specific knee extension range cut-off values to identify knee-specific hypermobility

Only 3 of 732 participants (0.4%) met the traditionally used >10° of knee extension cut-off to determine knee-specific hypermobility. If

TABLE 2 Ethnicity-specific knee extension range, Beighton score, and KOOS domains[a]

Outcome measure	Ethnicity		
	Cau (n = 588)	NCau (n = 144)	*P*
Mean age, y	57.2 (19.6)	35.1 (16.0)	<.001**
Beighton score, /9	0.4 (1.0)	1.1 (1.3)	<.001**
Knee extension range, degrees	0.6 (2.4)	1.6 (2.4)	<.001**
KOOS, all/100			
Symptom	90.3 (12.3)	92.9 (8.8)	.005*
Pain	93.8 (10.4)	96.7 (7.1)	<.001**
ADL	96.4 (7.9)	98.3 (4.7)	<.001**
Sport/rec	89.7 (16.8)	93.1 (14.5)	.016*
QoL	87.1 (17.1)	91.6 (14.4)	.002*

Abbreviations: ADL, activities of daily living; Cau, Caucasian cohort; KOOS, Knee Injury and Osteoarthritis Outcome Score (higher score indicates better knee health); NCau, non-Caucasian cohort; QoL, quality of life; Sport/rec, sports and recreation.

[a]Values in this table are mean (*SD*).

*Level of significance $P < .05$.

**Level of significance $P < .001$.

TABLE 3 Identified age- and gender-specific knee extension range of motion cut-off values used for the knee-specific hypermobility (KSH) criterion

Age group, y	Knee extension range cut-off value (°)	
	Male	Female
18-29	6	6
30-39	6	6
40-49	5	5
50-59	3	5
60-69	3	2
70-101	1	2

we extend the cut-off to >7°, to allow for measurement error associated with long-arm goniometry, the prevalence was 1.5%. The range of knee extension that placed a participant in the 5% most mobile for their age and gender was calculated to provide the knee-specific hypermobility criterion (Table 3).

3.4 | Prevalence of generalized joint hypermobility and knee-specific hypermobility across ethnicities and gender

A summary of the proportion of the 732 participants who met the 2 criteria for generalized joint hypermobility and that for knee-specific hypermobility is found in Table 4. Both the ≥4/9 Beighton and the age- and gender-specific Beighton criteria identified a higher prevalence of generalized joint hypermobility in non-Caucasians than Caucasians. A higher proportion of female than male participants were identified with generalized joint hypermobility when the ≥4/9 Beighton criterion was used. Prevalence of knee-specific hypermobility did not significantly differ between ethnic groups.

4 | DISCUSSION

In this large sample of healthy Australian adults, both generalized joint hypermobility and knee-specific hypermobility as determined by 3 criteria were generally not associated with poorer self-reported knee health. However, knee-specific hypermobility was found to be very weakly associated with lower knee-related, QoL in male participants. Novel age- and gender-specific knee extension range cut-offs to define knee-specific hypermobility were established which are lower than the widely accepted 10° of knee hyperextension. The prevalence of generalized joint hypermobility was significantly greater in non-Caucasians (regardless of the Beighton cut-off criteria used) and in females, while no differences were found in knee-specific hypermobility between 2 ethnic and gender cohorts.

Both generalized joint hypermobility and knee-specific hypermobility are predominantly not associated with poorer self-reported knee health. Knee-specific hypermobility had a significant negative influence on only the knee-related QoL domain among males who did not report significant knee-related issues such as pain, stiffness, ADL or sport/rec limitations. Such sparse and unremarkable associations between generalized joint hypermobility and knee health were also noted in other recent studies.[18,29,30] However, our study is in stark contrast to that of a large Danish study.[12] They found that those with knee-specific and generalized joint hypermobility were twice as likely to report symptoms, poorer QoL, and functional limitations. There are multiple reasons for the difference in findings. First, the Danish study used self-reported outcome measures for both knee-specific and generalized joint hypermobility. Knee-specific symptoms were reported using questions selected from the Standardized

TABLE 4 Prevalence of generalized joint hypermobility and knee-specific hypermobility across ethnicity and gender[a]

Joint hypermobility criterion	Caucasian	Non-Caucasian	*P*	Male	Female	*P*
≥4/9 Beighton	2.0%	5.5%	.034*	1.1%	4.4%	.007*
AGS Beighton	5.6%	17.4%	<.001**	N/A	N/A	N/A
KSH	8.7%	6.9%	.501	7.9%	8.7%	.688

Note: AGS Beighton: age- and gender-specific Beighton score cut-off criteria for generalized joint hypermobility;[23] KSH, age and gender knee extension range cut-offs used as per Table 3.

[a]Values are the percentage of cohort with joint hypermobility.

*Level of significance $P < .05$.

**Level of significance $P < .001$.

Nordic Questionnaire that was designed for an occupational setting. Second, the generalized joint hypermobility measure was the 5-part questionnaire developed by Hakim and Grahame (2003) which incorporates historical hypermobility questions about childhood hypermobility and a question about their knee-specific hypermobility ("Can you hyperextend one or both of your knees?").[31] Lastly, the age distribution differed between the studies, and ethnicity was not reported.

Given that our study found only 1 very weak correlation between QoL and knee-specific hypermobility, it is our preliminary impression that the presence of generalized joint hypermobility and/or knee-specific hypermobility are not detrimental to self-reported knee health in an otherwise healthy population. Therefore, clinicians should reassure their patients who exhibit generalized joint hypermobility or knee-specific hypermobility that these are not associated with lower knee health. Perhaps of greater importance and clinical usefulness is to determine whether a patient's hypermobility is related to any joint instability (ie, patient's report of giving way, weakness, unable to control into the hypermobile range during activity). The association between knee hypermobility and instability is yet to be established and warrants future research.

Non-Caucasians had a higher prevalence and extent of generalized joint hypermobility than Caucasians, which is consistent with the majority of existing research and current clinical understanding.[8] Even using a Beighton cut-off method limiting generalized joint hypermobility prevalence across a whole cohort to the most mobile 5%, non-Caucasians were over-represented with 17.4% exhibiting generalized joint hypermobility. Although this was an expected result as ethnicity is a known factor that influences the prevalence of joint hypermobility,[8] being over 3 times higher was somewhat unanticipated. Of interest, a recent study of 1987 Americans has challenged the aforementioned notion, finding lower prevalence of generalized joint hypermobility among African-Americans than Caucasians when using the ≥4/9 Beighton criterion.[30] Discrepancies between these findings warrant future research into the prevalence and effects of hypermobility in specific ethnic groups.

Non-Caucasians reported significantly better mean self-reported knee health than Caucasians across all KOOS domains (Table 2). This finding is contrary to the American study that found a higher proportion of middle-to-older-aged African-Americans reporting knee symptoms compared to Caucasians.[30] Two possible explanations are that our study included various ethnicities (typical of multicultural Australia) confounding the influence of specific ethnicities on joint hypermobility and therefore comparability to other studies, and/or that our non-Caucasian cohort is younger than our Caucasian group. Nonetheless, the statistical significance found here may have little clinical significance since the accepted minimal perceptible clinical change for the KOOS is between 8-10,[27] and our largest difference was 4.5/100 in the KOOS QoL measure.

Females had a higher prevalence of both generalized joint and knee-specific hypermobility. This is consistent with most studies on the epidemiology of joint mobility between genders.[14,15,17]

This study established novel knee extension range cut-off values to identify knee-specific hypermobility across the adult lifespan. For example, both male and female participants aged 18-49 years would be considered to have knee-specific hypermobility if they demonstrated knee extension range of 6° or more (Table 3). These clinically applicable cut-offs adhere to the recent consensus that those who have knee-specific hypermobility must demonstrate a statistically extreme knee extension range with respect to their age and gender.[23,32] In this study, we identified those participants whose knee extension range places them over the 95th percentile.[24] Such a methodology standardizes the proportion of people with joint hypermobility and can benefit the conduct of *a priori* power analyses in future studies. The cut-off ranges to identify knee-specific hypermobility decreased with age regardless of gender. Clinically, the criterion to define knee-specific hypermobility has been set at >10° as evidenced by its incorporation into the Beighton scoring system. As our proposed extension cut-off values for adults, regardless of age or gender, were all below 10°, the findings of this study suggest that the current clinical criterion grossly underestimates the prevalence of knee-specific hypermobility and therefore generalized joint hypermobility. We recommend clinicians take caution in using a strict >10° knee extension as a measure of hypermobility.

Novel knee extension range cut-off values established in this study, along with previously developed age- and gender-specific Beighton score cut-offs,[23] may be more useful approaches to identify knee-specific hypermobility and generalized joint hypermobility. These values could better assist the identification of ethnic and

gender differences in joint hypermobility. Upon validation of such methodology, future studies should adopt the use of the age- and gender-specific knee extension range cut-off values, as it may better assist clinicians in assessing and ultimately addressing knee mobility-related disorders.

A key strength of this study lies in the use of a large representative sample of the Australian population. The use of age- and gender-specific Beighton score[23] and knee extension range criteria to identify the most hypermobile 5% of participants with generalized joint hypermobility and knee-specific hypermobility respectively is another fundamental strength of this study. Hypermobility may contribute significantly to musculoskeletal complaints,[8] regardless of age. The use of age- and gender-specific cut-off values maintains the sensitivity in discerning hypermobility in older populations with naturally reduced joint mobility;[23] such cut-offs accurately identify hypermobile individuals across the lifespan and permit us to better investigate the relationship of joint hypermobility with mobility-related musculoskeletal disorders. Further, this study adopted the KOOS as its primary outcome measure. A patient-report measure, the KOOS has demonstrated good validity, test-retest reliability, responsiveness to clinical change,[33] assesses knee-related QoL,[27] and provides patient insight in domains of health that cannot be assessed clinically or radiographically.

The use of a cross-sectional study design limits our ability to observe the temporal relationship between joint mobility and knee health, nor can it attribute causality. The validity of the Beighton score has only been established in a single Caucasian pediatric population;[34] its use in the adult population has yet to be validated. Despite the relatively large sample of metropolitan Australians, results of this study may only be relevant to adults who self-identify as healthy. There was a higher proportion of non-Caucasians in the younger compared to the older groups. Ethnicity was not stratified in the recruitment to the study and it is possible that the higher prevalence of generalized joint hypermobility and knee-specific hypermobility in non-Caucasians was confounded by age. Future studies of this type would benefit from such stratification and prevalence estimates in specific ethnicities. Lastly, this study cannot fully determine the lack of association between self-reported knee health and knee-specific hypermobility as knee hypermobility in the coronal and horizontal planes were not investigated.

In conclusion, neither generalized joint nor knee-specific hypermobility were associated with self-reported knee health in this large Australian sample, with the exception of males with knee-specific hypermobility who reported lower knee health in the QoL KOOS domain. This suggests that joint hypermobility is not a significant determinant of knee health. Given this, our recommendation for health professionals is to carefully weigh and address all determinants of knee joint health including, but not limited to, lower limb strength and control, biomechanics/alignment and psychosocial factors, and not to overly attribute joint hypermobility to presenting knee symptoms and dysfunction.[35,36] The cut-off scores for knee-specific hypermobility established here indicate that previous prevalence estimates of knee hypermobility in adults are indeed under-estimations.

ACKNOWLEDGEMENTS

This study was funded by the National Health and Medical Research Council of Australia – Centre for Research Excellence and Neuromuscular Disorders (#1031893) and the Australian Podiatry Education and Research Foundation. The authors would also like to thank the volunteers who participated in the 1000 Norms Project.

AUTHOR CONTRIBUTIONS

All authors acknowledge they have contributed significantly, were involved in the conceptualization and methodology of the study and are in agreement with the content of the manuscript. Participants recruitment and data collection were conducted by Dr Marnee McKay and Dr Jennifer Baldwin. Dr Cliffton Chan, A/Prof Leslie Nicholson and Haiwei Qi were responsible for writing the original draft and formal analysis, but Dr Marnee McKay, Dr Jennifer Baldwin and Prof Joshua Burns played a role in reviewing the manuscript. Prof Joshua Burns was responsible for funding acquisition.

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SUPPORTING INFORMATION

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

How to cite this article: Chan C, Qi HH, Baldwin JN, McKay MJ, Burns J, Nicholson LL. Joint hypermobility and its association with self-reported knee health: A cross-sectional study of healthy Australian adults. *Int J Rheum Dis*. 2021;24:687–693. https://doi.org/10.1111/1756-185X.14096

Received: 27 June 2020 | Revised: 5 November 2020 | Accepted: 17 November 2020

DOI: 10.1111/1756-185X.14097

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases | WILEY

Frequency of Growth Differentiation Factor 5 rs143383 and asporin D-repeat polymorphisms in patients with hand and knee osteoarthritis in Kurdistan province, Iran

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Funding information
Kurdistan University of Medical Sciences, Grant/Award Number: 1394.87

Abstract

Aim: Osteoarthritis (OA) is the most common chronic joint disorder, resulting from the breakdown of joint cartilage. It occurs in the knees, hands, and hips, leading to pain, stiffness, inflammation, and swelling.

Methods: In this study, 100 hand and knee OA patients, meeting the American College of Rheumatology criteria were included in the case group, and 100 healthy individuals were allocated to the control group. Blood samples were collected from the participants. After DNA extraction, genotyping was carried out for *GDF5* rs143383 C/T polymorphism by allele-specific polymerase chain reaction (ASPCR) and for D-repeat alleles of asporin (ASPN) by conventional PCR assay.

Results: The results showed that the frequency of the D14 allele of ASPN was significantly higher than other alleles in the case group ($P = .0001$). Also, the frequency of the D14 allele among women was significantly higher than in men ($P = .004$). Moreover, the frequency of the TT allele in *GDF5* rs143383 C/T polymorphism was significantly higher than the CC and CT alleles in the case group, compared with the control group ($P = .001$). A significant difference was found between the TT allele and other alleles in female and male patients compared with the control group ($P = .02$).

Conclusions: The D14 allele of the ASPN gene and TT allele of the *GDF5* gene (rs143383 + 104T/C) are associated with hand and knee OA in the Kurdish population, indicating that these alleles could be risk factors for OA, at least in our populations.

KEYWORDS

asporin, growth/differentiation factor-5, osteoarthritis, polymorphism

1 | INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease, which occurs when the cartilage or cushion between the joints breaks down, leading to pain, stiffness, and swelling. OA is characterized by the degeneration of articular cartilage.[1] Several factors lead to the development of OA, including excess weight, injury, aging, and hormonal, environmental, and genetic factors, which are recognized as major risk factors for OA.[2] This disease occurs when mechanical stress and inflammatory mediators cause an imbalance in anabolic and catabolic processes of carcinogenesis.[3,4]

Symptomatic knee OA occurs in 10% of men and 13% of women aged 60 years or above.[5] Knee OA is a common condition in Asian countries. In Iran, the incidence of knee OA is estimated at 19.4%

in rural areas and 15.3% in urban regions; also, it is more prevalent among women than men.[4] The prevalence of hand OA in the Community-Oriented Program for Control of Rheumatic Diseases (COPCORD) study was reported to be 2.66%.[6] The prevalence of knee and hand OA in the COPCORD study was 18.8% and 3.9% in Sanandaj, Iran, respectively.[7] There are many risk factors for knee OA in different communities, such as obesity, aging, race, bone density, hormonal imbalance, occupation, and housing status.[8] Symptomatic hand OA has both clinical and public health aspects. The prevalence of OA in the general population of adults ranges from 3% to 8% in different countries.[9,10] Gender, race, education, obesity, history of a hand injury, and occupational hand activities are the six known or potential risk factors for OA of the hands.[5,11]

Genomic studies are used to determine the possible relationships between genetic variants and diseases. They mainly focus on the identification of polymorphisms associated with the disease.[12,13,14] Growth/differentiation factor 5 (GDF5) is a member of the transforming growth factor-β (TGF-β) superfamily and is closely related to bone morphogenetic protein. It plays an important role in the morphogenesis of tendons, ligaments, and bones.[12-15] It also contributes to the regulation of chondrogenesis significantly. GDF5 mutation may be correlated with abnormal joint development, the progression of OA, and various forms of chondrodysplasia, joint symphalangism, and brachydactyly type C.[16,17] The *GDF5* gene is expressed in both normal and osteoarthritic articular cartilages.[7,15] The 5′ untranslated region (UTR) of rs143383 + 104T/C *GDF5* core promoter is a single-nucleotide polymorphism (SNP), contributing to transcriptional activity.[18] T allele, a non-ancestral allele related to OA susceptibility, is associated with a decrease in transcriptional activity.[16] Moreover, studies on the Japanese and Han Chinese populations have shown the association of T allele with knee OA.[3,19] However, some reports from Spain and Greece have reported inconsistent results, and some found no significant association.[20] In a recent meta-analysis of the association between SNP rs143383 and OA, the association between rs-143383 SNP and OA was confirmed globally.[13,21]

Recently, it has been reported that a protein from an extracellular matrix, called asporin (ASPN), is associated with OA of the hands and knees.[22,23] The *ASPN* gene decodes proteins in cartilaginous cells. These proteins can regulate cartilage formation by attaching to TGF-β_1 and inhibiting its expression.[24] ASPN has a sequence of aspartic acid (D) and residual (D-repeat) strands in the N-terminal region of adult proteins.[25] Previous studies have shown that ASPN can bind to TGF-β_1 and interfere with type II receptors of TGF-β, thereby inhibiting TGF-β/Smad signaling and reducing chondrogenesis.[26,27] The repeat poly-mutation (D-repeat) in ASPN was first defined in 2005 as a knee OA-related polymorphism.[28] The D14 allele of *ASPN* was found in patients with knee OA. The inhibition of TGF-β_1 in the D14 allele is greater than the D13 allele, which is recognized as a common allele.[29] In Japanese and Chinese populations, the D14 allele was associated with the risk of knee OA, whereas the D13 allele was found to be a protective agent against OA in some Japanese people.[30,31] Although the D15 allele is considered a risk factor in the Greek population,[32] a study by Jazayeri et al, conducted in Tehran, Iran on patients with knee OA, showed that the D15 allele was a risk factor for knee OA only in women.[33]

Research shows that the *GDF5* gene and *ASPN* are related to knee OA. Considering the lack of research on this relationship and evidence of significant differences between ethnic groups, the present study aimed to investigate and compare *GDF5* and *ASPN* polymorphisms in patients with hand and knee OA and a healthy group of individuals, referred to the Department of Rheumatology of Tohid Hospital, Sanandaj, Iran, in 2016.

2 | MATERIALS AND METHODS

2.1 | Sample preparation

This study was approved by the Ethics Committee of the Faculty of Medicine of Tohid Hospital (ethics code: IR.MUk.REC.1394.87). Both the control and case groups belonged to the same ethnic group and geographical area. In this case-control study, the frequency of polymorphisms was investigated in patients, who were referred to the rheumatology clinic of Tohid Hospital in 2016 and volunteered to participate in the study. Patients who were referred to the rheumatology clinic and diagnosed with both hand and knee OA were included in the case group, whereas those who were referred to the hospital without OA were allocated to the control group. The two groups were matched in terms of age and sex.

A total of 100 patients, who met the American College of Rheumatology criteria for hand and knee OA were included in the case group, and 100 healthy individuals were included in the control group. Overall, 50 participants were male and 150 were female. The control group was selected from among individuals who did not show any symptoms of OA and all of them we clinically examined by our colleague (NM), who is Board-certified in Internal Medicine and Rheumatology. There was no parental or blood relationship between cases and controls.

Inclusion criteria for the case group were as follows: (a) age above 50 years; (b) clinical and radiological symptoms (eg, chronic pain and motor limitations); and (c) knee or hand OA. Exclusion criteria were as follows: (a) history of the inflammatory articular disease; (b) post-infection or post-traumatic arthritis; (c) joint dysplasia; (d) congenital anomaly; (e) crystallopathy; (f) malignancy; and (g) vascular necrosis.

The sample size, including 100 cases and 100 controls (a total of 200 individuals), was obtained based on the following formula considering $p_A = 0.26$ and $p_B = 0.15$ considering $\alpha = 0.05$ and $\beta = 0.2$ people and a total of 200 people.

$$n_A = n_B = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2[p_A(1-p_A) + p_B(1-p_B)]}{(p_A - p_B)^2}$$

where, nA= sample size of case group, nB= sample size of control group, Zα=Type 1 error, Zβ=TYPE 2 error, P= Prevalence of OA in population.

The power association was calculated by http://osse.bii.a-star.edu.sg/. Based on minor frequencies of T allele in both the case (26%) and the control (6%) groups, the sample size of 200 (100 samples for each), and the desired 5% significant level, the power was 97.6%.

A total of 200 peripheral blood samples were collected from the participants, and DNA was extracted using a Qiagen extraction kit (Qiagen, Valencia, CA, USA). The *GDF5* rs143383 C/T polymorphism was examined using allele-specific polymerase chain reaction (ASPCR), and the D-repeat allele of ASPN was determined by conventional PCR assay. The demographic information, including age, education, occupational status, body mass index, and sex, are summarized in Table 1.

2.2 | DNA extraction

DNA extraction was carried out for each sample. By centrifuging 2 mL of whole blood, a leukocyte cell pellet was obtained from the buffy coat samples. This cell pellet was used for DNA extraction. The Qiagen DNA blood mini-kit (Qiagen) was used to collect genomic DNA, according to the manufacturer's instructions. The DNA purity and concentration were determined using a Synergy 2 multi-mode reader (BioTek® Instruments Inc., Winooski, VT, USA).

2.3 | Conventional PCR assay

For the assessment of repeat polymorphisms of *ASPN* gene, according to a study by Mustafa et al,[34] the exact number of microsatellites and D-repeats was determined, using a forward primer (5′-GCTTTGTGCTCTGCCCAAACCC-3′) and a reverse primer (5′-CACTGACATCCAAATGGACAC-3′5).

TABLE 1 Demographic characteristics of the patients with OA and control individuals

Variables	Classification dimensions	Frequency	Percent
Age, y	50-60	81	40.5
	61-70	79	39.5
	More than 70	40	20
Education	Literature	81	40.5
	Elementary education	39	19.5
	Diploma	69	34.5
	University education	11	5.5
Job	Unemployed	104	52
	Worker	45	22.5
	Business	41	10
	Retired	10	15.5
Body mass index, kg/m^2	Less than 25	31	15.5
	25-30	82	41
	More than 30	87	43.5
Sex	Male	50	25
	Female	150	75

2.4 | ASPCR assay

The ASPCR method was performed according to a study by Yaku et al.[35] In their study, detectable amounts of PCR products were obtained when primers, forming a single or two mismatch pairings at the 3' end, were used. Their results indicated that a primer with a 3'-terminal nucleotide, which identifies the SNP nucleotide and the next two nucleotides forming mismatch pairings with the template sequence, can be used as an allele-specific primer to eliminate the pseudo-positive problem. In the present study, we designed three primer sets of C and T alleles for the identification of the rs143383 polymorphism (Figure 1A), according to a study by Liu et al.[36] We selected two allele-specific primers that were the most effective primers in detecting rs143383 polymorphism in two separate PCR tubes for each C allele and T allele (Figure 1B).

Briefly, after DNA extraction, forward and reverse primers were used in two separate PCR tubes for each sample to reproduce a 197-bp fragment of the *GDF-5* gene. Next, ASPCR primers were used to reproduce 109-bp fragments for each T or C allele (Table 2). Each allele-specific primer was designed according to the specificity-matching principle at the 3' end of candidate primers (Figure 1). In this study, the β-actin gene was used as a positive control. Also, gradient PCR was performed to determine the optimum annealing temperature for each allele-specific primer. After PCR amplification, electrophoresis was performed on a 2% agarose gel. The interpretation and comparison of the results were performed in SPSS version 20 (IBM, Armonk, NY, USA).

2.5 | Statistical analysis

Data were analyzed in SPSS version 20 using chi-squared and Fisher's exact tests. The odds ratio and its corresponding 95% confidence interval were calculated where necessary. The unpaired *t* test was used for continuous data and chi-squared test was used for categorical data. Allele frequencies, odds ratio, and the probability for Hardy-Weinberg equilibrium were estimated and analyzed as previously described.[37]

3 | RESULTS

The mean age of the participants was 59.49 ± 13.08 years in the case group and 57.21 ± 11.93 years in the control group. In terms of age, the case and control groups were classified into three groups of 50-60 years (40.5%), 61-70 years (39.5%), and ≥70 years (20%). In terms of literacy, 40.5% of the participants were illiterate; 19.5% had elementary education, 34.5% had a high-school diploma, and the rest (5.5%) had a university education. Regarding occupation, 52% of

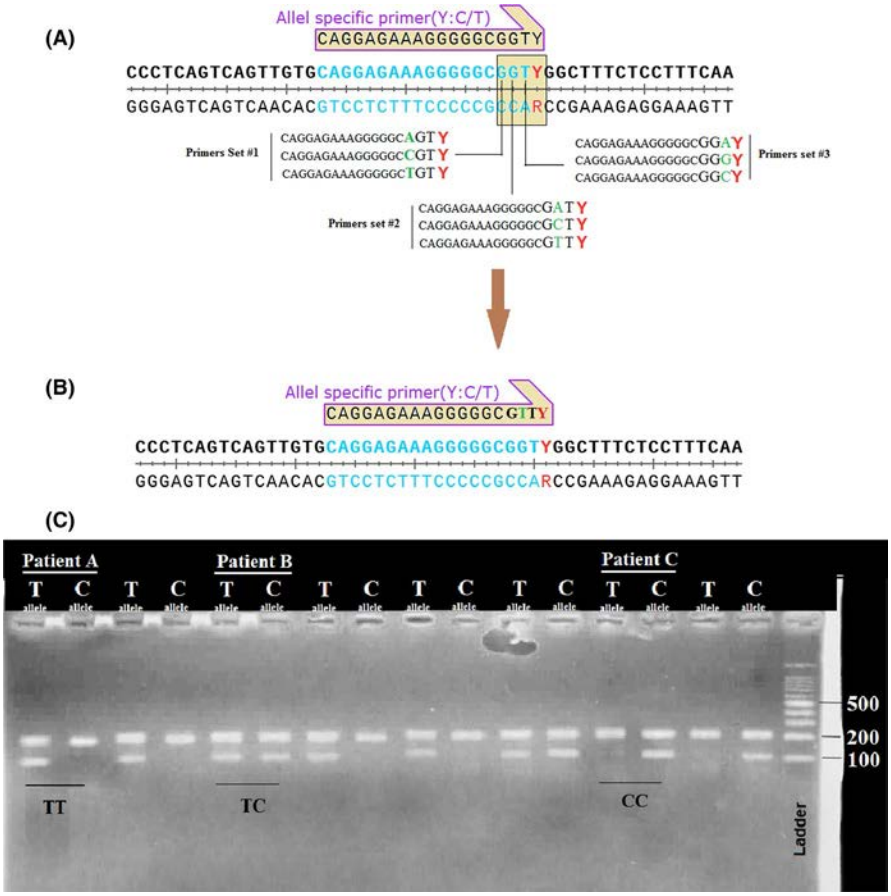

FIGURE 1 The ASPCR method for detecting *GDF5* rs143383 + 104T/C polymorphism. (A) The designed allele-specific primer based on the study by Liu et al.[37] There were three sets of primers, and each primer was tested for differentiation power between T and C alleles by gradient PCR (G=±3C°). (B) The sequence of allele-specific primers was selected and used with a mismatch in the third nucleotide at the 3′ end (G → T). (C) The 3% agarose gel electrophoresis in ASPCR; each patient had two PCR tubes, each containing one form of rs143383 polymorphism (C or T). Right to left: 100 bp ladder in patient C undergoing conventional PCR (197 bp); only C allele (109 bp) was reproduced (CC genotype). Patient B undergoing conventional PCR (197 bp); both C allele (109 bp) and T allele (109 bp) were reproduced (TC genotype). Patient A undergoing conventional PCR (197 bp); only T allele (109 bp) was reproduced (TT genotype)

TABLE 2 The sequence of primers for allele-specific polymerase chain reaction (PCR)

Primer name	Primer sequences	Melting temperature	PCR product length
PCR primer	F: 5′-AGCACAGGCAGCATTACG-3′ R: 5′-CCAGTCCCATAGTGGAAATG-3′	TM = 58 C	197 bp
Allele-specific PCR primer	F: 5-CAGGAGAAAGGGGGCGTTT-3 F: 5-CAGGAGAAAGGGGGCGTTC-3	TM = 59 TM = 62	109 bp

TABLE 3 Relationship between asporin polymorphic alleles in patients with hand and knee osteoarthritis

	The *CC* allele		The *TC* allele		The *TT* allele	
Aspirin allele	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
OA patients vs controls	0.19 (0.52-1.01)	.22	0.89 (0.52-1.12)	.21	0.47 (0.13-0.99)	.0001
Female patients vs female controls	0.77 (0.34-1.22)	.47	0.66 (0.44-1.09)	.33	0.69 (0.43-0.83)	.004
Male patients vs male controls	1.09 (0.38-1.27)	.55	0.29 (0.09-0.43)	.18	0.38 (0.16-0.73)	.27

Abbreviations: CI, confidence intervals; OA, osteoarthritis; OR, odds ratio.

the participants were unemployed, 22.5% were simple workers, 10% were business people, and 15.5% were retired (Table 1).

The D13, D14, and D15 alleles of the *ASPN* gene and also rs143383 + 104T/C polymorphism of the *GDF5* gene were investigated in hand and knee OA patients in the case group and compared with the control group. Based on the results, the frequency of rs143383 + 104T/C *GDF5* and *ASPN* polymorphisms in the control group was not significantly different between men and women. There was a significant difference between the D14 allele and other alleles ($P = .0001$). There was also a significant difference between the D14 allele and other alleles in female patients, compared with women in the control group ($P = .004$; Table 3). The D14 alleles of

ASPN were significantly different between the OA patients and the control group ($P = .0001$). We also observed that the frequency of this allele was significantly higher in the female and male patents ($P = .004$). However, the D13 and D15 alleles had no significant differences in total frequency or in female and male comparison groups ($P > .05$).

In this study, we aimed to introduce the ASPCR method as a fast, low-cost, and precise method for detecting rs143383 + 104T/C *GDF5* polymorphism as an important risk factor for OA of the hands and knees, using a primer (5'-CAGGAGAAAGGGGGCGTTY-3'). The present results revealed that the frequency of the TT allele of rs143383 polymorphism was significantly higher among patients with hand and knee OA in the case group, compared with the control group. The frequencies of genotype in our patients were TC 34%, TT 52%, and CC 14%; whereas these frequencies were TC 46%, TT 12%, and CC 42% in the control individuals. The total allele frequencies of T allele in patients and controls were 69% and 33%, respectively. Moreover, the total frequency of C allele was 31% in the patient group and 67% in the control group. As shown in Table 4, in comparison with the control group, the difference of TT genotype, but not CC and CT, was significant in patients with OA ($P = .001$). Moreover, this association was not gender-dependent and the TT genotype frequency was significantly higher in both men and women of the case group than the control group ($P < .05$; Table 4).

4 | DISCUSSION

The knee and hand OA is one of the most common articular diseases, and we have previously reported that the prevalence of knee OA and hand OA in the COPCORD study was 18.8% and 3.9% in Sanandaj, Iran, respectively.[7] Several factors, including age, obesity, race, genetics, and sex hormones, have been associated with knee OA, and factors, such as age, obesity, excessive use of joints, and physical activity, have significant effects on hand OA. Previous studies have shown that ASPN, a leucine-rich repeat protein, plays a major role in the pathogenesis of OA.[28,38] The *ASPN* gene also regulates the production of an extracellular cartilage protein, belonging to the leucine-rich proteoglycan family.[39] It has a variable number of aspartic acid residues in the N-terminal end. This enriched protein (D-repeat) is guided by the microsatellite polymorphism.[29] It can also prevent the TGF-β_1 signaling pathway, which plays an essential role in nearly every aspect of cartilage formation and maintenance.[22]

In the present study, D13, D14, and D15 alleles of the *ASPN* gene were investigated in the case and control groups, and a significant correlation was found between the *ASPN* polymorphic alleles (D14) and knee/hand OA, compared with the control group ($P = .0001$). The *ASPN* gene alleles (D14) were significantly different in women ($P = .004$), whereas no significant difference was observed in men. In this regard, Kizawa et al reported a significant correlation between the aspartic acid repeat polymorphism (D14) and knee and hip OA.[30] Song et al found a significant relationship between *ASPN* D14 polymorphism and lumbar disk disease in Asians.[40] Mustafa et al, in a study investigating the alleles of aspartic acid (D allele) in white British people with OA, reported that the D13 allele was more common in the controls. They also found that the D14 allele was more common in male patients; this trend was only significant in men who had undergone hip replacement.[34]

A recent systematic review and meta-analysis revealed that there is a significant relationship between *ASPN* D14 polymorphism and knee OA.[41] Taipale et al reported that the carriage of the *ASPN* D15 allele is associated with the increased risk of symmetrical hand OA.[22] Moreover, the D14 repeat polymorphism of the *ASPN* gene was found to be associated with primary OA of the knees in a Mexican Mestizo population.[42] All of these studies are consistent with our study, showing that D14 alleles are associated with OA. However, the results of the present study, unlike the study by Mustafa et al,[34] showed that the frequency of D14 alleles was significantly different between female and male patients. In contrast to the results of our study, a study conducted in Iran by Jazayeri et al concluded that the D15 allele of the *ASPN* gene could only be considered a significant allele in women.[33] This is unlike the results of this study, which depicted that the frequency of the D14 allele in the *ASPN* gene was significantly higher in hand and knee OA patients, compared with the control group in the Kurdish population. Moreover, there was a significant relationship between the D14 allele and hand and knee OA in women. Although both of these studies were carried out in Iran, the results are not consistent. It seems that genetic and environmental differences of ethnic groups in Iran may play a role in the etiology of OA as a complex condition in a human outbred population.

TABLE 4 Relationship between *GPF5* polymorphic alleles in patients with hand and knee osteoarthritis

	CC allele		TC allele		TT allele	
GDF-5 genotypes	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
OA patients vs controls	0.53 (0.34-0.90)	.28	1.27 (0.59-1.49)	.46	0.34 (0.21-0.76)	.001
Female patients vs female controls	1.34 (0.68-1.57)	.53	0.83 (0.51-1.16)	.12	0.56 (0.23-0.93)	.001
Male patients vs male controls	0.89 (0.53-1.08)	.31	0.48 (0.29-0.73)	.27	0.49 (0.23-0.80)	.02

Abbreviations: CI, confidence intervals; OA, osteoarthritis; OR, odds ratio.

In the current study, we also investigated the rs143383 + 104T/C polymorphism of the *GDF5* gene, as an important risk factor for hand and knee OA. This gene accounts for a wide range of skeletal disorders in humans.[43] However, Daans et al, by studying a Gdf5(Bp-J/+) mouse model, suggested that the decreased level of GDF5 in mice might contribute to OA through different mechanisms, including altered loading and subchondral bone changes.[44] On the other hand, studies by Miyamoto et al on Chinese and Japanese populations,[18] Southam et al on a European population,[4] and Valdes et al on a Caucasian population [14] found that the *GDF5* rs143383 polymorphism was associated with OA of the knees. Several other studies have reported a significant relationship between the *GDF5* gene and knee, hip, and hand OA.[16,22,45] In this study, we tried to present ASPCR as a fast, low-cost, and precise method for detecting *GDF5* rs143383 + 104T/C polymorphism as an important risk factor for OA of the hands and knees, using a primer (5'-CAGGAGAAAGGGGGCGTTY-3'). Our findings revealed that the frequency of the TT allele of rs143383 polymorphism was significantly higher in hand and knee OA patients (case group), compared with the control group. We also observed that there was a significant difference in both men and women of the case group, compared with the control group. Based on the results of the above-mentioned studies, the rs143383 polymorphism may play an important role in increasing the risk of knee and hand stiffness and OA in the Kurdish population, as has been reported in other populations in different parts of the world. It can be concluded that there is a significant association between the TT allele of GDF-5 rs143383 polymorphism and OA in Kurdish women. On the other hand, we have to consider that the sample size of our study was smaller than previous studies. Based on our knowledge, there are no studies showing the frequencies of these alleles in other ethnic groups in Iran, which needs to be further investigated. However, the population of this study seems to be homogeneous and the distribution between the case and control groups was in Hardy-Weinberg equilibrium. Overall, this study is the first report of an association between the *GDF5* polymorphism and OA of the hands and knees in the Kurdish population.

5 | CONCLUSIONS

It seems that the TT allele of the *GDF5* gene and the D14 allele of the *ASPN* gene are alleles indicating risk for hand and knee OA in Kurdistan. The rs143383 polymorphism may play an important role in increasing the risk of knee and hand stiffness and OA in the Kurdish population. However, further studies enrolling a larger number of patients are required.

ACKNOWLEDGMENTS

This work was supported by a grant (MUk.1394.87) from Kurdistan University of Medical Sciences to NM and AJ. The authors would like to thank Dr Khaled Rahmani and Mr Afshin Saaidi for statistical assistance.

CONFLICT OF INTERESTS

The authors declare that they have no competing financial interest and nothing to disclose.

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How to cite this article: Moghimi N, Nasseri S, Ghafouri F, Jalili A. Frequency of Growth Differentiation Factor 5 rs143383 and asporin D-repeat polymorphisms in patients with hand and knee osteoarthritis in Kurdistan province, Iran. *Int J Rheum Dis*. 2021;24:694–700. https://doi.org/10.1111/1756-185X.14097

Received: 1 January 2021 | Revised: 15 February 2021 | Accepted: 1 March 2021

DOI: 10.1111/1756-185X.14102

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases

WILEY

Evaluating the strength of spinal and proximal girdle muscles in patients with axial spondyloarthritis: Correlation with activity, disability, and functionality

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Abstract

Aim: To compare the muscle strength of muscle groups in axial spondyloarthritis (axSpA) patients with the muscle powers of healthy volunteers and to examine the relationship of muscle strengths with disease activity, functionality, and disability.

Method: One hundred males (50 axSpA, 50 healthy) were included in the study. Bath Disease Activity Index (BASDAI), Functional Index (BASFI), and Health Assessment Questionnaire-Disability Index (HAQ-DI) scores were recorded. The maximum (max) and mean cervical flexion, extension, lateral flexion (CF, CE, CLF), truncal flexion, extension (TF, TE), root joint flexion, extension, abduction, internal and external rotation (SF, SE, SAB, SIR and SER for the shoulder; HF, HE, HAB, HIR and HER for the hip) muscle strengths of the patients in both groups were measured by a handheld dynamometer. Total muscle strength (CT, TT, ST, HT) was found according to the sum of the max and mean values for each region.

Results: All muscle strengths were lower in the axSpA group compared to the healthy volunteers. The symptom duration was found to have a weak-moderate negative correlation with CT, TT, ST, HT and all individual muscle strengths except for the TE, CF, HIR, and HER. BASDAI and HAQ-DI had weak-moderate negative correlations with HIR and HER. BASFI had a weak-moderate negative correlation with cervical measurements, TE, TF, SF, SER, SIR, and hip measurements.

Conclusion: All muscle strengths were lower in patients compared to healthy volunteers. Strengthening specific muscle groups for the desired goal can be a reasonable strategy. The study is prospectively registered and available at www.clinicaltrials.gov (NCT04435860).

KEYWORDS

ankylosing spondylitis, handheld dynamometer, manual muscle tester, muscle power

1 | INTRODUCTION

Axial spondyloarthritis (axSpA) refers to a group of systemic inflammatory rheumatic diseases that cause inflammation and stiffness, particularly in the spine, resulting in pain and limitations in physical efficiency as well as quality of life. The spine is primarily affected, and there is milder peripheral involvement.[1] Ankylosing spondylitis (AS) constitutes the prototype of these diseases.[2]

Decreased muscle strength has been reported in axial SpA patients compared to healthy controls.[3-5] In addition, some studies have found a decreased lean mass and increased fat mass in axSpA patients compared to controls.[5,6] AxSpA is a disease in which abnormal proinflammatory cytokines are secreted by innate immune cells, and the overexpression of tumor necrosis factor and other cytokines in this disease may cause the inhibition of the pathways that lead to muscle hypertrophy as well as the activation of proteolytic pathways in the muscle.[7-9] According to another perspective, inflammation, pain, joint stiffness, and enthesitis observed in axSpA patients may lead to inactivity, fatigue, and muscle weakness. Inactivity can cause the instability of the joint and can affect the muscle strength secondarily.[10] In the literature, the studies on muscle involvement in this patient group have focused on sarcopenia.[11,12] Although muscle mass is a factor affecting muscle strength, there may be factors other than muscle mass, which affect muscle strength and, therefore, functionality in inflammatory rheumatic diseases. Therefore, measuring muscle mass may be insufficient for determining how the functionality of the patient is affected.

The European League Against Rheumatism (EULAR) published physical activity recommendations for patients with SpA and focused on 4 types of activity (aerobics, flexibility, strengthening, and neuromotor exercises).[13] Also, physical activity is strongly recommended for active axSpA patients in the recommendations of the American College of Rheumatology (ACR)/Spondyloarthritis Research and Treatment Network (SPARTAN) in 2019.[14] The importance of exercise for axSpA in treatment is generally accepted; however, specific exercise programs have not been established for this disease in the literature. In these recommendations, there is no clear consensus on the most effective exercise type, the intensity and frequency of the exercises to be performed, and the muscle groups that should be addressed more intensely.[15,16]

In patients with SpA, the muscles, particularly the paravertebral muscles, are affected. Restriction in spinal movements is a well-known consequence of the disease process, and paravertebral muscle atrophy is observed due to this restriction.[17,18] In extraspinal involvement in this group of diseases, the root joints (shoulder and hip) are usually involved, and enthesitis is common in these joints.[19] Even though this involvement is assumed, no study has ever evaluated the strength of the main involved spine and root joint-related muscles neither separately nor in one study so far. In this context, our aim is to determine how the spine-related muscle groups and proximal girdle muscle strengths are affected in axSpA patients compared to healthy volunteers and to determine the relationship of the affected muscle groups with disease activity, functionality, and disability.

2 | METHODS

2.1 | Study design and patient enrollment

A total of 100 volunteers (50 with axSpA, 50 healthy), who presented to the outpatient clinic of the corresponding author, were included in this cross-sectional study. The study was approved by the institutional ethics committee (No: 08/115), and written informed consent was obtained from the participants. The study was carried out per the "Declaration of Helsinki, Ethical Principles for medical research involving human subjects." The study is prospectively registered and available at www.clinicaltrials.gov from 17 June, 2020 (ID: NCT04435860). Inclusion criteria for the axSpA group were as follows: (a) having been diagnosed with axSpA according to the 2010 criteria of the Assessment for Spondyloarthritis International Society (ASAS) (1); (b) to be of the male gender; (c) to be aged ≥ 18. Exclusion criteria were as follows: (a) presence of severe cardiac/pulmonary/renal disease; (b) accompanying fibromyalgia; (c) severe psychiatric disorder; (d) other diseases that may cause a restriction in the spine, pelvic, and shoulder girdle; (e) myopathy, neuropathy, and radiculopathy that may cause deficits in muscle strength; (f) use of high-dose corticosteroids; (g) endocrinological disorder (such as thyroid, parathyroid disorder); (h) malignancy. The control group was composed of healthy volunteers who did not meet the exclusion criteria. Since there was no similar study to the present study, effect sizes were calculated based on the data after the preliminary analysis of 10 patients for each group. A minimum of 50 patients was considered sufficient for each group (for maximum truncal extension strength [TE_{max}]), with 99% power, 0.91 standard effect size, and .05 significance. TE_{max} was selected for presenting the most significant dispersion.

Demographic data of patients and healthy individuals, laboratory parameters of patients were recorded by the same physician, and patients filled out clinical assessment questionnaires. Then, muscle strength measurements were made by a different evaluator. All evaluations were made on the same day. Data analysis and interpretation were performed by a 3rd physician.

2.2 | Clinical assessments

Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), and Health Assessment Questionnaire-Disability Index (HAQ-DI) obtained from the patients were recorded. The BASDAI consists of a 0 through 10 scale with 6 questions.[20] On the other hand, BASFI examines the functional status of the patient by Numerical Rating Scale with 10 questions. The mean of the 10 answers yields the BASFI score, which is a value between 0 and 10.[21] A worsened patient condition

was indicated with a higher Bath index. HAQ-DI is a 20-item questionnaire in which daily life activities are examined and is generally used in rheumatic diseases. The mean of the responses is calculated, and the total score varies between 0 and 3. A high score indicates a high level of disability.[22]

2.3 | Muscle strength measurements

Muscle strength was measured with a manual muscle tester (Lafayette Instrument Company, Lafayette, IN, USA). This device can measure the maximum and the mean force in kilograms that can be applied in 10 seconds. The patients were asked to perform the desired movement with the maximum force they could apply for 10 seconds. To illustrate with an example, the supervisor described each movement on the patient once before the measurement and allowed the patient to rehearse. Next, the patient performed the movement. Measurements were made with 3 isometric contractions for each region, and there was a minimum of 30 seconds between measurements.[23] The maximum (max) and the mean value of 10 seconds were obtained for each measurement, and the highest of the 3 measurements was recorded. In order to determine the total max and mean muscle strength of the relevant joint region, the max and mean muscle strengths of all muscles in that region were summed up; thus, the HT, ST, TT, and CT values were determined. The mean of both sides was recorded in bilateral measurements, and the muscle strengths on the dominant side were tested in unilateral measurements.

Isometric muscle strength measurements of the cervical region were performed in a sitting position. The measurement cap of the dynamometer was placed in the middle of the forehead slightly above the eyebrows for CF, slightly above the external occipital protuberance for CE, and on the lateral aspect of the head above the ear for CLF[23] (Supplement 1). Isometric TF was measured by placing the patient in the supine position with the hip at 30 degrees and the knees in a straight position, and the measurement cap was placed on the sternum just below the suprasternal notch. Isometric TE was measured when the patient was in the prone position, with the hips and knees in the neutral position, and the measurement cap was placed at the level of the T4 vertebra[24] (Supplement 1). The measurement positions and methods, which were described by Katoh et al.,[25] were used for the measurements of the shoulder girdle, and the SF, SE, shoulder abduction (SAB), internal rotation (SIR) and external rotation (SER) values were obtained (Figure 1). The patient was placed in a sitting position with the hip and knee at 90 degrees, and the measurement cap was placed 5 cm above the patella on the anterior part of the distal thigh for HF. For HE, the measurement cap was placed 5 cm proximal to the knee behind the thigh when the patient was in the prone position. For hip abduction (HAB), the measurement was performed by placing the measurement cap on the lateral malleolus when the patient was in the supine position. For the internal rotation (HIR) and external rotation (HER) of the hip, the patient was placed in the prone position with the knees at 90 degrees of flexion. The cap was placed 5 cm proximal to the medial and lateral malleolus[26] (Supplement 2). The techniques described in the previous studies and proven for reliability with a handheld dynamometer were preferred for the measurements.

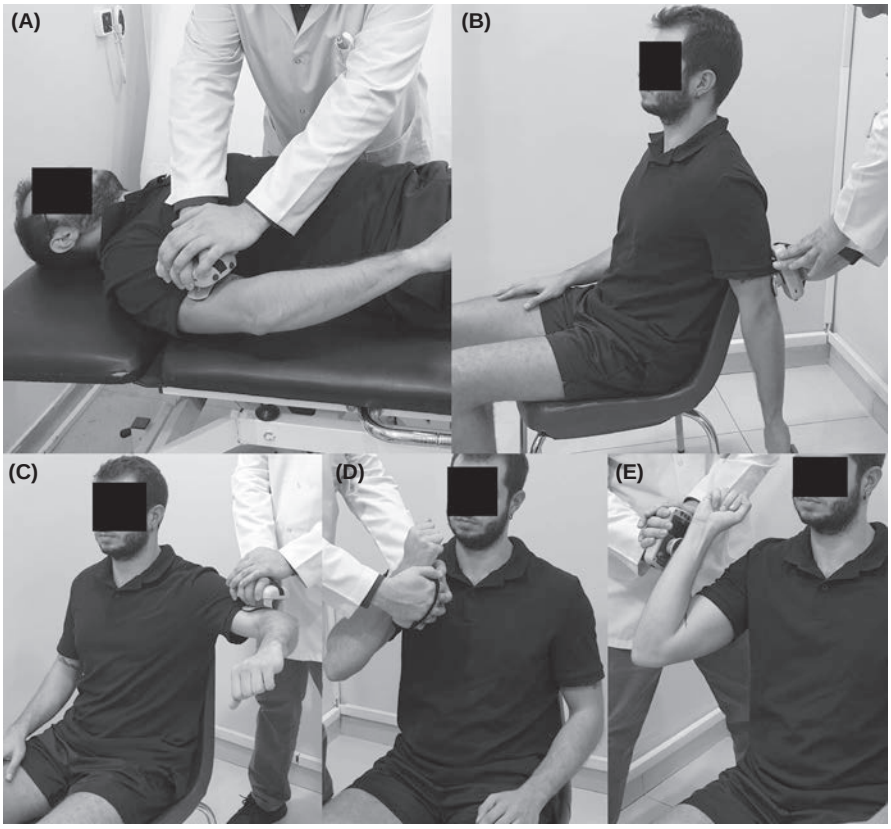

FIGURE 1 Shoulder girdle measurements (A) flexion, (B) extension, (C) abduction, (D) internal rotation, (E) external rotation

2.4 | Statistical analysis

The statistical analysis of the data was performed using the IBM Statistical Package for Social Sciences (SPSS) v23.0 (Armonk, NY, USA). The data distribution was analyzed for normality using the Shapiro-Wilk test. The descriptive statistics of the data were presented as mean ± SD (median [min-max]) for the continuous data and as frequency and percentage (n [%]) for categorical variables. In the comparison of 2 independent groups, the independent samples *t* test was used for normally distributed continuous data, and the Mann-Whitney *U* test was used for non-normally distributed continuous data. Pearson Chi-square and Fisher exact tests were used in the analysis of the categorical variables. Correlation analysis with Pearson correlation coefficient was used to determine the relationship between 2 independent variables in normally distributed continuous data. The relationships were interpreted as strongly, moderately, weakly, and negligibly correlated when $r \geq .70$, $r = .40$-$.69$, $r = .10$-$.39$, and $r \leq .10$, respectively.[27] The level of significance was determined as $\alpha = .05$.

3 | RESULTS

The study included 50 volunteers with axSpA (19 with non-radiographic axSpA and 31 with AS) and 50 healthy volunteers. The mean age was 39.88 ± 11.85 years for the axSpA group and 39.4 ± 10.52 years for the control group. Body mass index (BMI) was 25.95 ± 4.66 kg/m^2 in the patient group, while it was 26.2 ± 2.74 kg/m^2 in the control group. The demographic data and the data of the patient and control groups are presented in Table 1. There was no

TABLE 1 Demographic and clinical characteristics of the study population

	axSpA (50)	Control (50)	
	Mean ± SD Median (min-max)/ n (%)	Mean ± SD Median (min-max)/ n (%)	*P* value
Age, y	39.88 ± 11.85	39.4 ± 10.52	.831(t)
	39 (18-68)	38 (20-67)	
Height, cm	176.2 ± 6.72	176.96 ± 5.65	.542(t)
	175 (162-189)	176 (167-187)	
Weight, kg	80.58 ± 15.27	82.0 ± 9.1	.312(m)
	80 (50-123)	80 (63-103)	
BMI, kg/m^2	25.95 ± 4.66	26.2 ± 2.74	.746(t)
	25.75 (17.3-37.65)	25.73 (19.02-31.1)	
Educational status			
Primary school	9 (18.0%)	9 (18.0%)	.594**
Secondary school	10 (20.0%)	5 (10.0%)	
High school	17 (34.0%)	16 (32.0%)	
University	12 (24.0%)	16 (32.0%)	
Postgraduate	2 (4.0%)	4 (8.0%)	
Hand dominancy			
Right	47 (94.0%)	46 (92.0%)	1**
Left	3 (6.0%)	4 (8.0%)	
Smoking at anytime			
No	32 (64.0%)	36 (72.0%)	.52*
Yes	18 (36.0%)	14 (28.0%)	
Alcohol consumption			
No	44 (88.0%)	43 (86.0%)	1*
Yes	6 (12.0%)	7 (14.0%)	
Exercise habit			
None	23 (46.0%)	27 (54.0%)	.676*
1-2/wk	15 (30.0%)	14 (28.0%)	
≥3/wk	12 (24.0%)	9 (18.0%)	

Note: (t) independent samples *t* test; (m) Mann-Whitney *U* test.

axSpA, axial spondyloarthritis; BMI, body mass index.

*Pearson Chi-squared test.

**Fisher exact test.

TABLE 2 Comparison of two groups in terms of muscle strength

	Mean ± SD / Median (min-max)				Mean ± SD / Median (min-max)		
	axSpA (n = 50)	Control (n = 50)	*P* value		axSpA (n = 50)	Control (n = 50)	*P* value
HAB_{mean}	17.06 ± 6.47 16.7 (7.4-35.3)	21.04 ± 5.66 20.05 (11.5-35.4)	.002(m)	SER_{max}	16.62 ± 6.21 16.75 (6.1-30.7)	19.93 ± 4.67 20.25 (9.5-29.9)	.003(t)
HAB_{max}	21.58 ± 8.88 20.05 (8.6-44.7)	27.37 ± 8.17 25.7 (14.7-47.8)	.001(m)	SF_{mean}	15.03 ± 6.98 14.3 (4.6-30.2)	19.75 ± 6.97 19.95 (8-36)	.001(m)
HE_{mean}	18.21 ± 7.27 17.85 (6.8-32.5)	25.0 ± 6.2 25.75 (8.5-34.4)	<.001(t)	SF_{max}	19.4 ± 9.44 18.35 (5.4-42)	26.66 ± 10.03 28.3 (9.7-47.8)	.001(m)
HE_{max}	22.99 ± 10.69 20.4 (2.5-43.6)	32.45 ± 9.02 34.9 (9.8-51.3)	<.001(t)	SIR_{mean}	14.3 ± 5.44 14.2 (4.4-28.5)	16.41 ± 4.13 16.35 (9.3-27.6)	.032(t)
HER_{mean}	9.95 ± 3.34 9.6 (1.7-19)	11.94 ± 2.84 11.85 (6.6-18.2)	.002(t)	SIR_{max}	17.74 ± 6.46 17.35 (4.8-31.6)	20.98 ± 5.58 20.8 (12.3-35)	.008(t)
HER_{max}	12.11 ± 4.09 11.25 (3.5-25.2)	14.76 ± 3.46 14.4 (8.2-24.3)	.001(t)	CE_{mean}	8.63 ± 4.03 8.45 (0.4-17.7)	11.69 ± 3.64 11.15 (6-24.9)	<.001(m)
HF_{mean}	21.73 ± 10.68 18 (7.9-42.6)	28.37 ± 8.92 28.3 (9.6-48.7)	.001(m)	CE_{max}	10.85 ± 4.86 10.9 (3-22)	14.35 ± 4.34 14.1 (6.8-29.2)	<.001(m)
HF_{max}	28.27 ± 14.28 24.75 (8.9-54.5)	37.03 ± 12.09 37.3 (12.2-58.8)	.001(m)	CF_{mean}	8.82 ± 3.17 8.65 (3.8-15.3)	10.66 ± 2.92 10.2 (6.8-21)	.004(m)
HIR_{mean}	12.78 ± 3.98 11.9 (5.8-21.2)	14.89 ± 3.25 14.9 (8.1-21.1)	.005(t)	CF_{max}	10.68 ± 3.84 10.75 (4.5-18.2)	13.27 ± 3.7 12.55 (7.4-23.8)	.002(m)
HIR_{max}	15.7 ± 4.68 15 (6.8-23)	19.2 ± 4.65 19.05 (9.5-34.1)	.001(m)	CLF_{mean}	8.55 ± 3.4 8.1 (3.45-16.2)	11.14 ± 2.66 10.85 (6.6-21.15)	<.001(m)
SAB_{mean}	13.92 ± 5.34 14.1 (4.3-24.1)	19.1 ± 5.71 18.25 (8.3-32.4)	<.001(t)	CLF_{max}	10.32 ± 4.17 9.4 (4.2-20.35)	13.29 ± 3.24 12.68 (8.1-25.55)	<.001(m)
SAB_{max}	17.49 ± 6.75 17.95 (5.1-33.8)	24.6 ± 7.95 24.45 (11.9-42.4)	<.001(t)	TE_{mean}	15.17 ± 7.03 15.4 (2.4-28.5)	20.11 ± 4.63 20.05 (12.1-32.4)	<.001(m)
SE_{mean}	12.69 ± 4.49 12.55 (5-22.5)	16.51 ± 5.07 16.3 (7.5-31.2)	<.001(t)	TE_{max}	19.12 ± 9.54 17.4 (6.7-37.4)	25.48 ± 6.95 25.05 (9.9-42.3)	.001(m)
SER_{max}	16.32 ± 6.23 15.9 (6-28.7)	20.73 ± 6.44 20.25 (9.6-37.3)	.001(t)	TF_{mean}	15.51 ± 7.0 15 (4.3-28.3)	18.93 ± 5.07 19.25 (10.4-28)	.008(m)
SER_{mean}	13.54 ± 4.84 13.25 (5.1-23.7)	16.51 ± 3.75 17.05 (7.9-23.7)	.001(t)	TF_{max}	19.73 ± 9.61 18.8 (4.9-39.7)	25.06 ± 7.17 25.9 (12.4-39.8)	.002(m)

Note: (t): independent samples *t* test, (m): Mann-Whitney *U* test.

axSpA, axial spondyloarthritis; max, maximum; CE, cervical extension; CF, cervical flexion; CLF, cervical lateral flexion; HAB, hip abduction; HE, hip extension; HER, hip external rotation; HF, hip flexion; HIR, hip internal rotation; SAB, shoulder abduction; SE, shoulder extension; SER, shoulder external rotation; SF, shoulder flexion; SIR, shoulder internal rotation; TE, truncal extension; TF, truncal flexion.

significant difference between the 2 groups in terms of age, weight, height, BMI, educational status, hand dominancy, smoking and alcohol use, and exercise habits. The clinical features of the axSpA patients were as follows: the symptom duration (months) = 57.47 ± 90.73 (24 [3-360]), BASDAI = 4.24 ± 2.22 (4.35 [0-9.3]), BASFI = 2.47 ± 2.05 (2.15 [0-7.1]), HAQ-DI = 0.26 ± 0.24 (0.25 [0-1]), erythrocyte sedimentation rate (ESR) (mm) = 13.24 ± 13.72 (9.5 [2-60]) (N: 0-15) and C-reactive protein (CRP) (mg/L) = 13.13 ± 19.43 (5.11 [0.2-78]) (N: 0-5). Human leukocyte antigen-B27 was positive in 58% of the patients.

When the measurements of the muscle strengths of the control and axSpA groups were compared, a significant decrease was found in the axSpA group compared to the control in all muscle groups (Table 2). In terms of the total muscle strengths of the relevant regions, a significant decrease was found in the axSpA group compared to the control group (Table 3).

When the relationship of the total muscle strengths with laboratory tests was examined, no muscle group was related to ESR and CRP. Symptom duration was found to have a moderate negative correlation with ST_{max}, ST_{mean}, HT_{max} and HT_{mean} (r = −.4, −.42, −.4

TABLE 3 Comparison of total muscle strength according to regions

	Mean ± SD Median (min-max)		
	axSpA (n = 50)	Control (n = 50)	*P* value
Hip $total_{mean}$	79.72 ± 28.24 77.2 (38.1-135.9)	101.24 ± 21.57 101.1 (46.2-147.3)	<.001(t)
Hip $total_{max}$	100.65 ± 37.74 95.9 (44-175.4)	130.8 ± 30.28 129.85 (56.7-193.1)	<.001(t)
Shoulder $total_{mean}$	69.48 ± 24.38 71.15 (24.3-115.8)	88.28 ± 23.14 90.05 (44-149.1)	<.001(t)
Shoulder $total_{max}$	87.56 ± 31.91 86.8 (28.5-151.6)	112.9 ± 31.27 112.45 (59.9-184.8)	<.001(t)
Cervical $total_{mean}$	26.01 ± 10.18 24.5 (10.05-48.5)	33.49 ± 8.21 32.03 (19.5-60.7)	<.001(m)
Cervical $total_{max}$	31.86 ± 12.32 30.08 (13.75-59.15)	40.9 ± 10.37 39.63 (23.2-73.9)	<.001(m)
Truncal $total_{mean}$	30.68 ± 13.47 29.1 (10.3-56)	39.04 ± 8.96 39.95 (25.4-58.6)	.002(m)
Truncal $total_{max}$	38.85 ± 18.52 35.95 (11.6-71.1)	50.54 ± 13.45 51.1 (23.6-76.5)	.001(m)

Note: (t) independent samples *t* test, (m) Mann-Whitney *U* test.
axSpA, axial spondyloarthritis; max, maximum.

TABLE 4 Correlations between clinical features and total muscle strengths

	r					
	ESR	CRP	Symptom duration	BASDAI	BASFI	HAQ-DI
TT_{max}	-.09	-.18	-.34*	-.19	-.41**	-.17
TT_{mean}	-.04	-.12	-.35*	-.2	-.4**	-.19
ST_{max}	.03	-.13	-.4**	-.16	-.32*	-.06
ST_{mean}	.05	-.11	-.42**	-.19	-.31*	-.09
HT_{max}	-.03	-.21	-.4**	-.21	-.43**	-.14
HT_{mean}	-.02	-.22	-.4**	-.24	-.45**	-.19
CT_{max}	.06	-.06	-.32*	-.23	-.35*	-.12
CT_{mean}	.05	-.07	-.31*	-.24	-.35*	-.13

Note: r, Pearson correlation test coefficient; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; max, maximum; TT, truncal total; ST, shoulder total; HT, hip total; CT, cervical total; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; HAQ-DI, Health Assessment Questionnaire-Disability Index.
*P value < .05.
**P value < .01.

and −.4, respectively); and a weak negative correlation with TT_{max}, TT_{mean}, CT_{max} and CT_{mean} (r = −.34, −.35, −.32 and −.31, respectively). In terms of clinical evaluation scales, BASFI was found to have a moderate negative correlation with TT_{max}, TT_{mean}, HT_{max}, and HT_{mean} (r = −.41, −.4, −.43 and −.45, respectively); and a weak negative correlation with ST_{max}, ST_{mean} CT_{max} and CT_{mean} (r = −.32, −.31, −.35 and −.35, respectively). BASDAI and HAQ-DI had no relationship with the total strength of any muscle groups (Table 4).

When the muscle groups were evaluated individually, no relationship was found in any muscle group with CRP and ESR. In terms of the symptom duration, TF_{max}, TF_{mean}, SF_{max} and SF_{mean} were correlated moderately and negatively, while all other shoulder girdle measurements were correlated weakly and negatively. No relationship was found with HIR_{max}, HIR_{mean}, HER_{max}, and HER_{mean}; however, a weak negative correlation was found with HE_{max} (r = −.39), and moderate negative correlations were found in all remaining

TABLE 5 Correlation between strength of individual muscle groups and clinical parameters

	r					
	ESR	CRP	Symptom duration	BASDAI	BASFI	HAQ-DI
TF_{max}	-.03	-.12	-.41**	-.14	-.36*	-.13
TF_{mean}	-.01	-.1	-.42**	-.18	-.37**	-.17
TE_{max}	-.15	-.24	-.25	-.22	-.44**	-.19
TE_{mean}	-.06	-.14	-.26	-.21	-.39**	-.2
SF_{max}	.01	-.13	-.4**	-.07	-.31*	-.02
SF_{mean}	0	-.15	-.43**	-.11	-.29*	-.05
SE_{max}	.06	-.1	-.34*	-.15	-.2	-.05
SE_{mean}	.08	-.09	-.36*	-.13	-.2	-.03
SAB_{max}	.08	-.05	-.37**	-.21	-.25	.03
SAB_{mean}	.06	-.03	-.38**	-.21	-.24	.01
SER_{max}	-.01	-.16	-.39**	-.18	-.36*	-.16
SER_{mean}	-.03	-.16	-.38**	-.21	-.36**	-.17
SIR_{max}	.01	-.17	-.29*	-.14	-.32*	-.11
SIR_{mean}	.11	-.05	-.32*	-.21	-.29*	-.2
HF_{max}	.01	-.14	-.41**	-.12	-.35*	-.05
HF_{mean}	.01	-.16	-.4**	-.14	-.38**	-.09
HE_{max}	-.11	-.2	-.39**	-.14	-.43**	-.06
HE_{mean}	-.08	-.19	-.4**	-.13	-.39**	-.07
HAB_{max}	0	-.18	-.51**	-.14	-.34*	-.08
HAB_{mean}	-.01	-.19	-.5**	-.2	-.37**	-.13
HIR_{max}	-.03	-.26	-.11	-.41**	-.46**	-.38**
HIR_{mean}	0	-.22	-.17	-.4**	-.46**	-.37**
HER_{max}	.05	-.26	0	-.34*	-.41**	-.38**
HER_{mean}	.02	-.35	-.08	-.41**	-.49**	-.45**
CF_{max}	-.01	-.09	-.22	-.19	-.37**	-.11
CF_{mean}	0	-.1	-.21	-.2	-.37**	-.13
CE_{max}	.04	-.05	-.29*	-.23	-.28	-.09
CE_{mean}	-.02	-.09	-.28	-.26	-.31*	-.11
CLF_{max}	.14	-.04	-.41**	-.25	-.38**	-.16
CLF_{mean}	.18	-.02	-.4**	-.22	-.35*	-.13

Note: r, Pearson correlation test coefficient; max, maximum; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; CE, cervical extension; CF, cervical flexion; CLF, cervical lateral flexion; HAB, hip abduction; HE, hip extension; HER, hip external rotation; HF, hip flexion; HIR, hip internal rotation; SAB, shoulder abduction; SE, shoulder extension; SER, shoulder external rotation; SF, shoulder flexion; SIR, shoulder internal rotation; TE, truncal extension; TF, truncal flexion; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; HAQ-DI, Health Assessment Questionnaire-Disability Index.

*P value < .05.

**P value < .01.

measurements of the hip. Also, symptom duration had a negative and moderate correlation with CLF_{max} and CLF_{mean}, and a weak negative correlation with CE_{max}. Regarding the relationship with BASDAI, a moderate negative relationship was found with HIR_{max}, $HIR_{mean,}$ and HER_{mean}, and a weak negative relationship was found with HER_{max}. BASFI had a moderate negative correlation with the TE_{max}, and a weak correlation with the remaining truncal muscle measurements. BASFI was determined to have a weak and negative correlation with flexor, internal rotator, and external rotator muscles of the shoulder girdle; however, no correlation was found with extensor and abductor muscles. A moderate negative correlation was determined between BASFI and HE_{max}, HIR_{max}, HIR_{mean}, HER_{max}, and HER_{mean} in the hip girdle, while weak and negative correlations were determined with other hip-related muscle groups.

BASFI had a weak negative correlation with CF_{max}, CF_{mean}, CE_{mean}, $CLF_{max,}$ and CLF_{mean} in the cervical region, and there was no correlation with CE_{max}. HAQ-DI was found to have a weak negative correlation with only HIR_{max}, HIR_{mean}, and HER_{max} and a moderate negative correlation with HER_{mean} (Table 5).

4 | DISCUSSION

Inflammation is the primary mechanism in patients with axSpA, and it leads to the loss of skeletal muscle secondarily by causing pain and stiffness.[5] It has been suggested that muscle strength in patients with axSpA would decrease due to the inflammatory process; however, the muscle strengths associated with the main involved regions have not been evaluated in a single study. Recommendations of exercise made on this matter are far from the specifications suggesting the effects of the strengths of individual muscle groups on disease parameters. Some of the studies conducted with patients with axSpA have evaluated muscle mass, namely sarcopenia, rather than muscle strength. In various studies, it has been found that sarcopenia is increased in AS patients.[11,12] In these studies, methods such as skeletal muscle index were used in the evaluation of sarcopenia.[28] These types of measurement methods are far from evaluating the muscle groups that are affected and determining the muscles that should be included in the strengthening program. It is difficult to determine the muscles that the muscle power distribution is in favor of and against in the total muscle mass. For this reason, we found it more appropriate to measure the muscle strengths of the muscle groups that could be measured objectively by handheld dynamometer; and we excluded females for the uniformity of the groups. We found that both the specific muscle groups and the total muscle strengths of the truncal and cervical muscles and root joints we measured were lower in patients with axSpA compared to the healthy volunteers. We found negative weak-moderate correlations between total muscle strengths and BASFI. Also, we determined weak-moderate correlations between individual muscle strengths and BASDAI, BASFI, and HAQ-DI.

Root joint involvement has been found to be effective upon functionality in patients with axSpA. It was observed that patients with clinical or radiological involvement in the hip joint had worse BASFI values.[29] In fact, BASFI does not contain questions about the hip joint directly; however, it is believed that the involvement of this joint indirectly affects the mobility of the spine and decreases spinal mobility.[30] One study suggested that hip involvement may reduce functional limitation more than spinal involvement by reducing the compensation of impaired spinal mobility.[31] With a similar mechanism, it can be thought that the shoulder girdle should be used more effectively to compensate for the limitation of motion in the thoracic and cervical regions. However, studies on how the muscle strength of the shoulder girdle is affected in patients with axSpA are limited. Hagberg et al.[32] compared the isometric maximum flexion muscle strength of the shoulder at 90 degrees with a strain gage instrument in 8 patients with AS and 10 healthy volunteers in their study, and they found no significant difference. In contrast to that study, we found a decreased muscle strength in patients with axSpA compared to the healthy volunteers in terms of the maximum shoulder flexion. We also found a significant decrease in muscle strengths of other shoulder girdle movements and in the total muscle strength of the shoulder. We observed this decrease both in maximum muscle strength and in the mean muscle strength that can be applied for 10 seconds. According to our results, there was also a weak negative correlation between functionality and total muscle strength of the shoulder.

Limitations of functionality in the hip and shoulder girdle can lead to decreased quality of life and loss of workforce.[30] In our study, weak-moderate correlations were found between hip girdle muscles on BASFI, supporting this statement. In the shoulder girdle muscles, functionality was weakly associated with flexors, internal and external rotators. These results may be obtained due to the fact that BASFI contained questions addressing all muscle groups of hip girdle such as leaning forward, getting up from the ground, walking, as well as the questions addressing flexor and rotator muscle groups of the shoulder girdle such as wearing socks and lying on a shelf. Nevertheless, it may be argued that priority should be given to these muscle groups, which are used more in functionality while strengthening root joints in patients. Interestingly, a negative and moderate relationship was found between BASDAI and the internal and external rotators of the hip. It appeared that only this muscle group was observed to be affected due to the increased disease activity. Further, only the same muscle group seemed to be related to HAQ-DI, which was an indicator of disability. The critical role of the hip rotators in daily living activities and walking has been determined.[33] Nonetheless, why other muscle groups do not exhibit this relationship is a question worth investigating.

In their study, Akgul et al.[34] evaluated the fatty degeneration in the paravertebral muscles semi-quantitatively by magnetic resonance imaging in 36 patients with axSpA (14 with non-radiographic axSpA and 22 with AS). They concluded that there was increased fatty degeneration in the paravertebral muscles of the patients with AS with longer symptom duration compared to patients with non-radiographic axSpA. In our study, we found that symptom duration had a moderate correlation with truncal flexion and cervical lateral flexion muscle strength. Although the mentioned study did not evaluate muscle strength as in our study, it is an important study in terms of demonstrating the histological influence of the paravertebral muscles, and the reflection of these findings to the clinic was also observed in the results of our study. The weak-moderate correlation of cervical and truncal muscle strengths with functionality reveals the importance of these muscle groups in patients with axSpA. The relationship between the duration of symptoms and especially hip, shoulder, and truncal flexors may be manifestations of the postural disorder that develops in the flexor direction on muscle strength.

Sahin et al.[3,4] determined the flexor and extensor muscle strengths of the ankle and knee in 26 patients with AS and 26

healthy controls using an isokinetic dynamometer. They found that muscle strength was lower in patients with axSpA compared to the control group. They found no correlation between muscle strength of the knee and BASFI; however, they found a low correlation between muscle strength of the ankle and BASFI. Although knee and ankle muscle strengths are important for walking, walking alone may not be suitable for evaluating everyday life activities and functionality. In addition, since the patients with axSpA have axial and root joint involvement rather than peripheral lower extremity muscle involvement, it seems more logical to evaluate these muscle groups. In this context, we compared muscle strengths in muscle groups related to the cervical, truncal, and root joints with healthy volunteers in our study, and we found a significant decrease in all muscle groups. We also found weak-moderate correlations with BASFI in various muscle groups we evaluated.

There were some limitations in our study. First of all, peripheral muscle involvement was not examined in this study. Considering that the peripheral joints were affected less than the axial spine without causing deformity in most of the patients, it was thought that the priority was to examine the axial and root joint involvement. However, the effect of particularly the upper extremity muscle strength should be analyzed in future studies. In addition, the lumbar muscle strength was not studied due to the technical limitation of the handheld dynamometer and the inseparability of the effect of the hip girdle. Further, the effects of the hip and shoulder adductors were not studied due to reliability concerns and technical difficulty. Nevertheless, even though disease activity scores varied from 0 to 9.3, the mean BASDAI score was 4.24 ± 2.22, indicating a high disease activity. A future study in patients with low disease activity may be conducted. Also, we did not seek the relationship between enthesitis and strength of related muscles, which may be subject to another study. Finally, the weak-moderate correlations found in the study may seem to be low; however, these correlations are considered to be clinically significant since functionality and disability are affected by many factors.

In conclusion, the strengths of the cervical, truncal, shoulder, and hip joint-related muscles were found to be decreased in patients with axSpA compared to healthy individuals. As the symptom duration increased, all spine and root joint muscles examined were affected at a low or moderate level, except for truncal extensors, cervical flexors, and the internal and external rotators of the hip. Disease activity and level of disability were found to be associated with the rotator muscles of the hip. Although functionality was found to be associated with many muscle strengths examined, it was mostly found to be associated with TE_{max}, HE_{max}, and the rotators of the hip, and not with shoulder extensors and abductors among the muscles that were evaluated. According to the data of our study, muscle strengthening exercises should be an essential part of the treatment in this patient group. In addition, strengthening specific muscle groups for the desired goal can be a reasonable strategy. Broader studies are needed on this subject matter.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: OVY, OEI; methodology: OVY, FB, OEI; formal analysis and investigation: OVY, MK, EK; writing - original draft preparation: OVY, MK, EK; writing - review and editing: FB, TA; supervision: OVY, TA. All authors have read and approved the final manuscript.

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SUPPORTING INFORMATION

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

How to cite this article: Yurdakul OV, Ince OE, Bagcier F, Kara M, Kultur E, Aydin T. Evaluating the strength of spinal and proximal girdle muscles in patients with axial spondyloarthritis: Correlation with activity, disability, and functionality. *Int J Rheum Dis*. 2021;24:701–710. https://doi.org/10.1111/1756-185X.14102

Received: 2 December 2020 | Revised: 20 February 2021 | Accepted: 6 March 2021

DOI: 10.1111/1756-185X.14103

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases

WILEY

Association of serum CCL20 levels with pulmonary vascular involvement and primary biliary cholangitis in patients with systemic sclerosis

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Funding information
Ministry of Health, Labor, and Welfare of Japan, Grant/Award Number: grant for Research in Intractable Diseases

Abstract

Aim: Systemic sclerosis (SSc) is a chronic autoimmune disease resulting in vasculopathy and fibrosis of the skin and major internal organs. Especially, interstitial lung disease and pulmonary arterial hypertension are the leading causes of mortality. C-C motif ligand 20 (CCL20) is known as a homeostatic and inflammatory chemokine, which is associated with fibrosis and angiogenesis and constantly expressed in organs involved in SSc. Therefore, we investigated the potential contribution of CCL20 to the development of SSc.

Method: We conducted cross-sectional analyses of 67 SSc patients and 20 healthy controls recruited in a single center for 9 years. Serum CCL20 levels were measured by enzyme-linked immunosorbent assay. Statistical analyses were performed with the Mann-Whitney *U* test, the Kruskal-Wallis test followed by Dunn's multiple comparison test, Fisher's exact probability test and the Spearman's rank correlation coefficient.

Results: SSc patients had significantly higher serum CCL20 levels than healthy controls. In SSc patients, serum CCL20 levels correlated inversely with the percentage of predicated diffusion lung capacity for carbon monoxide and positively with mean pulmonary artery pressure (mPAP). In addition, SSc patients with increased serum CCL20 levels had anti-mitochondrial antibody M2 titer significantly elevated relative to those with normal levels, and SSc patients with asymptomatic primary biliary cholangitis (PBC) possessed higher serum CCL20 levels than those without. Importantly, serum CCL20 levels were associated positively with mPAP values and PBC presence by multivariate regression analysis.

Conclusion: Serum CCL20 levels may be involved in the development of pulmonary vascular involvement leading to pulmonary arterial hypertension and asymptomatic PBC in SSc patients.

KEYWORDS
anti-mitochondrial M2 antibody, CCL20, primary biliary cholangitis, pulmonary artery hypertension, systemic sclerosis

1 | INTRODUCTION

Systemic sclerosis (SSc) is a chronic autoimmune disease with unknown specific etiology. Fibrosis, vasculopathy and immune abnormalities cause various complications, such as skin fibrosis, interstitial lung disease (ILD), pulmonary artery hypertension (PAH), cardiac fibrosis, gastroesophageal reflux disease, scleroderma renal crisis, and so on.[1-3] In addition to SSc-related complications, other organ-specific autoimmune diseases, such as Hashimoto's thyroiditis and primary biliary cholangitis (PBC), are frequently concomitant with this disease.[4,5] Under the developmental process of these complications, there seems to be an SSc-specific orchestrated network of cytokines, growth factors, chemokines and cell adhesion molecules, leading to the activation of various cell types, including fibroblasts, endothelial cells and immune cells.[6,7]

Chemokines are substances which attract immune cells to appropriate body parts under physiological and pathological conditions. It is now well known that chemokines affect numerous biological processes other than chemotaxis, such as cell growth, autoimmunity, inflammation and angiogenesis.[8,9] In SSc, chemokines play a part in promoting the earliest perivascular infiltration of mononuclear cells after initial vascular injury.[10] This earliest lesion subsequently triggers autoimmune and inflammatory reactions by attracting much more immune cells.[10,11] So far, various chemokines have been reported to contribute to the early, active and late stages of SSc.[10] For example, C-C motif ligand 3 (CCL3) and CCL5 are elevated in very early stage, in which monocytes and T-helper (Th) cells are recruited and cause tissue damage.[10] In later stage, CCL2, CCL7, C-X-C motif ligand 10 (CXCL10) and CXCL12 are involved in fibroblast recruitment and activation.[10] Moreover, various chemokines have been shown to be related to major organ involvement: (a) CCL2 and CXCL4 for skin sclerosis; (b) CCL2, CCL18, CXCL4 and CXCL10 for ILD; (c) CXCL4 for PAH.[10,12] Also, CXCL4 and CXCL8 can serve as general prognostic indicators of SSc.[13,14] Thus, chemokines have been already recognized as a member of disease-driving molecules in SSc.

In this study, we focused on CCL20 (also known as macrophage inflammatory protein-3α or liver activation regulated chemokine), which belongs to the CC chemokine group and binds to CCR6 alone. It is a homeostatic and inflammatory chemokine attracting mainly immature dendritic cells, effector/memory T cells and B cells.[9] It has a quite important role to keep steady immune cell trafficking and to initiate T cell-dependent inflammation.[9,15] CCL20 is steadily expressed in the skin, lungs, liver, mucosal surfaces, appendix, lymph nodes and peripheral blood lymphocytes, and can be upregulated when inflammation occurs in those organs.[9] Since CCR6 is expressed on Th17 cells and regulatory T cells, CCL20 attracts them to inflammation sites,[16-18] suggesting that CCL20 controls appropriate amount of inflammation by maintaining delicate balance of offensive and defensive immunity.[17] On the other hand, CCL20 contributes to liver fibrosis[19,20] and angiogenesis in malignant tumors.[21-23] Thus, CCL20 appears to be involved in autoimmunity/inflammation, vasculopathy and tissue fibrosis, which are the 3 cardinal pathological features of SSc.[2,3,24]

Based on these backgrounds, we examined if CCL20 is involved in the development of SSc-associated symptoms and complications. To this end, we investigated relations between serum CCL20 levels and clinical symptoms in SSc patients.

2 | MATERIALS AND METHODS

2.1 | Patients

Patients who visited our hospital from February 2010 to May 2019 and met 2013 American College of Rheumatology / European League Against Rheumatism (ACR/EULAR) SSc classification criteria were included in this study.[25] Patients were excluded if they were treated with corticosteroids, other immunosuppressants or bosentan before or at the time of blood sampling. This study included 15 SSc patients with ILD and 4 SSc patients with borderline PAH, but blood samples were drawn from these patients before starting any specific treatments for these complications. Healthy controls were selected from individuals without any history of illness who visited us for the diagnosis of benign skin tumors, such as nevus cell nevi. Written informed consent was taken from all participants. This study was approved by ethics committee (University of Tokyo Graduate School of Medicine). The entire procedures were in accordance with the Declaration of Helsinki. Serum samples were obtained from 67 SSc patients (60 women, 7 men; median [25-75 percentiles], age, 59 years [48.0-68.0]; disease duration, 3.1 years [1.0-9.6]) and 20 healthy controls (17 women, 3 men; age, 52 years [38.3-67.5]). Ages of two groups were not statistically different ($P = .36$). All sera were stored at −80°C. Patients were categorized by LeRoy's classification system:[26] 38 diffuse cutaneous SSc (dcSSc) and 29 limited cutaneous SSc (lcSSc).

2.2 | Clinical assessment

Disease onset was defined as the first clinical event of SSc other than Raynaud's phenomenon. Disease duration was defined as an interval between the disease onset and the time of blood sampling. Skin score was measured by modified Rodnan total skin thickness score (mRSS).[27] ILD was defined as the presence of ground glass

opacity and/or reticular pattern on high-resolution computed tomography. Severity of ILD was evaluated by pulmonary function tests: the percentage of predicted vital capacity (%VC) and the percentage of predicated diffusion lung capacity for carbon monoxide (%DL_{CO}). Serum Krebs von den Lungen 6 (KL-6) levels and serum surfactant protein D (SP-D) levels were determined by chemiluminescent enzyme immunoassay kits, which were purchased from FUJIREBIO (Tokyo, Japan) and Hitachi chemical diagnostic systems (Tokyo, Japan) respectively, with the blood samples used for CCL20 measurement. PAH was determined based on mean pulmonary artery pressure (mPAP), pulmonary artery wedge pressure and peripheral vascular resistance obtained by right heart catheterization. Results of right heart catheterization were available from 21 patients (13 dcSSc and 8 lcSSc). Esophageal involvement was defined as distal esophageal hypomotility on barium-contrast radiography and gastroesophageal reflux disease. Hepatobiliary involvement was defined as serum liver enzyme abnormalities. The definition of PBC was described previously.[28] Anti-mitochondrial antibody M2 (AMA-M2) titer was measured by fluorescence-enzyme immunoassay.

2.3 | Measurement of serum CCL20 levels

Enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) was used to measure serum CCL20 levels (analytical range, 7.8-500 pg/mL; intra-assay coefficient of variation, 2.8%; inter-assay coefficient of variation, 8.9%; average recovery, 103%). Briefly, a polystyrene 96-well plate coated with anti-CCL20 antibodies was incubated with 2-fold diluted serum (linearity of the kit at 1:2 diluent; average % of expected, 104%; range, 98%-107%) for 2 hours at room temperature. The wells were washed and incubated at room temperature for 2 hours with horseradish peroxidase-conjugated anti-CCL20 antibodies. After the wells were washed again, they were impregnated with tetramethylbenzidine and incubated at room temperature for 30 minutes. Finally, sulfuric acid was added to terminate the reaction. The absorbance was measured at 450 nm. Serum CCL20 levels were calculated from a standard curve.

2.4 | Statistical analysis

Statistical analysis was done with the Mann-Whitney *U* test to compare 2 unpaired data with skewed distribution. Comparison between more than 3 groups with skewed distribution was conducted with the Kruskal-Wallis test, and multiple comparisons were done with the Dunn's multiple comparison test. The data with skewed distribution were shown as median with 25-75 percentiles. Fisher's exact probability test was carried out for frequency analyses. Spearman's rank correlation coefficient was used for clinical correlations. Statistical significance was defined as a *P* value of <.05.

3 | RESULTS

3.1 | Association of serum CCL20 levels with clinical and laboratory findings in SSc patients

Serum CCL20 levels were significantly higher in SSc patients than in healthy controls (15.89 pg/mL [9.79-33.33] vs. 9.27 pg/mL [6.36-12.46], $P < .001$). When compared among dcSSc, lcSSc and healthy control participants, dcSSc and lcSSc patients had higher serum CCL20 levels than healthy controls (14.21 pg/mL [8.36-34.0] for dcSSc, 20.56 pg/mL [12.38-32.75] for lcSSc), while there was no significant difference between SSc subtypes (Figure 1). We also assessed the association of serum CCL20 levels with age because the median of age was a little bit higher in the SSc patient group than in healthy controls (59 years [48.0-68.0] vs. 52 years [38.3-67.5], $P = .16$), but age did not affect serum CCL20 levels (supplementary Figure S1).

Demographic characteristics, clinical findings and laboratory variables were compared between SSc patients with increased serum CCL20 levels and those with normal levels (Table 1). Cut-off value was set at 23.49 pg/mL which equals to mean + 2 SDs, calculated from serum CCL20 levels of healthy controls. There was no significant difference in gender, age, disease duration and the ratio of

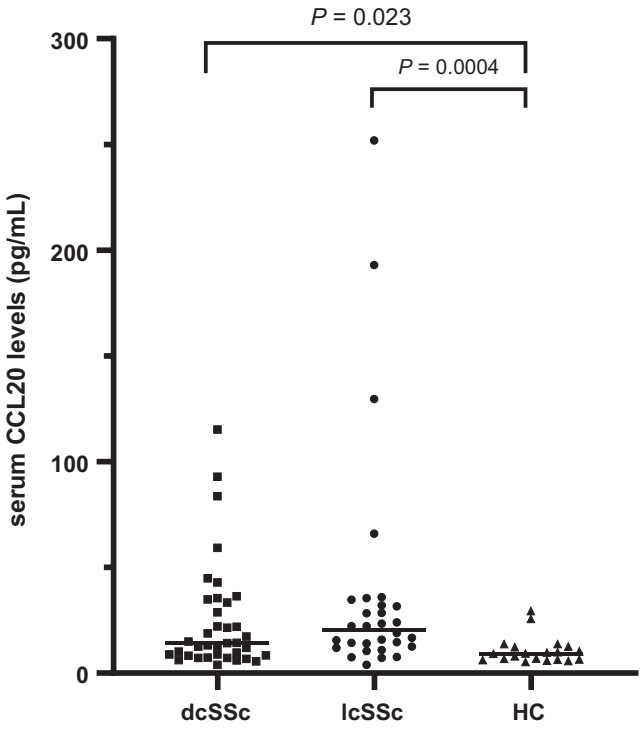

FIGURE 1 Serum C-C motif ligand 20 (CCL20) levels of systemic sclerosis (SSc) patients and healthy controls. Serum CCL20 levels were measured by enzyme-linked immunosorbent assay. Those of diffuse cutaneous SSc (dcSSc) (n = 38), limited cutaneous SSc (lcSSc) (n = 29) and healthy controls (HC) (n = 20) were compared. Horizontal bars represent median of each group. Statistical analysis was conducted with the Kruskal-Wallis test. Dunn's multiple comparison test was used to compare each group

TABLE 1 Association of CCL20 levels with demographic features, clinical manifestations and laboratory data in SSc patients

	Patients with elevated CCL20 levels (n = 23)	Patients without elevated CCL20 levels (n = 44)	*P* values
Demographic features			
Gender, male: female	1:22	6:38	.41
Age, y	66.0 [54.0-69.0] (n = 23)	58.0 [45.3-68.0] (n = 44)	.29
Disease duration, y	4.0 [1.0-14.0] (n = 23)	3.1 [1.0-7.0] (n = 44)	.24
dcSSc:lcSSc	11:12 (n = 23)	26:18 (n = 44)	.44
Clinical findings			
mRSS	6.0 [2.0-10.75] (n = 20)	9.0 [6.0-13.0] (n = 43)	.07
Raynaud's phenomenon	91.3 (21/23)	93.1 (41/44)	>.99
Nailfold bleeding	69.6 (16/23)	79.6 (35/44)	.38
Telangiectasia	38.9 (7/18)	33.3 (11/33)	.76
Pitting scar	36.4 (8/22)	31.0 (13/42)	.78
Digital ulcers	21.7 (5/23)	13.6 (6/44)	.49
Laboratory findings			
%VC	90.0 [74.0-106.0] (n = 23)	94.5 [78.3-102.9] (n = 44)	.49
%DLco	82.0 [61.0-101.0] (n = 23)	87.5 [77.0-100.8] (n = 44)	.17
KL-6, U/mL	541.0 [252.0-969.0] (n = 23)	402.5 [246.3-755.8] (n = 44)	.57
SP-D, ng/mL	113.3 [57.35-182.0] (n = 20)	100.7 [54.13-195.1] (n = 40)	.78
mPAP, mm Hg	18.5 [16.8-21.3] (n = 10)	12.0 [12.0-16.0] (n = 11)	.007
AMA-M2 titer	5.0 [3.4-8.6] (n = 20)	2.4 [1.1-6.8] (n = 44)	.03
Decreased %VC, <80%	43.5 (10/23)	27.3 (12/44)	.27
Decreased %DLco, <70%	39.1 (9/23)	13.6 (6/44)	.03

Note: For CCL20 comparison, median [25-75 percentiles] (number of patients) is shown for each group. For frequent analyses, percentage (number of patients applicable/number of population) are shown.

mRSS, modified Rodnan total skin thickness score; dcSSc, diffuse cutaneous systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; KL-6, Krebs von den Lungen 6; SP-D, surfactant protein D; mPAP, mean pulmonary artery pressure; AMA-M2, anti-mitochondrial M2 antibody; %VC, percentage of predicted vital capacity; $\%DL_{co}$, percentage of predicated diffusion lung capacity for carbon monoxide.

disease subtypes. Modified Rodnan Skin Score (mRSS) values and the frequencies of cutaneous vascular complications, such as Raynaud's phenomenon, nailfold bleeding, telangiectasia, pitting scars and digital ulcers, were comparable between the 2 groups. Although the values of %VC, %DLco, KL-6 and SP-D were not different, mPAP values were significantly higher in SSc patients with increased serum CCL20 levels than in those with normal levels. Additionally, SSc patients with increased serum CCL20 levels had AMA-M2 titer significantly elevated as compared to those with normal levels.

We also conducted frequency analyses (the 2 rows at the bottom of Table 1). The frequency of patients with decreased %DLco was higher in patients with elevated serum CCL20 levels than in those with normal levels. Meanwhile, the frequency of patients with decreased %VC was comparable in the 2 groups.

We further assessed the impact of each clinical symptom on serum CCL20 levels (Table 2). We evaluated %VC/%DLco ratio that enabled us to detect early PAH or to predict future progression to PAH.[29,30] Patients with %VC/%DLco ratio ≥ 1.4 had higher serum CCL20 levels than the others. In addition, patients with hepatobiliary involvement had significantly higher serum CCL20 levels than those without. The hepatobiliary involvement was concomitant with PBC alone in this study.

3.2 | Correlations of serum CCL20 levels with PAH-related parameters in SSc

We further examined if serum CCL20 levels correlate with numeric parameters of SSc-associated complications. Novel relations were

TABLE 2 Comparison of serum CCL20 levels between patients with organ involvement/laboratory findings and those without

	Serum CCL20 levels (pg/mL)		
	Patients with symptoms	Patients without symptoms	*P* values
Organ involvement			
Esophageal dysfunction	14.7 [8.9-31.6] (n = 55)	22.0 [13.3-35.5] (n = 12)	.32
Interstitial lung disease	14.7 [8.9-33.7] (n = 15)	14.5 [11.4-33.8] (n = 52)	.42
Decreased %VC, <80%	22.7 [9.9-37.7] (n = 14)	14.7 [9.4-28.4] (n = 53)	.22
Decreased %DLco, <70%	31.6 [14.4-44.8] (n = 15)	14.5 [9.1-27.3] (n = 52)	.06
Increased %VC/%DLco ratio ≥1.4	22.2 [20.4-88.3] (n = 9)	14.4 [8.8-32.4] (n = 58)	.02
Hepatobiliary involvement	65.9 [17.0-183.5] (n = 5)	15.3 [8.9-31.7] (n = 62)	.04
Laboratory findings			
Increased KL-6, ≥500 U/mL	18.8 [8.9-35.5] (n = 31)	15.7 [10.6-28.5] (n = 36)	.73
Increased SP-D, ≥110 ng/mL	14.4 [86.6-35.4] (n = 29)	14.7 [10.6-33.3] (n = 31)	.96

Note: Median [25-27 percentiles] (number of patients) is shown for each group.

%VC, percentage of predicted vital capacity; $\%DL_{co}$, percentage of predicated diffusion lung capacity for carbon monoxide; KL-6, Krebs von den Lungen 6; SP-D, surfactant protein D.

found in PAH-related parameters. Serum CCL20 levels negatively correlated with $\%DL_{CO}$ in SSc patients ($r = -.26$, $P = .04$; Figure 2A). Furthermore, %VC/%DLco ratio was positively correlated with serum CCL20 levels ($r = .27$, $P = .03$; Figure 2B). Moreover, serum CCL20 levels had a strong positive correlation with mPAP ($r = .68$, $P < .001$; Figure 2C). Importantly, 4 of 21 cases were officially diagnosed as having borderline PAH (mPAP: 21-24 mm Hg), and the remaining had normal mPAP values, namely, no patients had definite PAH. In contrast, serum CCL20 levels did not correlate with %VC, KL6, or SP-D (data not shown).

3.3 | Serum CCL20 levels are associated positively with mPAP values and PBC presence in SSc patients

As described above, serum CCL20 levels correlated with a variety of SSc-related clinical data. To more clearly evaluate the role of CCL20 in SSc, we conducted multivariate regression analysis with stepwise procedure using serum CCL20 levels as the dependent variable and clinical symptoms and data with *P* values of <0.05 in Tables 1 and 2 as independent variables. Anti-M2 antibody titers were not included because there is generally a close correlation between PBC and anti-M2 antibody titers. To convert qualitative data to quantitative values, we employed dummy variables, namely, patients were given a value of 1 if they had the symptom or a value of 0 if they did not have the symptom. First, we conducted the analysis with total SSc patients using %DLco values, %VC/%DLco values and PBC presence as independent variables, showing that %VC/%DLco values and PBC presence were identified as explanatory variables, but adjusted R^2 was low (supplementary Table S1). Second, we carried out the analysis with 21 SSc patients who underwent right heart catheterization, in which %DLco values, %VC/%DLco values, PBC presence and mPAP values were used as independent variables. Of note, we identified mPAP values and PBC presence as explanatory variables ($P = .032$ and $P = .012$, respectively), in which adjusted R^2 was 0.47

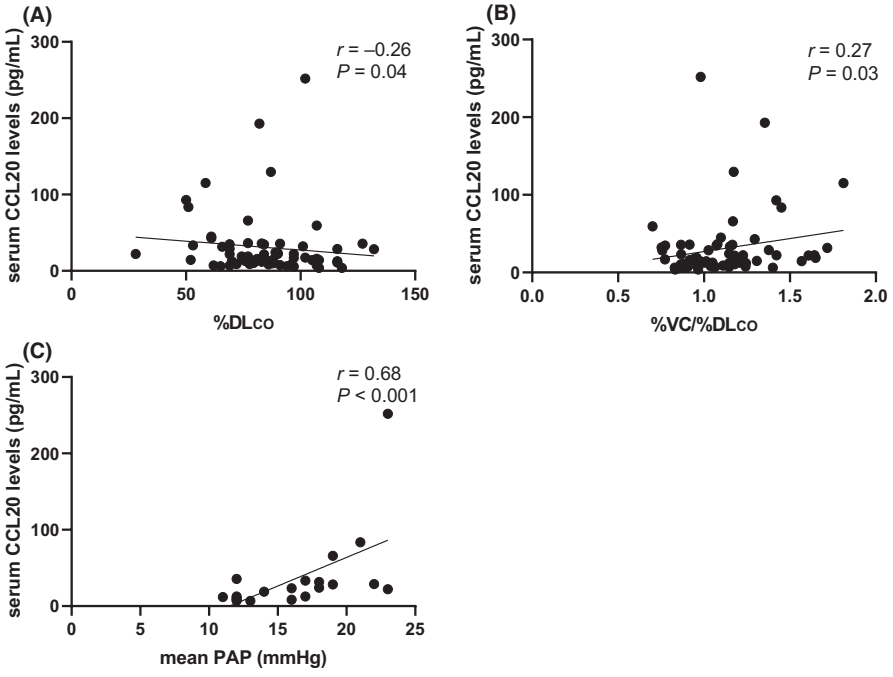

FIGURE 2 Correlation of serum C-C motif ligand 20 (CCL20) levels with clinical parameters in systemic sclerosis (SSc) patients. Serum CCL20 levels of SSc patients were negatively correlated with percentage of predicated diffusion lung capacity for carbon monoxide ($\%DL_{CO}$) (A). Serum CCL20 levels of SSc patients were positively correlated with percentage of predicted vital capacity (%VC)/$\%DL_{CO}$ ratio (B). Serum CCL20 levels of SSc patients were positively correlated with mean pulmonary artery pressure (mPAP) (C). The solid lines represent regression lines

TABLE 3 Factors predicting serum CCL20 levels determined by multiple regression analysis

	Estimate	SE	*P* value
Intercept	-61.62	37.07	.11
mPAP	5.34	2.30	.032
PBC presence	72.11	25.69	.012

Note: Dummy variables were assigned for PBC (the presence of diagnosis = 1, the absence of diagnosis = 0). The multiple regression equation predicting serum CCL20 levels is as follows: serum CCL20 levels = −61.62 + (5.34 × mPAP value) + (72.11 × dummy variable of PBC presence). N = 21, adjusted $R^2 = 0.47$, $P = .0012$.

Abbreviations: CCL20, C-C motif ligand 20; mPAP: mean pulmonary artery pressure, PBC: primary biliary cholangitis.

(Table 3). Therefore, the increase in CCL20 expression may be mainly included in the developmental process of pulmonary vascular involvement leading to PAH and PBC in SSc patients.

4 | DISCUSSION

CCL20 has a wide variety of effects on immune cells, and its abnormality plays a part in autoimmune and inflammatory diseases, such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease and psoriasis.[9,31] With respect to SSc, an excellent study by Tao et al. demonstrated the potential contribution of CCL20 to the development of SSc.[11] According to their study, CCL20 and CCR6 are elevated in the involved skin of early SSc patients, and CCL20 released from cytokine-activated dermal fibroblasts promotes the recruitment of mononuclear cells. Thus, CCL20 seems to be related to the development of SSc. To further understand the contribution of CCL20 to SSc complications, we examined the association of serum CCL20 levels with SSc-related clinical and laboratory findings in this study.

A novel finding of this study is the close relationship between serum CCL20 levels and SSc-association PAH (SSc-PAH). Most strikingly, serum CCL20 levels showed a strong positive correlation with mPAP measured by right heart catheterization. Relevant to this, serum CCL20 levels of SSc patients were correlated negatively with %DLco and positively with %VC/%DLco ratio, and SSc patients with increased %VC/%DLco (≥1.4) had higher serum CCL20 levels than those without. These results are plausible because %DLco usually starts to fall during the early pathological vascular change leading to PAH.[29] To our best knowledge, this is the first report demonstrating the potential association of CCL20 with SSc-PAH. Although only 4 of 21 patients had mPAP high enough to meet the diagnostic criteria of borderline PAH, our results suggest that CCL20 up-regulation may be involved in the development of pulmonary vascular involvement leading to PAH in SSc patients.[32]

Recently, it was reported that CCL20 plays a part not only in inflammation, but also in angiogenesis.[21,33] For instance, human umbilical vein endothelial cells develop more capillary-like structures *in vivo* through the CCL20 activity.[21] Also, CCL20 increases messenger RNA and secretion of vascular endothelial growth factor in breast epithelial cells and subsequent microvessel sprouting.[33] In addition, there are other studies demonstrating CCL20 expression in vascular endothelial cells with or without stimulation.[9,34,35] Given that vascular change leading to SSc-PAH is profoundly attributed to impaired vascular remodeling, such as dysregulated angiogenesis and defective vasculogenesis,[36] our current results may reflect the contribution of CCL20 to dysregulated small vessel angiogenesis underlying SSc-PAH.

Another critical finding of our study is the link between serum CCL20 levels and PBC. As far as we know, this is the first report regarding serum CCL20 levels in patients with PBC, but a previous study demonstrated similar results in alcohol-induced hepatitis. Circulating levels of CCL20, possibly derived from macrophages and hepatic stellate cells, are increased in patients with alcohol-induced hepatitis and correlated with the degree of fibrosis and portal hypertension.[19] This observation suggests the involvement of CCL20 in hepatic fibrosis. Supporting this notion, the following data have been reported: (a) periductal Langerhans cells and biliary epithelial cells produce CCL20 in the context of PBC;[37] (b) Th17 cells attracted by CCL20 to the liver promote the interleukin-17-dependent proliferation of hepatic stellate cells, leading to the development of hepatic fibrosis.[20] Thus, CCL20 appears to be a critical profibrotic factor in the developmental process of PBC at least partially by serving as a chemoattractant for Th17 cells. In SSc, AMA antibody is positive approximately in 25% of patients, but many of them are asymptomatic.[38,39] However, early detection and management of PBC is quite important to reduce the risk of liver dysfunction induced by drugs used for the treatment of SSc complications, such as bosentan.[40] Therefore, from the perspective of concomitant PBC, it can be beneficial to further evaluate whether elevated serum CCL20 levels predict the development of hepatobiliary involvement in SSc patients with AMA antibody and normal liver function.

A limitation of this study is the small number of SSc patients who underwent right heart catheterization for the diagnosis of PAH. Among them, no patients met the diagnostic criteria of PAH, while 4 patients had borderline PAH, namely, these patients likely had early pulmonary vascular involvement leading to PAH. Therefore, we need to confirm the association of serum CCL20 levels with mPAP values in the large number of SSc patients with definite PAH. Another limitation is that only a cross-sectional analysis was carried out due to the lack of longitudinal data in each SSc patient included in this study. To further make the current findings more convincing, a further observational study with a second population is required in the future.

In summary, this is the first report demonstrating that serum CCL20 levels may be involved in the development of pulmonary vascular involvement leading to PAH and asymptomatic PBC. Our current results further strengthen the canonical idea that chemokines contribute to a variety of SSc-related disease processes and can be potential therapeutic targets of this intractable disease.

ACKNOWLEDGEMENTS

This work was supported by a grant for Research in Intractable Diseases from the Ministry of Health, Labor, and Welfare of Japan. The funder is not involved in study design, data collection, data analysis, manuscript, preparation and publication decisions.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Conception and design: Tetsuya Ikawa, Yoshihide Asano, Shinichi Sato; acquisition of data: Tetsuya Ikawa, Takuya Miyagawa, Yuki Fukui, Shun Minatsuki, Hisataka Maki, Toshiro Inaba, Masaru Hatano, Satoshi Toyama, Jun Omatsu, Kentaro Awaji, Yuta Norimatsu, Yukuke Watanabe, Ayumi Yoshizaki; analysis and interpretation of data: Tetsuya Ikawa, Yoshihide Asano, Shinichi Sato; funding acquisition: Yoshihide Asano, Shinichi Sato; supervision: Yoshihide Asano, Ayumi Yoshizaki, Shinichi Sato. All authors have read and approved the manuscript.

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SUPPORTING INFORMATION

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

How to cite this article: Ikawa T, Miyagawa T, Fukui Y, et al. Association of serum CCL20 levels with pulmonary vascular involvement and primary biliary cholangitis in patients with systemic sclerosis. *Int J Rheum Dis*. 2021;24:711–718. https://doi.org/10.1111/1756-185X.14103

Received: 23 February 2021 | Revised: 8 March 2021 | Accepted: 8 March 2021

DOI: 10.1111/1756-185X.14106

International Journal of Rheumatic Diseases

WILEY

APLAR IMAGE IN MEDICINE

Ballet foot in a boy

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KEYWORDS: clinical aspects, rehabilitation

A previously healthy 10-year-old boy presented with a 2-month history of pain and marked non-reducible plantar flexion of the right foot. Symptoms occurred after a mild injury to his right foot and progressively had increased during the last month, persisting all day long, and worsening with movements, limiting daily activities including school attendance. Prolonged bed rest, and treatment with different types of non-steroidal anti-inflammatory drugs was ineffective. A similar, but milder and transient episode had occurred a year earlier. His family history was unremarkable.

On physical examination the patient presented in good general health. His right foot was in a fixed posture, with non-reducible plantar flexion of ankle and toes (Figure 1A). Mild swelling was palpable on the dorsal area of the foot with preserved skin color. His ankle joint mobility was completely restricted, and the boy was unable to bear weight on the affected side. Extreme sensitivity to light touch was appreciable, and pain assessment by visual analog scale was referred by the child to be 8 out of 10.

- What is the possible diagnosis?
- Which test can confirm the diagnosis?
- What is the first treatment step?

Answers on page 2.

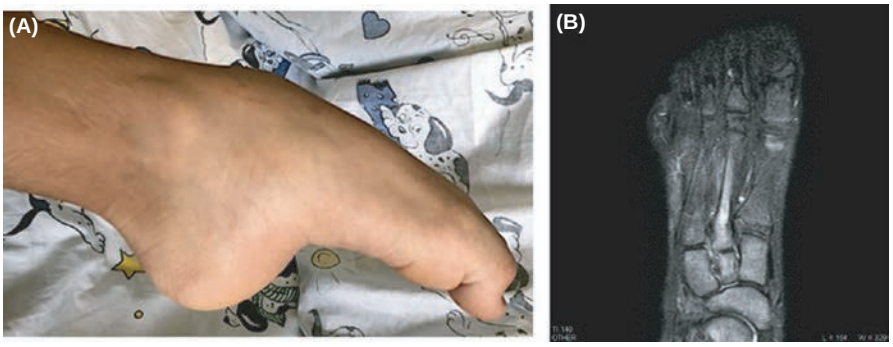

FIGURE 1 (A) Clinical aspect of right foot, which is kept in forced full plantar flexion. Ankle, midfoot, and toes are contracted, and the flexion is non-reducible for marked opposition by the child. Under general anesthesia the contracture could partially be reversed, but returned to original position upon awakening. (B) Magnetic resonance imaging of the foot showing bone marrow edema of second metatarsal bone [Colour figure can be viewed at wileyonlinelibrary.com]

1 | "BALLET FOOT" IN A BOY: PART 2

1.1 | What is the possible diagnosis?

- Complex regional pain syndrome (CRPS) type I triggered by a minor, accidental injury.

1.2 | Which test can confirm the diagnosis?

- CRPS is a clinical diagnosis, there is no specific single test to confirm it. In this boy, as expected, routine laboratory exams resulted within normal range.

Classic radiography may support the diagnosis with suggestive but non-specific features such as soft tissue swelling, periarticular, and patchy osteopenia; magnetic resonance may add some information as bone marrow edema, muscle atrophy, joint effusion and synovial hypertrophy may be present. In this patient axial short tau inversion recovery magnetic resonance imaging of the right foot showed a bone marrow edema at the right second metatarsal (Figure 1B).

1.3 | What is the first treatment step?

- Treatment should provide an early multidisciplinary management, initially based on physical/occupational, and psychological therapy and on educational strategies.

In our case multiple cycles of physical therapy, massage, transcutaneous electrical nerve stimulation and mobilization, as well as different pharmacological treatments including gabapentin, amitriptyline, and pamidronate were unsuccessfully tried. Due to the persistence of the pain and the impossibility to return to routine daily activities, we treated him with parenteral ketamine; however, after 5 infusions (1 mg/kg) only a partial benefit was obtained. Only after 3 days of continuous sciatic nerve block, an initial, clear improvement was achieved. Immediately a tight, and well-coordinated multidisciplinary meeting program involving neuropsychiatrist, physical therapist, pediatric rheumatologist, and anesthesiologist was organized. The boy gradually returned to school over a 2-month period.

Key learning points

- CRPS is part of the larger pediatric chapter defined as amplified musculoskeletal pain syndromes.
- CRPS type I follows days to a month after trauma (often of mild entity), in the absence of nerve injury.
- Distal extremities are the typically involved sites.
- Diagnosis is one of exclusion, it is mostly based on clinical signs such as disproportionate pain intensity and duration, allodynia, autonomic disturbances, and severe disability.[1]
- Magnetic resonance may show skin thickness, subcutaneous edema, muscle atrophy, bone marrow edema and, in chronic cases, bone mineral loss.
- Treatment requires a multidisciplinary, and patient-centered approach.[2,3]
- Second line treatments such as bisphosphonates, opioids, botulinum toxin injections, ketamine, or sympathetic nerve block should be considered in severe, unresponsive cases.[4,5]

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How to cite this article: Giani T, Gattinara M, Cimaz R. Ballet foot in a boy. *Int J Rheum Dis*. 2021;24:719–720. https://doi.org/10.1111/1756-185X.14106

Received: 23 October 2020 | Revised: 2 February 2021 | Accepted: 6 March 2021

DOI: 10.1111/1756-185X.14099

NOVEL HYPOTHESIS

International Journal of Rheumatic Diseases | WILEY

Musculoskeletal ultrasound as a diagnostic tool for eosinophilic fasciitis and correlation with magnetic resonance imaging findings

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Keywords: eosinophilic fasciitis, MRI, ultrasound

1 | INTRODUCTION

Eosinophilic fasciitis (EF) presents with pain and induration of the skin. Currently, clinical diagnosis is based on typical physical findings and magnetic resonance imaging (MRI) enhancement of the fascia and confirmed by tissue histology. We suggest that fascia thickness can be measured with use of musculoskeletal ultrasound. We describe a cohort of patients with musculoskeletal ultrasound and corresponding MRIs supporting the diagnosis of EF compared to controls.

2 | METHODS

Our study was determined exempt by the Mayo Clinic Institutional Review Board. Seven patients seen in our rheumatology clinic underwent musculoskeletal ultrasound of upper or lower extremities that exhibited findings of induration suggestive of EF. Ultrasound was performed using a 12 to 18 MHz linear array transducer to visualize muscle and fascia in the area of pain and induration. A measurement of fascial thickness was recorded in all patients. Musculoskeletal ultrasound was also performed in 7 healthy controls. Patients subsequently underwent MRI of the same region, and full-thickness skin-to-muscle biopsy was performed to confirm diagnosis in 6 (1 patient refused biopsy). Initial laboratory work-up with serum eosinophils were recorded. None of the patients had Raynaud disease or showed clinical or laboratory findings of scleroderma.

3 | RESULTS

Four women and 3 men were included, with a mean age of 43.5 years (range 34-58). Absolute eosinophil values ranged from 1051 to 4780/μL. Mean thickness of the fascia was 0.43 cm (range 0.21-0.7 cm) versus 0.14 cm (range 0.11-0.19 cm) in controls (Table 1 and Figure 1). All patients had MRI with contrast, with evidence of thickened and enhanced fascia of the same region (Figure 2). Diagnosis of EF was confirmed with tissue histology in all 6 patients who agreed to biopsy.

4 | DISCUSSION

In 1974, Shulman[1] described an uncommon scleroderma-like disorder with features of indurated skin, peripheral eosinophilia, hypergammaglobulinemia, and elevated erythrocyte sedimentation rate as EF. Since then, over 300 cases have been reported in the literature, but no international consensus regarding diagnostic criteria has been established.[2] An average age of onset ranging between

Presented at American College of Rheumatology Annual Meeting in San Diego, California, November 3-8, 2017.

Portions of this manuscript have been published in abstract form: Berianu F, Narula N, Abril A. Musculoskeletal Ultrasound as a Diagnostic Tool for Eosinophilic Fasciitis and Correlation with MRI Findings. *Arthritis Rheumatol.* 2017; *69* (suppl 10).

TABLE 1 Patient characteristics

Age	Gender	Areas involved	Biopsy positive	Peripheral eosinophil count: absolute values (cells/μL)	mean fascial thickness on MSK US (cm)	MRI supporting findings for EF
68	F	Arms, legs, back	+	1,051	0.21	+
42	F	Legs	+	1,121	0.39	+
29	M	Arms, chest	+	4,780	0.36	+
38	F	Arms, legs	+	2,023	0.40	+
52	M	Legs	+	3,001	0.70	+
30	M	Arms, abdomen	+	1,781	0.41	+
46	F	Arms, legs	N/A	1,985	0.51	+

Abbreviations: EF, eosinophilic fasciitis; F, female; M, male; MRI, magnetic resonance imaging; MSK US, musculoskeletal ultrasound; N/A, not available.

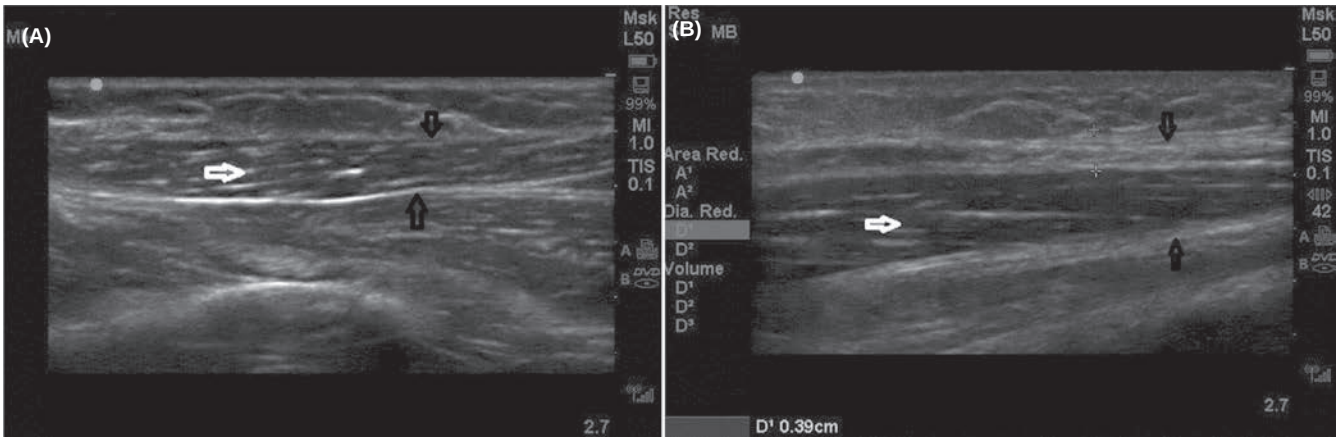

FIGURE 1 Musculoskeletal Ultrasound. (A) Transverse view of normal forearm muscle. White arrow indicates normal muscle; black arrows, normal fascia. (B) Transverse view of forearm muscle with eosinophilic fasciitis. White arrows indicate normal muscle; black arrows, thickened fascia

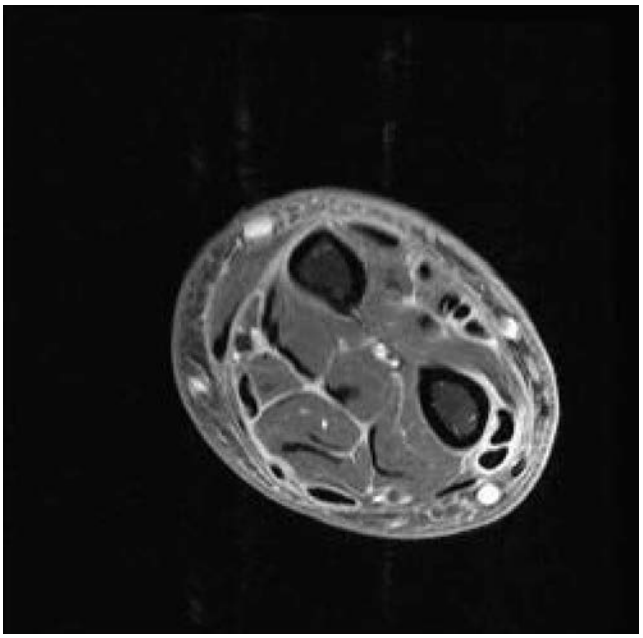

FIGURE 2 Magnetic resonance imaging with contrast forearm: enhanced fascia surrounding muscles

40 and 50 years has been noted, with inciting factors such as strenuous physical activity, trauma, medications (eg, statins, phenytoin, ramipril, subcutaneous heparin), and bacterial infection (eg, borreliosis, mycoplasma).[2-5] The hypothesis is that circulating T cell clones and interleukin-5 may cause release of eosinophilia in the fascia, leading to accumulation of cationic granules, which may have fibrogenic properties. In early disease there is edema of the fascia and subcutaneous tissue with a lymphocytic infiltrate containing plasma cells and eosinophils. With time the collagen becomes thickened and sclerotic with extension in subcutaneous fibrous septa.[6,7]

Clinical features range from edema of the skin, mainly in the limbs, to skin induration, also known as *orange peel* or *cobblestone*.[3] Groove sign has been noted in half of the patients, occurring due to venous furrowing along the veins in the infiltrated areas, and is highly suggestive of fasciitis or deep fibrosis.[2] Thickened fascia of up to 15 times the normal proportion with lymphocytic and plasma cell infiltration on full-thickness wedge biopsy, which includes skin and muscle, is the more acceptable method for clinical diagnosis; however, eosinophils may or may not be present on biopsy.[8]

In clinical practice, early diagnosis apart from tissue histology is made with MRI. Baumann et al.[9] showed MRI as an ideal imaging modality for diagnosis and monitoring of the disease.

Kirchgesner et al.[10] recently reviewed imaging modalities in EF, and described MRI as the best modality to identify the site for biopsy, as well as to differentiate EF from other forms of deep and superficial tissue diseases that may produce abnormal intensity in the fascia. MRI with gadolinium of both limbs is most often used for diagnostic purposes. Before injection, MRI typically shows a thickened deep fascia (peripheral deep fasciae and, more rarely, intermuscular fasciae) on T1-weighted sequences, which appears with a relatively higher signal intensity than that of muscle tissue on fat-suppressed or fat-saturated T2-weighted sequences.[7] Following injection of a gadolinium-based contrast agent, thickened fasciae appear with markedly enhanced signal intensity, thereby helping in diagnosis and establishing the biopsy site.[10,11] Baumann et al.[9] also projected MRI as a tool for prognostic purposes, but there is a lack of randomized controlled prospective studies and established guidelines.

Changes in health care have given rise to the need for more cost-effective techniques, and advances in ultrasound help meet this goal. It remains a more accessible, noninvasive modality for immediate, point-of-care imaging in rheumatology.[12] Kissin et al.[13] did note that using the high-frequency probe in ultrasound (high-frequency probe: more than 10 MHz has shorter wavelengths and allows a better description of superficial structures including skin, muscle, fascia) showed changes in compressibility of the subcutaneous tissue due to excess collagen deposition. One noted exception was atrophied dermis under 4 mm thickness.[13]

The mainstay of initial treatment in EF is glucocorticosteroids at doses of 0.5-1 mg/kg; however, steroid-sparing agents, like methotrexate, have been promising.[3-5,8] Lebeaux et al.[5] continued corticosteroid therapy for an average of 45 months (*SD* 31 months), and adjunctive immunosuppressive therapy was required in 44% of patients and lasted, on average, 24.7 months (*SD* 23.3 months).

To our knowledge, ours is the first study to compare patients diagnosed with EF to controls. For each of our 7 patients, diagnosis of EF was established with MRI, and the corresponding ultrasound showed a mean thickness of the fascia of 0.43 cm (range 0.21-0.7 cm), supporting the clinical use of ultrasound in patients with suspected EF. Ultrasound also helped us locate the optimal site for muscle biopsy, and 6 of 7 patients who agreed to biopsy had histologic evidence of inflammatory changes with infiltrates of eosinophils, confirming the diagnosis.

Musculoskeletal ultrasound may be a quick, safe, inexpensive, and reliable diagnostic method for patients with suspected EF. It can also help locate the best site for biopsy. Further randomized controlled studies of larger patient populations and controls are needed to establish the role of musculoskeletal ultrasound in the diagnosis of EF.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

NGN contributed to conception and design, experiments; collection, analysis, and interpretation of data, and drafting of the manuscript. AA contributed to conception and design, figures/table, and critical revision of the manuscript. KTC contributed to conception and design and critical revision of the manuscript. FB contributed to conception and design, experiments, collection, analysis, and interpretation of data, figures/table, and drafting and critical revision of the manuscript. All authors approved the final draft.

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How to cite this article: Narula NG, Abril A, Calamia KT, Berianu F. Musculoskeletal ultrasound as a diagnostic tool for eosinophilic fasciitis and correlation with magnetic resonance imaging findings. *Int J Rheum Dis*. 2021;24:721–723. https://doi.org/10.1111/1756-185X.14099

Received: 23 February 2021 | Revised: 27 February 2021 | Accepted: 27 February 2021

DOI: 10.1111/1756-185X.14098

COCHRANE CORNER

International Journal of Rheumatic Diseases

WILEY

What are the benefits and harms of anti-cytokine targeted therapies for adults with anti-neutrophil cytoplasmic antibody-associated vasculitis? - A Cochrane Review summary with commentary

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The aim of this commentary is to discuss the published Cochrane Review "Anti-cytokine targeted therapies for ANCA-associated vasculitis"[a] by Bala et al.,[1] under the direct supervision of the Cochrane Musculoskeletal Group. This Cochrane Corner is produced in agreement with the *International Journal of Rheumatic Diseases* and by Cochrane Rehabilitation.

1 | BACKGROUND

The anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAVs) refer to a group of rare, systemic, small-vessel vasculitis. These conditions are immune-mediated and involve loss of tolerance to myeloperoxidase or proteinase 3. They are characterized by endothelial injury, blood vessel inflammation (capillaries, arterioles and venules, in particular), and tissue/organ damage.[2] AAV includes microscopic polyangiitis, granulomatosis with polyangiitis, and eosinophilic granulomatosis with polyangiitis.[3]

This variable disease group can be unpredictable with potential life-threatening consequences. According to the latest European League Against Rheumatism (EULAR)/European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) recommendations for the management of AAV, new-onset organ-/life-threatening AAV should be treated with a combination of glucocorticoids and either cyclophosphamide or rituximab. For patients with rapidly progressive renal failure or severe diffuse pulmonary hemorrhage, plasma exchange should be considered. On the other hand, non-organ threatening AAV can be managed by methotrexate or mycophenolate mofetil in combination with glucocorticoids.[4]

The etiopathogenesis of AAV is multifactorial and influenced by the innate and adaptive immune system responses. Certain cell types and numerous circulating factors such as cytokines are involved in the pathophysiology.[5] Given the pivotal role of cytokines, biological anti-cytokine therapies (ie, abatacept, mepolizumab and alemtuzumab) have been used for the treatment of refractory AAVs.[1] In this regard, a comprehensive look at the effectiveness and safety of anti-cytokine therapies in AAVs would be of value.

2 | ANTI-CYTOKINE TARGETED THERAPIES FOR ANCA-ASSOCIATED VASCULITIS

(Bala MM, Malecka-Massalska TJ, Koperny M, Zajac JF, Jarczewski JD, Szczeklik W, 2020).

2.1 | What is the aim of this Cochrane Review?

The aim of this Cochrane Review was to evaluate the benefits and harms of anti-cytokine targeted therapies for adults with AAV.

[a]This summary is based on a Cochrane Review previously published in the Cochrane Database of Systematic Reviews 2020, Issue 9, Art. No.: CD008333, https://doi.org/10.1002/14651858.CD008333.pub2 (see www.cochranelibrary.com for information). Cochrane Reviews are regularly updated as new evidence emerges and in response to feedback, and Cochrane Database of Systematic Reviews should be consulted for the most recent version of the review.

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

2.2 | What was studied in the Cochrane Review?

The population addressed in this review was adults (18 years or older) with AAV. Randomized controlled trials (RCTs) and controlled clinical trials were considered for inclusion. The intervention (anti-cytokine therapy) was compared to placebo, standard therapy or another modality. Major benefit outcomes were mortality, remission, durable remission and disease flare/relapse. Major harms outcomes were total adverse events, serious adverse events and withdrawals due to adverse events. Minor outcomes included treatment response, health-related quality of life, control of asthma/sinonasal disease and disease damage.

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

The review authors searched for studies that had been published up to 16 August 2019. Literature search was performed through Cochrane Central Register of Controlled Trials (2019, Issue 7) and MEDLINE (OVID), Embase (OVID). Further, ongoing trial registries were searched on 28 August 2019 through ClinicalTrials.gov, European Trials Register, International Standard Randomised Controlled Trial Number Registry and World Health Organization trials portal. The authors searched the web sites of regulatory agencies on 3 September 2019 in order to assess adverse events. The reference lists of all primary studies and review articles were checked to identify any additional references. Related manufacturers' web sites were searched for trial information.

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

The review included 4 RCTs (a total of 440 patients). Three RCTs compared anti-cytokine therapies (belimumab, mepolizumab and etanercept) with placebo, while 1 RCT compared 2 pharmacological agents (rituximab vs infliximab).

2.4.1 | Comparison of mepolizumab to placebo in adults with relapsing or refractory eosinophilic granulomatosis with polyangiitis

Only 1 study with 136 participants reported the following outcomes.

- During 52 weeks follow-up, 1 death was observed in the mepolizumab group, while no deaths in placebo group (Peto odds ratio [OR] 7.39, 95% confidence interval [CI] 0.15 to 372.38). The low-certainty evidence suggests that mepolizumab results in little to no difference in mortality.
- In the mepolizumab group, more patients achieved remission for at least 1 week (risk ratio [RR] 2.77, 95% CI 1.62 to 4.74) and more had ≥24 weeks of accrued remission (28% vs 33%; RR 9.5, 95% CI 2.3 to 39.2) compared to the placebo group. The low-certainty evidence suggests mepolizumab results in a large increase of the probability of accruing at least 24 weeks of remission over a 52-week period.
- Durable remission rate within the first 24 weeks that was sustained until week 52 was also higher in the mepolizumab group than the placebo group (13 vs 1 participant; RR 13.0, 95% CI 1.75 to 96.33; low-certainty evidence). The low-certainty evidence suggests that mepolizumab results in a large increase of the probability of durable remission within the first 24 weeks, sustained until week 52.
- Mepolizumab probably results in a reduction in disease relapse. Fewer patients relapsed in the mepolizumab group than in the placebo group (56% vs 82%; RR 0.68, 95% CI 0.53 to 0.86; moderate-certainty evidence).
- Mepolizumab may not increase adverse events. The proportion of patients with any adverse event was similar between groups (97% vs 94%; RR 1.03, 95% CI 0.96 to 1.11; low-certainty evidence).
- Mepolizumab results in little to no difference in serious adverse events. The percentages of patients with any serious adverse events were 17.7% versus 26.5% in mepolizumab and placebo groups, respectively (RR 0.67, 95% CI 0.35 to 1.28; low-certainty evidence).

2.4.2 | Comparison of active drug (etanercept or belimumab) with standard therapy to placebo with standard therapy in adults with granulomatosis with polyangiitis and microscopic polyangiitis

- Active drug (etanercept or belimumab) added to standard therapy does not increase or reduce mortality. Mortality rate was similar between active (etanercept or belimumab) and placebo groups (3.4% vs 1.4%; Peto OR 2.45, 95% CI 0.55 to 10.97; 285 participants, 2 studies; low-certainty evidence).
- Etanercept may have little or no effect on remission. In the etanercept and placebo groups, 80 and 84 participants achieved remission, respectively (RR 0.97, 95% CI 0.89 to 1.07; 180 participants, 1 study; low-certainty evidence).
- Etanercept may have little or no effect on durable remission. The number of patients achieving durable remission (≥6 months) was 62 versus 64 patients in the etanercept and placebo groups, respectively (RR 0.93, 95% CI 0.77 to 1.11; 174 participants, 1 study; low-certainty evidence).
- Moderate-certainty evidence from 180 participants in 1 study showed that etanercept probably does not reduce disease flares. The proportion of patients with no disease flares and the number of any disease flares per 100 person-years were similar between groups (RR 0.98, 95% CI 0.76 to 1.27 and HR 0.89, 95% CI 0.62 to 1.28, respectively).
- Active drug (etanercept or belimumab) may result in little or no difference in severe or serious adverse events. With low-certainty evidence, the frequency of severe and/or serious adverse events

was similar in both groups (RR 1.0, 95% CI 0.8 to 1.27; 285 participants, 2 studies).

- Active drug (etanercept or belimumab) combined with standard therapy may result in a slightly increased withdrawal rate due to adverse events (11.2% vs 4.2%, RR 2.66, 95% CI 1.07 to 6.59; low-certainty evidence).

2.4.3 | Comparison of infliximab versus rituximab added to steroids and cytotoxic agents in adults with refractory granulomatosis with polyangiitis

Only 1 study with 17 participants was studied in this comparison.

- Mortality rate during 12 months (Peto OR 0.88, 95% CI 0.05 to 15.51) and during the additional follow-up was similar between groups. Very low-certainty evidence shows that we are uncertain about the effect of infliximab when compared to rituximab on mortality.
- We are very uncertain about the effect of infliximab when compared with rituximab on remission at 12 months (22% vs 50%, RR 0.44, 95% CI 0.11 to 1.81; very low-certainty evidence).
- Moderate-certainty evidence from 180 participants in 1 study showed that etanercept probably does not reduce disease flares. The proportion of patients with no disease flares and the number of any disease flares per 100 person-years were similar between groups (RR 0.98, 95% CI 0.76 to 1.27 and HR 0.89, 95% CI 0.62 to 1.28, respectively).
- We are very uncertain about the effect of infliximab when compared with rituximab on durable remission during additional follow-up beyond 12 months (11% vs 50%, RR 0.22, 95% CI 0.03 to 1.60; very low-certainty evidence).
- There is very uncertain evidence regarding any severe adverse events (22.3% in the infliximab group vs 12.5% in the rituximab group; RR 1.78, 95% CI 0.2 to 16.1; very low-certainty evidence).

2.5 | What did the authors conclude?

In patients with relapsing or refractory eosinophilic granulomatosis with polyangiitis, there is moderate-certainty evidence that mepolizumab probably decreases disease relapse compared to placebo and with low-certainty evidence mepolizumab may increase disease remission. Low-certainty evidence showed that total and serious adverse events maybe similar in mepolizumab and placebo groups. In patients with granulomatosis with polyangiitis and a small subgroup of microscopic polyangiitis, low-certainty evidence suggests that etanercept added to standard therapy when compared with standard therapy with placebo has little or no effect on sustained remission and probably does not reduce the number of flares. Adding active drug (etanercept or belimumab) to standard therapy may increase the risk of withdrawal due to adverse events, may have little or no impact on serious adverse events, durable remission and major relapse. The effects of infliximab, as compared to rituximab, in the management of patients with refractory granulomatosis with polyangiitis, is uncertain. Given the small number of studies, as well as concerns about the risk of bias and small sample sizes, the authors were not able to draw reliable conclusions on the benefits and harms of anti-cytokine therapy in AAV.

3 | WHAT ARE THE IMPLICATIONS OF THE COCHRANE EVIDENCE FOR PRACTICE IN RHEUMATOLOGY?

The management of rheumatic diseases is a complex issue. Pharmacological therapies and non-pharmacological treatment modalities are used for the management of non-inflammatory and inflammatory/autoimmune rheumatic diseases.[6] Over the last decade, there has been a shift to biological therapies in inflammatory and autoimmune disorders. However, short- and long-term treatment with biological disease-modifying antirheumatic drugs might be related to some adverse effects.[7] In this regard, the present Cochrane Review provided evidence and guidance in terms of the potential benefits and harms of anti-cytokine therapies in patients with AAV.

Different aspects of anti-cytokine therapies including their effects on mortality, remission, durable remission, disease flares, as well as adverse events and withdrawals due to adverse events were studied. The Cochrane Review showed low- to moderate-certainty evidence on the clinically relevant effect of mepolizumab in adults with relapsing or refractory eosinophilic granulomatosis with polyangiitis. The evidence was downgraded due to risk of bias, limited number of studies and relatively small sample sizes. Further research using well-designed and adequately powered studies is required to provide more reliable evidence on the benefits or risks of anti-cytokine medicines in AVVs.

ACKNOWLEDGEMENTS

The author thanks Cochrane Rehabilitation and Cochrane Musculoskeletal Group for reviewing the contents of the Cochrane Corner.

CONFLICT OF INTEREST

The author declares no conflicts of interest.

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DOI: 10.1111/1756-185X.14116

International Journal of Rheumatic Diseases

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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.