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Production Editor

Aishwarya Radhakrishnan (APL@wiley.com)

Editorial Assistant

Lalitha Pari (IJRD.EO@wiley.com)

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EDITORIAL

"A singular property in my hands"

The use of laying on of hands is ancient and was described by Hippocrates as "as singular property in my hands" (cited by Tardy[1]). Has it a place in modern medicine? The paper by Zacaron et al[2] tested laying on of hands as an adjunctive complementary therapy to a kinesiotherapy program in the treatment of older women with osteoarthritis of the knee. At the outset, we should emphasize that it is a randomized controlled trial with sound methodology. Triple blinding was in place for participants, assessors and statisticians, and participant blinding was tested and demonstrated to be present. There was a very low research and treatment attrition rate (11% and 8% respectively) and comprehensive reporting of primary and secondary outcomes. However, the methodology is challenging with respect to the aim to test efficacy of a religious-based therapy, namely *Spiritist passe*.[3] The control groups are probably "as good as could be" designed, first laying on of hands not by a spiritual healer, and second, no laying on of hands but with a person in the room simulating the presence of a therapist. (Just for the reader, note in the therapy in this paper the hands hover 1-2 inches above the patient's body, ie they do not touch the body.)

Spiritist passe is a widely used and apparently accessible therapy in Brazil where Spiritism is the third largest religious denomination.[3] Forms of spiritist therapy include prayer, laying on of hands with and without touch to enable good Spirit energy transmission, magnetized water, volunteerism, and spiritual education for a moral life according to Christian principals, and dispossession of spirit release therapy. All of these have been studied in clinical trials, mostly for mental health problems such as depression or anxiety or psychosomatic illness such as chronic pain.[3] Many of these arguably are diagnosed with self-reported symptoms and may have "soft" subjective outcomes where the placebo effect can be high. However, in this paper[2] *Spiritist passe* had positive outcomes in a disorder such as osteoarthritis where there is well defined pathology, and better results than laying on of hands without Spiritism for some functional outcomes.

Despite the care taken to control for non-specific and mediating effects of therapy (such as expectancy and improved mood/anxiety) it is difficult to dismiss these as a factor in the work by Zacaron et al.[2] Much research has demonstrated positive effects on well-being and mood from various religious activities and interventions such as prayer. This encompasses both "mainstream" Western religions as well as traditional religious such as those of the Sami peoples of Scandinavia.[4] Does this imply God or spirits are "real"? Or is it part of a broader phenomenon of "belief"? For example, Farias et al. have shown that secular belief in science improves mental health.[5] Beliefs as such are regarded as strongly influenced by emotion and may remain impervious in the face of information or facts.[6]

If faith or belief impacts emotion in a positive or negative direction, then that emotion can result in physical or biological change. Since the seminal work of Bartrop et al[7] there is now a large body of knowledge around the effects of emotional stress on human biological functions such as the immune system. Zacaron et al[2] acknowledge as a limitation that it was not possible to blind the laying on of hands therapists (or the control "non-therapists") to the treatment group. The "positive energy" from the therapy may have been from the beliefs of the providers in their approach – a potent albeit non-specific feature of psychological therapies.[8] This is one explanation for the findings, that is therapist effects may have resulted in reduced depression anxiety (which did occur[2]) which then mediated the improvements in pain and subsequently physical function.

It should be acknowledged that another important aspect critical to building evidence about a new intervention is the replicability of the findings by other researchers from different institutions. The authors of the current trial are affiliated with the same group of researchers involved in several studies assessing the efficacy of *Spiritist passe* in several clinical conditions.[9-11] Thus, it is necessary to have other studies conducted by different investigators from other groups and even from other countries. Replication of findings is indeed a problem in the psycho-behavioral sciences[12] and many useful therapy manuals gather dust (or in this age disappear into computer 'ether') for lack of replication and dissemination.

The absolute "truth" about *Spiritist passe*' efficacy is likely elusive. However, the present study raises questions that are intriguing. At the least, it supports further research exploring mediators of such observed effects of faith-based interventions on mental and physical health and quality of life.

CONFLICT OF INTEREST

PH is a person of faith.

Phillipa Hay[1,2]
Jose Carlos Appolinario[3]

[1]*Translational Health Research Institute, School of Medicine, Western Sydney University, Sydney, New South Wales, Australia*
[2]*Camden and Campbelltown Hospitals, SWSLHD, Campbelltown, New South Wales, Australia*

[3]Group of Obesity and Eating Disorders, Institute of Psychiatry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Correspondence

Phillipa Hay, School of Medicine, Western Sydney University, Locked Bag 1797 Penrith South, New South Wales, 2751, Australia.

Email: p.hay@westernsydney.edu.au

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EDITORIAL

COVID-19 and rheumatology: Reflecting on the first wave and preparing for the second wave

Cases of COVID-19 and associated hospitalizations are rising again, and we are at the start of a "second wave". As we prepare for the second wave, we must reflect on what we have learned from the first and how we are going to effectively manage rheumatology patients going forward.

Rheumatology patients were thought to be at a higher risk of contracting COVID-19 due to their disease and associated immunosuppressive treatments. In March, the British Society of Rheumatology developed a risk stratification tool to identify patients who were to shield during the height of the pandemic.[1] Shielding precautions included staying home or within 2 m of other individuals when in public. Patients deemed to be at high risk were those on high-dose corticosteroids, cyclophosphamide and 2 immunosuppressive agents.

Although shielding can reduce the risk of contracting COVID-19 we must also consider the psychosocial impact it has. Shielding renders patients to extreme isolation and rheumatology patients are already at higher risk of mental health disorders due to the challenges and chronicity of their disease. Superimposed social restrictions make them even more vulnerable to loneliness, depression and anxiety.[2] In addition, denying them access to gyms and swimming pools which is a key part of managing arthritis, can cause exacerbation of symptoms.

Another vital part of management are immunosuppressant drugs. When the pandemic began there was a theoretical risk that these drugs could increase the risk of developing severe COVID-19. Therefore, there was a hesitation in the rheumatology community to initiate disease-modifying antirheumatic drugs (DMARDs) in newly diagnosed rheumatic patients. However, since the start of the pandemic now pathophysiology of COVID-19 has come to light. It is thought the virus drives a "cytokine storm" leading to a hyper-inflammable state observed in conditions such as rheumatoid arthritis and lupus.[3] It is therefore postulated that some of the immunosuppressive therapies used to treat rheumatic conditions are protective against COVID-19.[4]

An observational study demonstrated that rheumatic patients did not have a higher risk of contracting COVID-19 and they did not suffer a more aggressive illness than the general population. Rather, outcome is more dependent on age and co-morbidities.[5] A case series revealed that baseline use of biologic therapy does not lead to worse outcomes compared to the general population.[6]

More recently, the RECOVERY trial in the United Kingdom has demonstrated that the use of steroid dexamethasone, reduces 28-day mortality in COVID-19 patients with an oxygen requirement.[7] The interleukin (IL)-6 inhibitor tocilizumab has shown some benefit in observational studies in reducing mortality and invasive ventilation and is currently part of RECOVERY trial phase 2. Cumulative evidence so far suggests there may be a role for tocilizumab in controlling the cytokine storm induced by COVID-19 and it can have a protective factor in the rheumatoid cohort, but research is still ongoing, and the definite effect of tocilizumab is still yet to be determined. Furthermore, cohort studies in France have shown that anakinra, an IL-1 receptor antagonist, reduces the need for invasive ventilation in COVID-19 patients.[8] Barcitinib, a Janus-activated kinase inhibitor and canakinumab, a monoclonal antibody of IL-1B have been shown to improve oxygenation in severe COVID-19 infection.[9,10] There is a wealth of data suggesting that immunosuppressive therapy may be influential in downregulating the cytokine storm and in turn be protective against severe infection.

Early aggressive treatment of inflammatory conditions, especially rheumatoid arthritis, leads to a better long-term prognosis and having untreated overt inflammation can itself cause immunocompromise.[11] Current practice involves discussing the risks and benefits of starting DMARDs with patients and if they are agreeable then to favor drugs that have a shorter half-life such as hydroxychloroquine or sulfasalazine.[12] For biologics, guidelines suggest switching from intravenous to subcutaneous or oral where possible to reduce hospital attendance. Additionally, they advise patients who have suspected or confirmed COVID-19, to continue hydroxychloroquine and sulfasalazine but suspend all other DMARDs.[13] However, for COVID-free patients who are already established on DMARDs, stopping treatment abruptly will lead to a disease flare which will inevitably impact on their function. Therefore, many centers continued therapy for stable patients throughout the first wave. The risk of abruptly stopping DMARDs could cause hospitalization and requirement for high-dose systemic steroids ultimately leading to poorer disease outcomes.[14]

As COVID-19 cases are rising and lockdown measures are being reintroduced, it is necessary to consider the long-term plan for rheumatology patients based on what we have learned from the first wave.

The drawbacks of shielding are extensive and there is no reproducible evidence that rheumatology patients are at increased risk of developing COVID-19. Patient factors such as age, body mass index, ethnicity, gender, and co-morbidities are proven risk factors for poorer outcomes.[15] Thus, the previous recommendations of shielding to rheumatic patients who are an extremely heterogeneous cohort is not appropriate. We recommend conducting an individualized risk assessment like the one undertaken for hospital staff to identify who is at high risk and would benefit from additional protective measures. Those with multiple risk factors along with immunosuppressive therapy are likely to be at higher risk than stable patients on DMARDs alone. We agree with recent recommendations that vulnerable patients at high risk (over 65 years, medical co-morbidities as well as rheumatic disease) should not shield in this "second wave" but will mostly benefit from taking particular caution: reducing the number of social interactions, working from home where possible and limiting the use of public transport. Local rheumatology centers should strive to identify and appropriately advise these patients. We suggest for lower risk rheumatic patients to follow government guidance with the general population and continue with their medication.

The mode in which we deliver care has drastically changed since the pandemic. Although the majority of new referrals are seen face-to-face following strict social distancing guidelines and utilizing appropriate personal protective equipment, some new patients are reviewed virtually. History and investigations may be all that's needed to reach a diagnosis or create a management plan for certain conditions for example, those referred for osteoporosis, fibromyalgia, or ankylosing spondylitis (AS) where the main bulk of information is obtained from history. Examination is still important and should not become obsolete, but it adds value only when objective assessment of joints are needed, for example those referred for inflammatory arthritis. Therefore, a triage system to differentiate who will benefit from a face-to-face review will be helpful as the pandemic continues. Follow up of existing patients has largely become virtual over the last 4 months. Data on patient experience have been analyzed in our center and there has been an overwhelming amount of positive feedback. Patients feel safer staying at home but still appreciate the opportunity to speak to their rheumatologist. They feel that virtual appointments are less stressful with no commuting, parking or waiting and therefore a lot of patients are happy to continue virtual clinics for the foreseeable future and even after the pandemic. We acknowledge that there are drawbacks to virtual clinics such as the patient feeling lonely and lack of interaction, there are also fewer support group meetings which can all make the patient feel isolated. Virtual clinics also rely on patients to carry out their own disease activity assessment; some can be reliably done such as the Bath AS Disease Activity Index but measures such as the Disease Activity Score of 28 joints will be difficult for patients to do accurately, but they can give some idea on the extent of disease severity and whether a remote consultation is suitable. Although patients seem to have a good experience with virtual consultations, the effect on clinical outcome is not yet known, whether they experience any adverse effects or suboptimal care will require a longitudinal study. Virtual consultations can take away from the holistic approach to care that a face-to-face review provides but weighing up the risks and benefits and as the pandemic continues, we feel it puts more onus on patients to manage their condition and provide a safer review of patients.

In addition, we observed that many drug monitoring blood tests took place in the community with primary care following up results. We have not seen any detriment from this and believe that stable patients can safely increase blood to from 3-monthly to 6-monthly.[12]

Our recommendations for the second wave

1. Most patients can be continued to be reviewed in virtual clinics, along with a defined triage system to reduce delay in diagnosis and management.
2. Rheumatology patients should have individualized risk stratification based on age, ethnicity, and burden of other medical co-morbidities.
3. Patients should be initiated early onto DMARD therapy as part of a swift treat-to-target approach and stable patients' blood monitoring can be predominately done in primary care 6-monthly.

Armin Fardanesh[1]
Swetha Byravan[2]
Arumugam Moorthy[2,3]
Hasan Tahir[1,4]

[1]*Royal Free London NHS Foundation Trust, London, UK*
[2]*University Hospitals of Leicester NHS Trust, Leicester, UK*
[3]*College of Life Sciences, University of Leicester, Leicester, UK*
[4]*Division of Medicine, University College London, London, UK*

Correspondence
Hasan Tahir, Royal Free London NHS Foundation Trust, London, UK.
Email: hasan.tahir@nhs.net

ORCID

Armin Fardanesh https://orcid.org/0000-0003-1076-4592
Arumugam Moorthy https://orcid.org/0000-0001-5903-1125

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REVIEW ARTICLE

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Clinical implications of synovial fluid specimen handling for crystal associated arthritides: A systematic review

Megan M. Meyer[1] | Lisa A. Marks[2] | Fawad Aslam[3]

[1]Department of Internal Medicine, Mayo Clinic, Scottsdale, AZ, USA

[2]Department of Medical Education and Medical Libraries, Mayo Clinic, Scottsdale, AZ, USA

[3]Division of Rheumatology, Department of Internal Medicine, Mayo Clinic, Scottsdale, AZ, USA

Correspondence
Fawad Aslam, Division of rheumatology, department of internal medicine, Mayo Clinic, 13400 E. Shea Blvd, Scottsdale, AZ, 85259, USA.
Email: aslam.fawad@mayo.edu

Abstract

Aim: To identify the appropriate methods of synovial fluid (SF) specimen storage, manipulation and handling for crystal associated arthritides (CAA) diagnosis.

Method: A systematic literature review was conducted using 5 medical databases to identify diagnostic studies assessing SF specimen handling for calcium pyrophosphate (CPP) and monosodium urate (MSU) crystals identification. All included studies were rated for quality using the Quality Assessment of Diagnostic Accuracy Studies 2.

Results: Fifteen studies, including 2 non-English language manuscripts, were included. Eight studies examined both types of crystals, while 3 studies examined CPP and 4 studies examined MSU crystals only. Overall, MSU crystals were more stable over time compared to CPP crystals. MSU stability was generally independent of time, preservative and temperature. CPP crystals deteriorated with time and were more stable if refrigerated. Ethylenediaminetetraacetic acid (EDTA) was a suitable preservative. Re-examining an initially negative SF sample at 24 hours facilitated detection of additional cases. Very few studies had an overall low risk of bias and applicability.

Conclusion: Monosodium urate crystals remain stable over time independent of storage time, temperature and preservative. CPP crystals are mostly stable for 24-48 hours but can deteriorate with time. Overall, SF crystal examination should ideally be done within 24-48 hours. They may be stored at room temperature without any preservative. Otherwise, refrigeration (4°C/39°F) and EDTA preservation is reasonable. Stored SF re-examination, at 24 hours, helps identify a small number of additional MSU and CPP cases. Centrifugation techniques allow better and easier crystal identification, particularly CPP. Most studies were of unclear or low quality.

KEYWORDS

calcium pyrophosphate, chondrocalcinosis, gout, specimen handling, synovial fluid

1 | INTRODUCTION

Synovial fluid (SF) analysis detected monosodium urate (MSU) and calcium pyrophosphate (CPP) crystals remain the gold standard for the diagnosis of gout and CPP deposition (CPPD) disease respectively.[1,2] SF analysis is especially important in the diagnosis of atypical presentations of these crystal associated arthritides (CAA) and otherwise unclassifiable cases of inflammatory arthritis.[2,3] Understandably therefore, SF analysis training is a requirement of rheumatology fellowship training.[4,5]

However, SF analysis utilization remains low and accounted for only 32% of gout diagnoses in rheumatology clinics.[6] Diagnostic ultrasound usage was 0.8% in that study and did not explain this low SF utilization. Expectedly, SF aspiration was only performed in 2% of suspected gout cases in the primary care setting.[7] For the standard patient, gout can still be reliably diagnosed by clinical criteria.[1,8] CPPD, on the other hand, remains a difficult and an underdiagnosed condition.[9,10] In the absence of specific clinical criteria for CPPD, SF based diagnosis is essential as it is for atypical gout.

Synovial fluid analysis is operator dependent and its interpretation remains challenging.[11] Moreover, SF analysis is impacted by its handling, that is, processing method, temperature, type of anticoagulant/preservative in the specimen container and time from aspiration to analysis. SF specimen handling methods remain controversial. Commercial laboratories in the United States exhibit a wide variation in the requirements of sample handling. Depending on the laboratory, a SF specimen at room temperature is thought to be unstable to stable for 48 hours.[12-14] Similar discrepancy affects the storage preservative, with 2 laboratories preferring liquid ethylenediaminetetraacetic acid (EDTA), the lavender-top bottle, while one categorically not preferring it for crystal analysis.

The optimal recommendation is to examine the SF promptly, ideally immediately after aspiration, for the highest yield,[15] a task that is not always possible. This is because a busy practicing clinician may be unable to analyze SF promptly at acquisition due to time constraints or due to lack of training or proper equipment. The relevant questions for such a clinical scenario are: how should the sample be collected and stored; is the sample still reliable for examination at the end of the day; and, if sent out for analysis, for how long does it remain reliable?

Several studies have looked at SF handling for CPP analysis in SF with discrepant results. These studies have not been systematically evaluated. MSU specimen handling was investigated in a systematic review in 2013[16] which examined 5 studies. Our review examines 12 MSU studies. Our paper systematically addresses the SF handling literature in CPP, and updates that for MSU. This systematic review aimed to address if certain methods of SF handling, compared to others, are more reliable at detecting MSU or CPP crystals in suspected cases of CAA.

2 | MATERIALS AND METHODS

2.1 | Protocol registration and eligibility criteria

This systematic review was conducted in accordance with the recommendation of Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA).[17] Studies fulfilling the following criteria were included: (a) complete studies with an objective of comparing methods of SF handling for CAA; (b) studies including, at least, some patients with a confirmed or suspected diagnosis of either gout or CPPD; (c) studies enrolling human subjects. Assessed specimen handling methods included storage temperature, storage preservative, specimen manipulation such as centrifugation, time to analysis. Studies focusing on staining methods were felt to be less clinically relevant and excluded.

2.2 | Search strategy and information sources

A literature search was conducted by a trained, experienced medical librarian utilizing medical subject heading (MeSH) and text words related to the study question. The following keywords and their combinations were used in the search strategy: "synovial fluid analysis"; "synovial fluid chemistry"; "chondrocalcinosis"; "cppd"; "calcium phosphate deposition"; "crystallization" and "calcium pyrophosphate". The following MeSH terms were used in conjunction with their keyword counterparts: synovial fluid; gout; chondrocalcinosis; crystallization and calcium pyrophosphates. Keywords and MeSH terms were combined using the Boolean operators "AND" and "OR". A sample Ovid MEDLINE search strategy is given as Table S1. Adaptations of this strategy were used to search other databases.

Searched databases included Ovid MEDLINE, Ovid EMBASE, Scopus, Web of Science and the Cochrane Database of Systematic Reviews. There were no language or publication period restrictions for the initial search. Non-English language studies without an abstract were excluded. Included non-English studies were translated using a professional translation service. Full-text of studies identified as conference abstracts were retrieved, if available. Case reports were excluded. Bibliographies of identified studies was scanned to identify further studies for inclusion. Additional relevant studies identified from review articles on topics of gout, CPPD and SF analysis were also included. Abstracts of the annual conferences of American College of Rheumatology from 2012-2019 and of the European League against Rheumatism from 2008-2019 were reviewed with the search term "synovial fluid".

2.3 | Study selection and data collection

EndNote library (version X9, Clarivate Analytics) was used for reference duplication assessment and data management. Two authors (FA and MM) independently scanned the identified abstracts for inclusion in the review. If an abstract was not available at this stage, full text was reviewed for inclusion determination. Study authors were not contacted for additional data. Full texts of the identified abstracts were then independently reviewed by the 2 authors for eligibility. Any conflicts were resolved by consensus. Reasons for exclusion of the full-text papers were recorded. Relevant information from the final included papers was extracted and recorded by one reviewer (FA) and re-examined by the second reviewer (MM) for accuracy. Pertinent data included study first author, publication year, study location, study design, subject number with confirmed and/or suspected CAA, SF specimen storage temperature, specimen storage preservative, specimen time(s) to analysis for presence of crystals, specimen manipulation, and

identification of CPP and/or MSU crystals. Due to expected study heterogeneity, summary measures were not calculated.

2.4 | Assessment of methodologic quality

Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) was used to assess risk of bias (ROB) in the included studies.[18] QUADAS-2 assesses ROB and concerns over applicability in the domains of patient selection, index test, and reference standard and only ROB in the "low and timing" domain. Each domain is rated as: high, low or unclear. A high rating indicates a higher ROB or greater concerns over applicability. Two authors (FA and MM) independently assessed the included studies and resolved any conflicts by consensus. Our signaling questions and definitions for quality assessment are given in the Appendix S1.

3 | RESULTS

A total of 737 citations were retrieved from the medical database searches. After removing duplicate references, 487 citations were reviewed based on inclusion and exclusion criteria. Conference abstract search did not identify any abstracts that were still unpublished. Fifteen studies were included in the final review including 1 study in French language and 1 in Bulgarian language. Study selection flowchart is shown in Figure 1.

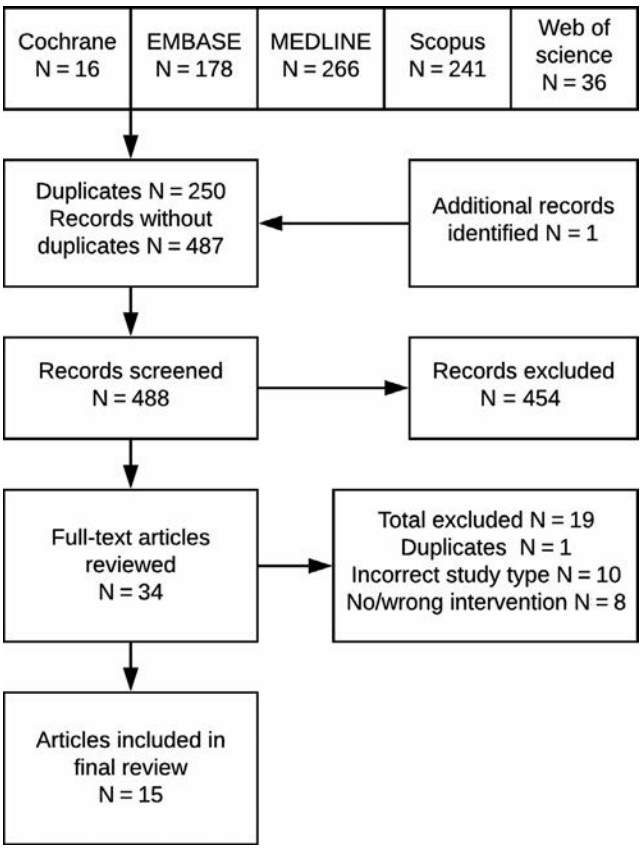

FIGURE 1 Flow diagram of study selection

Eight studies examined both types of crystals, while 3 and 4 studies each examined only CPP and MSU crystals respectively. Study details, results and conclusions are presented in separate tables for CPP (Table 1) and MSU (Table 2) crystals. Some studies assessed both crystals, but we are presenting the results separately for each crystal, providing greater clarity and easier clinical interpretation. Detailed study results are provided in the tables and major findings are given below. Seven studies used compensated polarized microscopy.[19-25] Two studies utilized only polarized microscopy.[26,27] In 6 studies, the method of polarized microscopy could not be determined.[28-33]

3.1 | Storage time

Of the 7 studies that examined MSU detection at 24 hours compared to immediate assessment, 4 found no change.[19,24,28,30] In 1 study the detection was 100% in EDTA tubes but dropped to 92% in heparin tubes.[27] Similarly another study showed 100% detection at 24 hours in EDTA tubes but it dropped to 94% and 97% in the heparin and no preservative groups respectively.[21] One study showed a decline only in the samples stored at −4C° at 24 hours but not in those stored at 3C° or room temperature.[23] Three studies examining the stability at 72 hours found no change[19,24,27] while in 1 study the detection dropped by only 3% in samples held in plain tubes.[21] Therefore, for the clinically relevant time of 72 hours, MSU crystals are mostly unaffected by storage time. Two studies looked at storage over several weeks. One study found 100% stability for up to 12 months but this was on samples that had been air dried and mounted on slides, and not on standard specimens.[30] In the other study that used preservative free vials, at 24 weeks, MSU was detected in 87%, 69% and 89% in the −20, 4 and 20°C group respectively.[33]

Seven studies examined CPP stability at 24 hours compared to immediate assessment. Of these, 5 did not identify any decline.[20,21,23,24,29] One study did not find any decline in samples stored in heparin tubes at 20°C but did find a decline by 17% in heparin at 4°C and EDTA held at either temperature.[27] One study by Kerolus et al[19] showed a rapid CPP decline in number within 24 hours, although crystals remained detectable. However, the authors later commented that their own additional data did not support that assessment.[29] Of the 4 studies assessing CPP stability at 72 hours, 1 did not show any deterioration.[24] One study showed a detection decline of 3%.[21] The study by Pastor et al[27] showed detection rates falling to 78% in EDTA tubes. The study by Kerolus et al, as expected, now showed crystals becoming undetectable. In the studies looking at storage for a week and beyond, 1 study each demonstrated no loss in CPP detection at 2 and 4 weeks.[20,29] In the Pastor et al study, CPP detection declined by 6%-18% with the worst detection of 67% in EDTA at 20°C.[27] In summary, unlike MSU crystals which remain detectable for 72 hours, CPP crystals do deteriorate with time. They should ideally be examined within 24 hours although they remain fairly detectable for up to 72 hours.

TABLE 1 Characteristics and results of studies evaluating CPP crystals

Study Country Year	N	Objective (s)	Temperature	Preservative	Time	Results	Conclusions	Comments
Kerolus[19] USA 1989	5	Effect of storage temperature and time on CPP crystals	4°C and 22°C	Heparin	Up to 8 wk	100% CPP crystals undetectable by 3-8 wk irrespective of temperature.[a]	CPP crystals began dissolving significantly within 24 h.	
McGill[20] Australia 1991	11	Effect of storage temperature and time on CPP crystals	−70°C, 4°C and room temperature	None	Up to 8 wk	100, 90 and 73% of CPP crystals were detectable at 8 wk in −70°C, 4°C and room temperature samples respectively.	CPP crystals remain highly detectable for 8 wk if stored at 4°C although with time the quantity decreases.	−70°C samples had no decline is cell count. A few samples stored in heparin behaved similarly. Only 1 (9%) spontaneous unidentified crystals formed.
McKnight[29] USA 1991	6	Stability of CPP crystals over time at room temperature	Room temperature	Unclear. Likely none	4 wk	100% CPP crystals detectable till 4 wk.	CPP crystals remain fully detectable for 4 wk.	CPP quantity did not decrease.
Galvez[21] Spain 2002	30	Effect of storage temperature and preservative on CPP crystals	−80°C and 4°C	EDTA, heparin or none at 4°C. No preservative for −80°C	24 and 72 h for the non-frozen and 18 wk for frozen samples	CPP detected in 100% of EDTA, heparin and no preservative at 24 h and 97% at 72 h in all tubes.	CPP is highly detectable up to 72 h if stored at 4°C, independent of preservative.	Over 2 mo, intra-cellular CPP crystals decreased while extra-cellular CPP remained stable. No spontaneous crystals formed in the control group.
Yuan[22] USA 2003	107	Do initially crystal negative SF samples become positive after 24 h?	4°C	Not specified.	24 h	2% became CPP positive at 24 h	When high clinical suspicion, reanalysis at 24 h may yield crystals.	Reanalysis was by a different observer.
Solakov[23] Bulgaria 2005	14	Effect of storage temperature on CPP crystals	−4°C, 3°C and 22-24°C (room temperature)	Heparin or none	24 h	CPP detected in 100% of samples at −4°C, 3°C or room temperature.	CPP crystals remain fully detectable up to 24 h independent of temperature or preservative.	
Robier[30] Austria 2012	10	Effect of cytospin slides on long-term storage of CPP crystals	Room temperature	None	Up to 12 mo	100% crystals remained detectable at 12 mo	Dried cytospin preparation are suitable for long-term storage and CPP crystal analysis.	No change in controls was noted ie, no false positive results.
Tausche[24] Germany 2013	6	Effect of storage temperature and preservative on CPP crystals	4°C and 20°C	EDTA or none	72 h	100% crystals remained detectable at 72 h	CPP is 100% detectable up to 72 h, if stored at 4°C or 20°C, independent of preservative.	No crystals appeared in the crystal negative specimens and crystal counts did not change.

(Continues)

TABLE 1 (Continued)

Study Country Year	N	Objective (s)	Temperature	Preservative	Time	Results	Conclusions	Comments
Robier[31] Austria 2014	50	CPP detection between regular smears and cytospin slides in low WBC SF	Room temperature	Heparin	Not specified but within 24 h	100% crystals detected with cytospin and standard smear.	Cytospin allows identification of a much higher number of CPP crystals per sample.	
Boumans[25] Netherlands 2017	16-11[b]	CPP detection between regular smears and regular centrifuged slides (not cytospin)	4°C	EDTA	Up to 5 d. Average 2 d	Additional 20% to 27%[b] CPP identified after centrifugation.	Centrifugation is of benefit in identifying additional CPP crystals.	Used 2 observers without reconciliation. Centrifugation made detection faster and easier.
Pastor[27] Spain 2020	18	Effect of storage temperature and preservative on CPP crystals	4°C and 20°C	EDTA or heparin	Up to 7 d	At d 3, CPP detection rate dropped to 78% in EDTA at 20°C. For all others, it dropped to an average of 91%.	CPP detection rate falls over time independent of temperature or preservative. The decline is greatest in EDTA storage at 20°C.	

Abbreviations: CPP, calcium pyrophosphate; EDTA, ethylenediaminetetraacetic acid; N, number; SF, synovial fluid; WBC, white blood cells.

[a]Kerolus et al revised their findings in a subsequent letter to the editor.

[b]Two observers.

TABLE 2 Characteristics and results of studies evaluating MSU crystals

Study Country Year	N	Objective (s)	Temperature	Preservative	Time	Results	Conclusions	Comments
Medicis[26] Canada 1979	6	Does initially crystal negative SF sample become positive based on storage conditions?	5°C and 25°C	Heparin. Also closed tubes, sealed and unsealed slides	0-15 d	Only 1 case of spontaneous crystal formation in samples in closed tubes at 5°C. Quicker formation if unclosed vs. closed coverslips.	Spontaneous crystal formation may not reflect gout but a consequence of hyperuricemia and storage conditions.	No exact numbers are given.
Bible[28] USA 1982	NS	Assess spontaneous formation of MSU crystals	Room temperature and refrigeration	Not specified.	4 and 24 h	Initially MSU positive samples remained positive independent of temperature.	MSU is fully detectable up to 24 h independent of temperature.	No spontaneous crystal formation was noted.
Kerolus[19] USA 1989	7	Effect of storage temperature and time on MSU crystals	4°C and 22°C.	Heparin.	Up to 8 wk	100% crystals remained detectable at 8 wk	MSU is completely detectable up to 8 wk, if stored at 4°C or 22°C.	The number of MSU crystals decreased with time, more in the 22°C group. Small number of spontaneous crystals developed over time.
Galvez[21] Spain 2002	31	Effect of storage temperature, time and preservative on MSU crystals	−80°C and 4°C	EDTA, heparin or none at 4°C. No preservative for −80°C.	24 and 72 h for the non-frozen items. Up to 18 wk for frozen	MSU detected in 100%, 94% and 97% of EDTA, heparin and no preservative at 24 h and 100%, 94% and 94% at 72 h respectively.	MSU is highly detectable up to 72 h if stored at 4°C, independent of preservative.	Heparin tubes retained 100% positivity at 72 h. In the frozen sample, slight increase in extra-cellular and decrease in intra-cellular. No spontaneous crystals formed in the control group.
Yuan[22] USA 2003	107	Do initially crystal negative SF samples become positive after 24 h?	4°C	Not specified.	24 h	3% became MSU positive at 24 h	When high clinical suspicion, reanalysis at 24 h may yield crystals.	Reanalysis was by a different observer.
Solakov[23] Bulgaria 2005	31	Effect of storage temperature on MSU crystals	−4°C, 3°C and 22-24°C (room temperature)	Heparin or none.	24 h	MSU detected in 94% initially. At 24 h, MSU detected in 100% at room temperature, 97% at 3°C and 77% at −4°C.	MSU is fully detectable up to 24 h at room temperature and highly detectable at 3°C. This is independent of preservative.	Additional MSU identified after 24 h of storage. Stored MSU morphology changed.
Robier[30] Austria 2012	10	Effect of cytospin slides on long-term storage of MSU crystals	Room temperature	None.	Up to 12 mo	100% crystals remained detectable at 12 mo	Dried cytospin preparations are suitable for long-term storage and MSU crystal analysis.	No change in controls was noted, ie no false positive results.

(Continues)

TABLE 2 (Continued)

Study Country Year	N	Objective (s)	Temperature	Preservative	Time	Results	Conclusions	Comments
Tausche[24] Germany 2013	16	Effect of storage temperature and preservative on MSU crystals	4°C and 20°C	EDTA or none.	72 h	100% crystals remained detectable at 72 h	MSU is 100% detectable up to 72 h, if stored at 4°C or 20°C, independent of preservative.	No crystals appeared in the crystal negative specimens and crystal counts did not change.
Robier[32] Austria 2014	17	MSU detection between regular smears and cytospin slides in low WBC SF	Room temperature	None	Not specified but within 24 h	100% crystals detected with cytospin and 71% with standard smear.	Cytospin is an appropriate method for detection of MSU in low WBC count SF.	Greater number of MSU crystals detected with cytospin.
Kienhorst[33] Netherlands 2015	10	Effect of storage temperature on MSU crystals	−20°C, 4°C and 20°C	None	Up to 24 wk	MSU detected in 87%, 69% and 89% in the −20°C, 4°C and 20°C groups respectively.	Storage at 20°C is reliable for MSU detection up to 24 wk.	Not all samples were positive at every time point. 20°C suffered from evaporation. Subjectively, number of MSU crystals was similar in 4°C and 20°C
Boumans[25] Netherlands 2017	27-32[a]	MSU detection between regular smears and regular centrifuged slides (not cytospin)	4°C	EDTA	Up to 5 d. Average 2 d	Additional 7% MSU identified after centrifugation.	Centrifugation allowed only minimal additional MSU detection.	Used 2 observers without reconciliation. Centrifugation made detection faster and easier.
Pastor[27] Spain 2020	12	Effect of storage temperature, time and preservative on MSU crystals	4°C and 20°C	EDTA or heparin	Up to 7 d	100% MSU detected at 4°C in EDTA at d 1. For all others, detection rate dropped to 92%.	MSU remains highly detectable till 7 d independent of preservative type used and temperature.	Difference in detection rate was not statistically significant.

Abbreviations: CPP, calcium pyrophosphate; EDTA, ethylenediaminetetraacetic acid; MSU, monosodium urate; N, number; NS, not specified; SF, synovial fluid; WBC, white blood cells.

[a]Numbers vary as 2 observers independently confirmed presence of crystals.

3.2 | Storage preservative

Four studies compared MSU detection in different preservatives or no preservative. One study found 100% MSU detection is samples stored in EDTA or no preservative independent of temperature.[24] The study by Galvez et al[21] compared preservatives at the same temperature of 20°C and found MSU detection of 100% in EDTA, 97% in no preservative and 94% in heparin. Another study did not give details but noted no effect of heparin versus no preservative on MSU detection.[23] Pastor et al compared EDTA with heparin at different temperatures (4°C and 20°C). MSU detection was 92% in both preservatives at 20°C and in heparin at 4°C but was 100% in EDTA at this temperature.[27]

The same 4 studies compared storage preservative in CPP detection. One study found 100% CPP detection in samples stored in EDTA or no preservative independent of temperature.[24] Another study did not give details but noted no effect of heparin versus no preservative on CPP detection.[23] The study by Galvez et al compared preservatives at the same temperature of 20°C and found CPP detection of 100% in EDTA, heparin and no preservative at 24 hours. This only dropped to 97% in all samples at 72 hours.[21] Pastor et al compared EDTA with heparin at different temperatures (4°C and 20°C). CPP detection was 100% in heparin at 24 hours at 20°C which dropped to 89% in samples at 4°C. Detection in EDTA was 89% and 83% at 20°C and 4°C respectively.[27]

Ethylenediaminetetraacetic acid is an excellent storage medium for MSU crystals. EDTA is also an appropriate storage medium for CPP. There is some evidence, although conflicting, that heparin may be slightly better for CPP. Several studies looked at SF crystal analysis results when submitted without any preservative, for example in a plastic vial or syringe. CPP crystal identification and MSU crystal identification were independent of the use of preservative for up to 72 hours.[21,23,24] In summary, crystal detection seems not to be highly influenced by the preservative used or if not used at all.

3.3 | Storage temperature

Six studies compared effects of storage temperature on MSU detection. All of these compared room temperature to refrigeration. Three found no difference in MSU detection at 24 hours, 72 hours and 8 weeks each.[19,24,28] One found only a decline of 3% in favor of room temperature.[23] Kienhorst et al found 89% detection at room temperature compared to 69% in refrigeration.[33] Pastor et al found 100% detection in refrigerated samples in EDTA at 24 hours. The value was 92% for refrigerated EDTA specimens as well in heparin samples stored at either temperature.[27]

For CPP crystals, 5 studies compared room temperature to refrigeration. In 3 studies, no difference was noted at 24 hours, 72 hours and 8 weeks each.[20,23,24] One study did not give exact number but crystals dissolved faster at room temperature. Pastor et al found 100% CPP crystals at 24 hours in heparin and 89% in EDTA at room temperature, and under refrigeration at 89% in heparin and 83% in EDTA.[27]

In summary, MSU remains very stable at room temperature for the clinically relevant duration of 72 hours. CPP is also very stable for 24-72 hours. Overall, it seems room temperature storage should be reasonable.

3.4 | Reanalysis of an initially negative SF sample

One study specifically addressed the question if an initially negative SF sample becomes positive 24 hours later and found 3% additional MSU cases and 2% additional CPP cases.[22] Some other studies noted their findings in this regard. In the study by Solakov et al,[23] at 24 hours, 94% MSU positivity changed to 100% positive. In the Pastor et al study, between SF analysis at 24 and 72 hours, MSU in heparin at 4°C increased from 92% to 100% and CPP increased from 89% to 100%.[27] In summary, a very small number of MSU and CPP crystals are identified on reanalysis, after 24 hours, of an initially negative SF in suspected CAA and reanalysis may be warranted in highly suspected cases of CAA.

3.5 | Centrifugation

Centrifugation and cytocentrifugation were the 2 techniques assessed. One study compared regular smears with centrifugation in which the latter allowed for identification of an additional 7% MSU cases and 20%-27% (2 observers) CPP cases.[25] One study compared regular smears with cytocentrifugation, in low inflammation SF, and identified 100% MSU crystals compared to 71% with regular smears.[32] They were not able to detect additional CPP crystals but found that cytocentrifugation allowed earlier and easier identification of CPP.[31]

3.6 | Quality assessment

From a ROB perspective, study quality was mixed. For the more important domains of patient selection and index test, ROB was generally high or unclear. ROB in "flow and timing" domain was low. Applicability results were mixed. Detailed results of QUADAS-2 are presented in Figure S1. Table S2 gives detailed scoring of individual domains.

4 | DISCUSSION

In the multiple factors governing an accurate diagnosis of CAA, specimen handling of SF is an important variable. This systematic review provides the current data on this topic. The most pertinent clinical applicability findings regarding SF sample handling are summarized in Table 3.

From a clinical perspective, a timeframe of up to 48 hours is most relevant as that is the usual window for a clinician to personally

TABLE 3 General recommendations for SF sample handling for the practicing clinician

1. Consult with the local laboratory for sample handling recommendations
2. SF should be examined immediately after aspiration for optimal results.
3. If immediate analysis is not possible, SF should be examined within 24 h.
Refrigeration is preferred (4°C/39°F), particularly for CPP crystals SF may be stored at room temperature (22°C/72°F)
4. For analysis beyond 24 h (usually 48-72 h), SF should be refrigerated.
Ideally sample should be stored in an EDTA tube, as this preserves white blood cells
If enough sample and CPP crystals suspected, some SF may be placed in a heparin tube
5. If available, request centrifugation in the following situations.
If suspecting CPP crystals and initial sample is negative
Cytospin if suspecting MSU in non-inflammatory SF and routine smear is negative
Routinely if cost-effective
6. If high clinical suspicion for CAA and initial sample is negative for crystals:
Re-examine refrigerated SF after 24 h

Abbreviations: CAA, crystal associated arthritides; CPP, calcium pyrophosphate; EDTA, ethylenediaminetetraacetic acid; MSU, monosodium urate; SF, synovial fluid.

analyze SF or to deliver the SF sample to an external laboratory. This is also the timeframe requirement of commercial laboratories. Therefore, outside of research settings, storage beyond 48 hours is likely of little clinical interest. Moreover, storing SF for months is neither clinically relevant nor feasible. MSU crystals remain stable over long periods of time, and definitely beyond this window of clinical significance. CPP crystals do remain very stable for at least 24 hours. CPP crystals also remain highly detectable for the first 72 hours, especially if refrigerated. Suffice to say, they can remain detectable for weeks although their quantities decline. This time-survival is more important in the emergency room settings, where the focus is on infection evaluation, and crystal analysis may not always be ordered initially. It may be important to re-examine the SF for crystalline presence in such settings when an infection is excluded.

Monosodium urate crystals remain stable independent of preservative. CPP crystals also remain quite stable irrespective of preservative, at least for up to 48 hours. However, 1 study suggested that heparin may be better for prolonged CPP stability.[27] Based on our systematic review, EDTA is the most practical storage medium for both CPP and MSU. In addition, to prevent WBC degradation, EDTA is preferred for more accurate SF white blood cell (WBC) evaluation.[34] EDTA is helpful because inflammatory SF can clot.[35] Therefore placing all the SF in EDTA is practically easier than splitting the sample and would give the highest yield. It is important to note that some labs may prefer SF for crystal analysis be submitted without any perseverative, for example in a syringe. Studies have established that both CPP and MSU crystals remain stable independent of preservative for 24 hours and up to 72 hours.[21,23,24] Therefore, if only a crystal analysis is required, the specimen may be submitted in a syringe.

Our systematic review found that both CPP and MSU crystals remain stable at room temperature (mostly defined as 22°C/72°F) and if refrigerated (mostly defined as 4°C/39°F). Therefore, both these methods are appropriate from a clinical perspective. An earlier study had reported disappearance of CPP crystals within 24 hours of aspiration.[19] This was later amended by further observations of the same authors.[29] However, what has not been studied is the influence of temperatures higher than 22°C (such as may be encountered during transport, hot climates or resource poor settings) on crystal stability in SF samples. Evidence suggesting MSU solubility increases at higher temperatures[36,37] may be extrapolated to SF specimen handling. Therefore, based on our review, refrigeration is recommended if SF cannot be analyzed immediately or requires any transport.

The utility of an initially negative SF reanalysis after 24 hours in suspected cases of CAA was specifically assessed by 1 study.[22] It led to identification of an additional 3% and 2% of cases of gout and CPPD respectively. The samples were refrigerated. No convincing evidence of spontaneous crystal formation was found. In another study, identification of an additional 3% MSU cases were identified on SF re-examination at 24 hours, at room temperature.[23] One study proposed that spontaneous formation of crystals can take place, in patients with different rheumatic diseases, if SF is not stored in a refrigerated and sealed tube.[26] However, their study premise, an interesting one, was that such crystal formation is actually indicative of hyperuricemia and may lead to an incorrect gout diagnosis.

Centrifugation was assessed by 1 study and enabled detection of an additional, approximately, 25% SF specimens with CPP crystals.[25] It also modestly enhanced MSU detection. Cytocentrifugation, also known as cytospin, is a specialized centrifugation technique that allows cells and crystals to concentrate on a slide, making detection easier. This method allowed reliable detection of CPP and MSU crystals, stored on SF slides, for up to a year at room temperature.[30] Cytocentrifugation had an advantage of detecting more MSU crystals than a standard smear in SF with low WBC count (defined as < 2000 WBC/μL).[32] This technique also facilitated easier CPP detection than a standard smear, although the overall detection rate was similar.[31] Centrifugation may be used in routine analysis of SF for crystals or at least in those cases with a high clinical suspicion of CAA. It is easier, less costly and more widely available than cytocentrifugation.

Our systematic review has several strengths. It is the largest systematic review of SF specimen handling for CAA. Our review strategy had no language restrictions and the only 2 foreign language studies identified were translated and included. Publication bias risk is a lesser concern, considering the study question, because even a negative result should not preclude study publication. We have focused on identifying findings that are most relevant to the practicing clinicians. Limitations of our study include the relatively high or unclear ROB in the categories of patient selection

and index test performance. This bias emanates from the lack of methodological details for rating ROB in general and absence of adequate blinding in most studies in particular. Many studies did not restrict recruitment to subjects suspected of having a CAA, which may limit applicability. Most studies, by design, needed larger SF sample volumes. It is unclear if those findings apply to small SF sample volumes.

In conclusion, SF should ideally be examined immediately after aspiration. If that is not possible, examination should preferably occur within 24-48 hours. For storage beyond 24-72 hours, the SF sample should be refrigerated and preferably placed in an EDTA tube. Stored SF re-examination, at 24 hours helps identify a small number of additional CPP and MSU cases. Centrifugation techniques help in a quicker and greater identification of both CPP and MSU crystals.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

FA conceived the study idea. FA and MM collected the data. LM conducted the literature search. All authors were involved in the writing of the final draft.

ORCID

Fawad Aslam https://orcid.org/0000-0001-6016-6123

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SUPPORTING INFORMATION

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

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REVIEW

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Interleukin-35 regulates the balance of Th17 and Treg responses during the pathogenesis of connective tissue diseases

Di Wang | Ling Lei

Department of Rheumatology and Clinical Immunology, The First Affiliated Hospital of Guangxi Medical University, Nanning, China

Correspondence
Ling Lei, Department of Rheumatology and Clinical Immunology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Province, China.
Email: leiling1972@aliyun.com

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Abstract

Interleukin (IL)-35 belongs to the IL-12 cytokine family and is a heterodimer of the p35 and Epstein-Barr virus-induced gene 3 (EBI3) subunits. Functionally, IL-35 can promote the proliferation and activation of regulatory T cells (Tregs) and suppress the function of T helper 17 (Th17) cells and other inflammatory cells to inhibit immune responses. In recent years, an abnormal IL-35 expression causing a Th17/Treg imbalance has been associated with the development and progression of several connective tissue diseases (CTDs), such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), dermatomyositis (DM)/polymyositis (PM), and primary Sjögren's syndrome (pSS). Here, we review the role of IL-35 in regulating the balance of Th17/Treg responses in different types of CTDs and provide new insights into the role of IL-35 in these diseases.

KEYWORDS
interleukin-35, dermatomyositis, polymyositis, rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis

1 | INTRODUCTION

Interleukin (IL)-35 is a member of the IL-12 family and is composed of a heterodimer of the p35 and Epstein-Barr virus-induced gene 3 (EBI3) subunits. IL-35 is mainly secreted by regulatory T cells (Tregs) and $CD8^+$ dendritic cells. Furthermore, IL-35 can also be secreted by regulatory B cells (Bregs).[1,2] Functionally, IL-35 promotes the proliferation of Tregs, induces IL-35-secreting B cell differentiation, and inhibits the activation and functional differentiation of effector T cells, such as $IL\text{-}17A^+$ T helper 17 (Th17) cells, thus inhibiting autoimmune inflammation.[3-7] Hence, IL-35 is an important regulator for maintaining the balance of Treg and Th17 responses during the inflammatory process.

A connective tissue disease (CTD) is a disease affecting the connective tissue, and many CTDs are characterized by the loss of self-tolerance and autoimmunity with circulating antibodies and self-reactive T cells. This autoimmunity against different body organs causes symptomatic manifestations. Aberrant autoimmunity, such as strong humoral responses, and downregulated Treg and Breg responses occur in patients with a CTD. It is well known that engagement of a T cell receptor by an antigen determinant and major histocompatibility complex molecules presented by antigen-presenting cells induces T cell activation. The activated T cells differentiate into different types of effector T cells, such as Th1, Th2, Th17, Tregs, and others, in a specific cytokine environment. These functional T cells secrete specific cytokines, including interferon (IFN)-γ, tumor necrosis factor (TNF)-α, IL-2, IL-4, IL-5, IL-17A, IL-10, IL-35, and transforming growth factor (TGF)-β1. Previous studies have shown that IL-35 is crucial for regulating both Th17 and Treg/Breg responses during the development and progression of CTDs.[2,7,8] Here, we update the present knowledge on how IL-35 regulates the balance of Th17 and Treg/Breg responses in individual types of CTDs.

2 | RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is an autoimmune disease affecting multiple joints. Pathologically, RA usually manifests as synovial hyperplasia, inflammatory cell infiltration, and bone erosion in the affected joints, accompanied by blood circulating anticitrullinated protein antibodies (ACPAs) and rheumatoid factors. Animal experiments have shown that ACPAs play an important role in the pathogenesis of RA and can activate osteoclasts, thereby increasing bone erosion before the onset of clinical joint inflammation.[9,10] Furthermore, a variety of immune cells infiltrate into the joint and are crucial for synovial inflammation and joint destruction. These inflammatory infiltrates can secrete cytokines that activate synovial fibroblasts, leading to sustainable inflammation as well as subsequent bone and cartilage damage.[11] Increased Th17 and decreased Treg responses lead to an imbalance, contributing to the pathogenesis of RA.[12-16] Th17 cells induce inflammation by secreting IL-17A, which can activate fibroblast-like synoviocytes and promote the maturation and function of osteoclasts[17,18] In addition, IL-17A can recruit and activate neutrophils, macrophages, and B cells, enhancing inflammation during the process of RA.[19,20] On the other hand, Tregs attenuate inflammation by secreting inhibitory cytokines, such as IL-10 and TGF-β1, to suppress the functions of effector T cells, natural killer cells, and antigen-presenting cells.[21] Adoptive transfer of Tregs protects against inflammation-related joint injury in rodents. Hence, maintenance of the balance of proinflammatory Th17 and anti-inflammatory Treg responses is important in the management of RA patients.

Bregs are important for the maintenance of self-tolerance and inhibition of autoimmunity,[22] and their deficiency is associated with the development and progression of autoimmune diseases, such as RA.[23,24] A decreased number of peripheral blood Bregs is detected in RA patients.[24,25] Functionally, Bregs can inhibit the differentiation of $CD4^+$ T cells into effector Th1 and Th17 cells, which are important pathogenic factors of RA, and promote the proliferation of Tregs.[1,26] Given that IL-35 can induce Breg development, IL-35 may be critical for maintaining the balance of pathogenic Th1/Th17 and Treg/Breg responses during the progression of RA.

There is still debate regarding the precise role of IL-35 in RA-related inflammation. Previous studies have shown that IL-35 is an important inhibitor of autoimmune inflammation and that it can inhibit the osteoclast formation and bone loss induced by TNF-α during the process of RA.[27,28] In addition, a decrease in the serum levels of the EBI3 subunit of IL-35 has been detected in RA patients, which may cause an insufficient IL-35 response.[29] Moreover, the serum levels of IL-35 have been demonstrated to be negatively associated with the active status and Disease Activity Score of 28 joints (DAS28) scores in RA patients, suggesting that IL-35 inhibits the pathogenesis of RA. Additionally, previous studies have revealed that IL-35 inhibits the production of IL-17A and IFN-γ by T cells in vitro[30] as well as the progression of collagen-induced arthritis by decreasing $CD4^+$ effector T cell proliferation and Th17 cell differentiation in rodents.[31] Furthermore, IL-35 can increase IL-10 production and promote Treg proliferation.[32] IL-35 can also minimize angiogenesis and vascular endothelial cell growth by inhibiting angiotensin (Ang) II expression and affecting Ang II/Tie2 signaling.[33] Thus, IL-35 can inhibit systemic inflammation of RA through different pathways, and IL-35 may be valuable to treat RA.[32]

However, there are different views on the role of IL-35 in RA development. The serum level of IL-35 is inversely correlated with disease severity in RA patients, suggesting that IL-35 acts as an anti-inflammatory factor.[34] In addition, high levels of IL-35 in synovial fluid are associated with disease activity in early RA patients, but the levels are reduced in these patients after standard treatment for 12 weeks, suggesting that IL-35 may promote inflammation.[35] Furthermore, IL-35 can promote the release of various proinflammatory cytokines by monocytes in vitro[36] and inoculation with the plasmid for IL-35 expression accelerates disease progression and increases Th17 responses in collagen-induced arthritic mice.[37] The increased levels of IL-35 may stem from the potential proinflammatory role of IL-35 or the presence of excess free p35 subunit.[37] Alternatively, the increased serum IL-35 levels may reflect a physiological compensation to downregulate severe inflammation during the process of active RA. Moreover, a previous study has reported that there was no significant difference in the serum levels of IL-35 between RA patients and healthy controls.[38] Given that IL-35 can be produced by different cell types, it is important to determine how these cells and their IL-35 secretion regulates the pathogenesis of RA.

3 | SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is an autoimmune disease found predominantly in women. Patients with SLE usually present blood circulating antinuclear antibodies, which result in immune complex deposition and complement consumption in multiple organs, leading to cytopenia, glomerulonephritis, rash, and serositis. Accordingly, antinuclear antibodies are crucial for the progression of SLE.[39] Many studies have shown that the imbalance of Treg and Th17 responses is crucial for the pathogenesis of SLE.[40-42] Th17 cells can induce vascular inflammation by increasing the secretion of IL-17A during the pathogenesis of SLE.[42] This, together with a decreased number and function of Tregs, contributes to the activity of SLE.[43,44]

IL-35 can inhibit the differentiation of Th17 cells and the pathogenesis of SLE. The serum levels of IL-35 and $CD4^+EBI3^+$ T cells are negatively correlated with disease activity in SLE patients, particularly in those with nephritis.[45,46] As a result, the decreased levels of IL-35 may increase Th17 responses, but reduce Treg responses, causing an imbalance of Th17 and Treg responses, thus contributing to the pathogenesis of SLE. In the clinic, treatment with methylprednisolone can mitigate the reduction in the serum levels of IL-35 in SLE patients,[45] indicating that IL-35 is an anti-inflammatory factor and potential biomarker for nephritis in SLE.[46] Another study has shown that the levels of plasma IL-35 and Bregs in newly diagnosed SLE patients are significantly reduced.[8] Additionally,Cai,Z et al.[47] have found that in the SLE mouse model, Bregs downregulated

autoimmune responses and had potential therapeutic effects. Moreover, treatment with recombinant IL-35 alleviated SLE in mice, suggesting that the increase in the serum IL-35 level may restore the balance of Th17 and Treg responses to re-establish self-tolerance in SLE mice.[47] Therefore, IL-35 inhibits inflammation during the process of SLE and is a potential biomarker for the diagnosis of SLE-related nephritis.

However, the precise role of IL-35 in the pathogenesis of SLE remains controversial. One study has indicated that serum IL-35 concentration is increased in active SLE patients and that it is decreased after high-dose prednisone treatment in remitting patients.[48] Another study has found that the increased serum levels of IL-35 did not correlate with the Systemic Lupus Erythematosus Disease Activity Index 2000 scores in active SLE patients.[49] Elevated serum levels of IL-35 may be associated with the promotion of inflammation, but they can also be a feedback compensation in response to severe inflammation. Alternatively, this discrepancy may stem from different patient populations with varying disease stages and genetic backgrounds. Thus, further studies are necessary to determine the role of IL-35 in the pathogenesis of SLE as well as its therapeutic potential for the treatment of SLE.

4 | SYSTEMIC SCLEROSIS

Systemic sclerosis (SSc) is characterized by the presence of autoantibodies and progressive tissue fibrosis, particularly in the skin, internal organs, and small blood vessels.[50] It is well known that autoimmunity-related inflammation and fibrosis are important for the pathogenesis of SSc.[51,52] Several studies have demonstrated that reduced numbers and function of Tregs, increased frequency of Th17 cells, and higher serum IL-17A levels are detected in SSc patients.[53-56] These data indicate that the imbalance of Th17 and Treg responses contributes to the pathogenesis of SSc.[57] In addition, pathogenic $CD8^+$ T cells may also participate in the pathogenesis of SSc by secreting type 2 proinflammatory cytokines,[58,59] which may explain the high fibrosis feature of SSc.

How IL-35 regulates the pathogenesis of SSc has not been clarified. IL-35 may inhibit inflammation by inducing the proliferation of Tregs and by inhibiting the differentiation and development of Th17 cells.[32] Evidently, decreased EBI3 expression is associated with reduced numbers of Tregs in the skin of SSc patients,[60] and IL-35 can inhibit type I and III collagen expression in mouse fibroblasts.[61] Induction of EBI3 overexpression in the skin has been demonstrated to improve skin fibrosis in mice, further suggesting that IL-35 may have a therapeutic potential to inhibit fibrosis during the process of SSc. Moreover, IL-35 can enhance Breg development to inhibit inflammation during the pathogenesis of SSc.[7] Hence, IL-35 may be valuable for the treatment of SSc.

However, other studies have shown that IL-35 can activate dormant fibroblasts and promote collagen production.[62,63] The serum levels of IL-35 are increased in active SSc patients, particularly for those with pulmonary fibrosis, and are decreased after standard treatment.[64,65] These data suggest that IL-35 may contribute to the pathogenesis of SSc. However, it is unclear whether aberrant IL-35 responses come from a feedback compensation to inhibit Th17 responses and simultaneously promote stronger Treg responses that secrete high levels of TGF-β1, enhancing fibrosis.[65] Alternatively, IL-35 may regulate the pathogenesis of SSc, depending on its stage of development.

5 | IDIOPATHIC INFLAMMATORY MYOPATHIES

Idiopathic inflammatory myopathy (IIM) is a rare autoimmune disease affecting the proximal muscles. Clinically, the major types of IIMs include dermatomyositis (DM) and polymyositis (PM). Although the pathogenesis of IIMs is currently unclear, B cell-mediated small vessel damage and $CD8^+$ T cell-mediated muscular injury contribute to the development of DM and PM, respectively.[66,67] Previous studies have shown that a decreased number of peripheral blood Tregs and an increased frequency of Th17 cells occur in patients with DM or PM,[38,68] indicating that the imbalance of Th17 and Treg responses is crucial for the pathogenesis of PM/DM. Furthermore, the serum levels of proinflammatory and anti-inflammatory cytokines are increased in patients with DM or PM.

How IL-35 influences the pathogenesis of PM and DM is unclear. Some studies indicate that serum IL-35 levels are increased in PM and DM patients, particularly in those with dysphagia,[38,68] and are moderately negatively correlated with the disease severity. Moreover, elevated serum levels of IL-35 in those with anti-3-hydroxy-3-methyl-glutaryl-coenzyme A reductase and anti-signal recognition particle antibodies, 2 key markers for severe necrotizing myopathy and dysphagia,[69] suggest that elevated serum levels of IL-35 may be a biomarker of dysphagia in IIM patients.[38] The serum IL-35 levels decrease after IIM remission; therefore, the serum IL-35 level may be a biomarker for evaluating disease activity.[38] In contrast, higher serum levels of IL-35 are detected in PM/DM patients after the initial treatment, compared to those with recurrent diseases, suggesting that IL-35 may act as an anti-inflammatory factor.[70] In addition, recombinant IL-35 treatment can significantly reduce the serum levels of IL-17A and TNF-α in patients with PM/DM.[70] The different results may stem from the limited number of studies reported in the literature, different stages of disease, and patients with varying genetic backgrounds. Hence, further studies with a larger population sizes, particularly for a prospective clinical trial, are necessary to determine the role of IL-35 in the pathogenesis of PM/DM and its potential as a treatment for PM/DM patients at different stages of disease.

6 | PRIMARY SJÖGREN'S SYNDROME

Primary Sjögren's syndrome (pSS) is an autoimmune disease that mainly affects middle-aged women.[71] pSS is characterized by B cell activation-mediated chronic inflammation in the exocrine glands,

TABLE 1 The role of interleukin (IL)-35 in the pathogenesis of connective tissue diseases

Disease	Function	Section References	Mechanism
Rheumatoid arthritis	Suppress inflammation	27-34	Th17/Treg ratio imbalance; Breg cell number decreases
	Promote inflammation	35-37	
Systemic lupus erythematosus	Suppress inflammation	8,45-47	Th17/Treg ratio imbalance; Breg cell number decreases
	Promote inflammation	48	
Systemic sclerosis	Suppress inflammation	7,32,61	Th17/Treg ratio imbalance
	Promote inflammation	62-64	
Idiopathic inflammatory myopathies	Suppress inflammation	70	Th17/Treg ratio imbalance
	Promote inflammation	38,68	
Primary Sjögren's syndrome	Suppress inflammation	71,77	Th17/Treg ratio imbalance; IL-12/IL-35 imbalance
	Promote inflammation	79	
Behçet's disease	Suppress inflammation	83	Th17/Treg ratio imbalance
	Promote inflammation	84	

leading to dry eyes and mouth.[66] Pathologically, inflammatory infiltrates, such as activated $CD4^+$ T cells, in the affected organs as well as serum autoantibodies are detected in pSS patients.[72] Further analysis has indicated an increased frequency of peripheral blood Th17 cells but reduced Tregs in pSS patients,[73,74] suggesting that the imbalance of Th17 and Treg responses contributes to the pathogenesis of pSS.[75] However, 1 study detected an increased frequency of Tregs in pSS patients.[76] Furthermore, the imbalance of IL-12 and IL-35 responses may be associated with the development of pSS,[77] and the frequency of peripheral blood Bregs is increased in pSS patients.[78] However, the precise mechanisms underlying the action of the imbalance of IL-12 and IL-35 responses and Bregs in the development and progression of pSS remain to be investigated.

IL-35 may inhibit inflammation during the process of pSS. Evidently, decreased serum levels of IL-35, p35, and EBI3 messenger RNA (mRNA) transcripts and $CD4^+EBI3^+$ T cells have been detected in pSS patients and inversely correlated with the erythrocyte sedimentation rate and the European League Against Rheumatism Sjögren's syndrome disease activity index scores in these patients.[71,77] These findings suggest that these measures may be valuable for evaluating disease activity. However, it has been reported that increased serum IL-35 levels were positively correlated with disease activity in pSS patients.[79] These conflicting data may be due to different populations of patients at varying stages of disease. Alternatively, the increased serum levels of IL-35 may reflect a negative feedback compensation to inhibit severe inflammation.[79] Given that there are few studies related to pSS, it is urgent to further investigate the role of IL-35 in regulating the pathogenesis of pSS and its potential therapeutic effect.

7 | BEHÇET'S DISEASE

Behçet's disease (BD) is a systemic vascular disease characterized by recurrent oral ulcers, genital ulcers, and intraocular lesions. Previous studies have shown that increased serum IL-17A levels and peripheral blood Th17 cells, together with impaired Treg function, are detected in BD patients,[80-82] indicating that the imbalance of Th17 and Treg responses contributes to the development of BD.

A few studies have demonstrated the role of IL-35 in the pathogenesis of BD. One study has pointed out that serum IL-35 levels are decreased but IL-17A levels are increased in BD patients and that the reduced levels of IL-35 responses may be insufficient to inhibit inflammation and attenuate Th17 responses, leading to the imbalance of Th17 and Treg responses.[83] Furthermore, the serum IL-35 levels are reduced after remission in BD patients following metformin treatment, which may reflect alleviation of the inflammatory responses.[84]

8 | CONCLUSION

IL-35 is a potent anti-inflammatory cytokine which acts by directly suppressing inflammatory Th17 and Th1 responses as well as indirectly inducing Treg responses. In different CTDs, IL-35 may play different roles (Table 1). In RA and SLE, IL-35 may play a protective role by inhibiting disease progression. In IIM and SSc, IL-35 also tends to inhibit inflammation. The role of IL-35 in complex CTDs, such as SSc and BD, remains controversial given that there is evidence of it inhibiting as well as promoting inflammation. In addition, high levels of IL-35 secretion and consequent strong Treg responses may enhance fibrosis, contributing to the pathogenesis of SSc. In some CTDs, the levels of IL-35 increase during active disease and decrease after treatment. These findings suggest that IL-35 may promote inflammation or may reflect compensative anti-inflammatory responses to severe inflammation. IL-35 may have therapeutic potential in the treatment of some CTDs, and its efficacy may depend on the disease type and stage. Moreover, whether endogenous and exogenous IL-35 have different effects on the development and progression of different types of CTDs still needs to be explored. Understanding such questions may aid in the design of new therapies for the treatment of CTDs in the clinic. Therefore, the immunosuppressive and

therapeutic effects of IL-35 on CTDs need to be further studied, especially in humans.

CONFLICT OF INTEREST

Neither of the authors have any conflicts of interest. There has been no interest or relationship with pharmaceutical agencies within the past 36 months.

AUTHOR CONTRIBUTIONS

Di Wang consulted the literature and wrote the manuscript. Lei Ling designed the study, reviewed the manuscript, and supervised the entire study. Both authors read and approved the final manuscript.

ORCID

Di Wang https://orcid.org/0000-0002-5342-4152

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REVIEW

A practical guide to interpreting and applying systematic reviews of qualitative studies in rheumatology

Daniel Sumpton[1,2,3] | Ayano Kelly[2,3,4,5] | David Tunnicliffe[2,3] | Jonathan C. Craig[3,6] | Chandana Guha[2,3] | Geraldine Hassett[4] | Allison Tong[2,3]

[1]Rheumatology Department, Concord Repatriation General Hospital, Sydney, NSW, Australia

[2]Sydney School of Public Health, The University of Sydney, Sydney, NSW, Australia

[3]Centre for Kidney Research, The Children's Hospital Westmead, Sydney, NSW, Australia

[4]Department of Rheumatology, Liverpool Hospital, Sydney, NSW, Australia

[5]Australian National University, Canberra, ACT, Australia

[6]College of Medicine and Public Health, Flinders University, Adelaide, SA, Australia

Correspondence
Dr Daniel Sumpton, MBBS, Rheumatology Department, Level 6 West, Concord Repatriation General Hospital, Concord, NSW, Australia 2139
Email: daniel.sumpton@health.nsw.gov.au

Abstract

While patient-centered care is widely advocated in the management of rheumatic diseases, it can be challenging to implement, particularly for patients with complex systemic conditions. Patient-centered care involves identifying and integrating the patient's experiences, attitudes, and preferences in decision-making. Qualitative research is used to describe patient perspectives and priorities that may not always be expressed in clinical settings. Systematic reviews of qualitative studies can provide new and more comprehensive evidence of patients' beliefs and priorities across different populations and healthcare settings and are increasingly being reported across medical specialties, including rheumatology. In rheumatology, they have been used to examine topics including medication-taking and adherence, coping with systemic sclerosis and conservative management and exercise in osteoarthritis. By referencing recent examples of systematic qualitative reviews in the rheumatology literature, this article will outline the methodology and methods used, and provide an approach to guide the appraisal of reviews. We aim to give the reader a practical understanding of systematic reviews of qualitative literature and elucidate how knowledge gained from such reviews can be applied to improve the care of patients with rheumatic conditions.

KEYWORDS

Qualitative research, Systematic review, Thematic synthesis

1 | INTRODUCTION

Patient-centered care is widely advocated and requires partnership between healthcare professionals and patients in decision-making to address the broader impacts of disease and treatments that are important to patients, but may not be considered by clinicians.[1,2] Such an approach is particularly relevant in rheumatology because patients with rheumatic disease often have unpredictable and debilitating symptoms impairing quality of life. Patient-centered care is crucial in the management of rheumatology conditions where the complexity and chronicity of disease mean that patients often experience care that is fragmented and inefficient.[1]

Patient-centered approaches hinge on a deep comprehension of the needs, beliefs, and values of patients living with rheumatic disease. Often such values remain unstated in time-limited clinical consultations, and patients may not feel comfortable expressing their concerns with healthcare providers. Qualitative research provides an opportunity for patients to articulate their priorities and reveal detailed insights into experiences of disease and treatment to inform patient-centered care. Systematic reviews of qualitative

studies combine and synthesize the findings from primary studies to offer more comprehensive knowledge of people's experiences and perspectives across a range of different health settings and populations.[3,4] They are used to develop new conceptual models, identify research and clinical knowledge gaps and provide evidence of the acceptability of healthcare interventions.[3] There is an increasing number of systematic reviews of qualitative studies published across medical specialties, including rheumatology.

In rheumatology, systematic reviews of qualitative studies have addressed clinically important challenges including medication-taking and adherence and patient attitudes to conservative treatment in osteoarthritis.[5,6] This article will briefly highlight examples of recent systematic reviews of qualitative studies in rheumatology and provide a practical guide to reading and understanding the methods of systematic reviews of qualitative studies in rheumatology literature.

2 | EXAMPLES OF SYSTEMATIC REVIEWS OF QUALITATIVE RESEARCH IN RHEUMATOLOGY

Systematic reviews of qualitative studies in rheumatology have described often under-recognized experiences and impacts of disease in people across a spectrum of rheumatic conditions. Knowledge gained from systematic reviews of qualitative studies is described below with examples from systematic reviews of medication-taking and adherence in inflammatory arthritis, systemic sclerosis, and osteoarthritis (Table 1). The examples described below were selected from 18 published systematic reviews of qualitative studies in rheumatology literature in the last decade (Table S2). The reviews were found through a search of MEDLINE (Table S1), a manual search of reference lists and search of top 5 rheumatology journals by impact factor. The following examples are not intended to represent a comprehensive synthesis of all systematic reviews of qualitative studies in rheumatology literature. The reviews included have been selected for their variable topics, to provide context to the application of such reviews to rheumatology practice and to guide discussion of the appraisal of systematic reviews of qualitative studies outlined at the end of this article.

2.1 | Medication-taking and adherence

Kelly et al[6] conducted a systematic review of patient experiences of taking disease-modifying antirheumatic drugs (DMARDs), involving 56 studies of 1383 patients with either rheumatoid arthritis or spondylarthritis. The main findings showed that patients with inflammatory arthritis are alarmed at the need to take DMARDs, viewing medications as a sign of deteriorating health and are daunted by the prospect of life-long treatment. Patients are distressed by potential side effects, uncertainties in treatment efficacy, and confused when they received conflicting medical advice. Patients wanted to be in control of decisions and make choices about DMARDs based on their life priorities. Of importance was the concept that

TABLE 1 Recent examples of systematic reviews of qualitative studies in rheumatology literature

Research topic	Methodology	Quality appraisal	Key findings	Implications
Patients' attitudes and experiences of DMARDs to inform strategies to improve medication adherence[7]	Thematic analysis	COREQ	Patients with inflammatory arthritis equate DMARDs with intensifying disease identity and distressing uncertainties and consequences	Patient-provider relationships play a vital role in DMARD acceptance. Patients felt more confident in medication-taking when guided by optimistic and knowledgeable clinicians who attempt to understand their practical needs and validate their fears
Experience of living with systemic sclerosis[14]	Thematic analysis	COREQ	Systemic sclerosis is disfiguring, unpredictable, undermining patients' sense of certainty and impairing their self-image and affecting normal social roles	A multidisciplinary approach needs to be undertaken to address psychological turmoil and enhance coping with illness. Strategies could include promotion of self-esteem, resilience training, coping with fatigue, and interventions to enhance return to work
Attitudes of people with osteoarthritis toward their conservative management[6]	Meta-ethnography	CASP	People with osteoarthritis delay their diagnosis, opting for self-management, and informal information gathering even after healthcare involvement. Diagnosis is sought at a "critical point," but symptoms are perceived to be trivialized by healthcare professionals	Healthcare providers should not trivialize osteoarthritis, and patients should be encouraged to continue self-management strategies. People caring for people with osteoarthritis should convey an optimistic perception of non-operative management to increase the adoption of conservative management strategies

Abbreviations: DMARDS, disease-modifying antirheumatic drugs; COREQ, Consolidated Criteria for Reporting Qualitative Health Research; CASP, Critical Appraisal Skills Program

patient-provider relationships play a critical role in DMARD acceptance and adherence. Patients felt more confident in medication-taking when guided by optimistic and knowledgeable clinicians who attempt to understand their concerns about medications. The need for more trusting relationships has implications for communication styles adopted by clinicians. Patients are likely to be more convinced to take medications by clinicians who not only provide information about medication side effects but who also validate their concerns and make attempts to understand their practical needs.

The systematic review of qualitative studies has implications for practicing clinicians and in further research of medication adherence. This study outlines a practical guide to discussion of disease-modifying antirheumatic drugs (DMARDs) to improve patient understanding of their medications, and potentially improve medication adherence. The guide outlines five key areas including: addressing initial patient concerns, assessing patient willingness to take DMARDs, advising on the medication choices including the impacts on lifestyle and risks, providing assistance in taking medication by providing support and arranging follow up to address the experience of medication-taking. The systematic review of qualitative studies has also been used in the development of an Outcomes Measures in Rheumatology (OMERACT) core domain set for interventions that aim to support medication adherence in rheumatology.[7]

2.2 | Systemic sclerosis

A systematic review by Nakayama et al described the experience of living with systemic sclerosis and included 26 studies involving 463 patients. Systemic sclerosis is experienced as a disfiguring and unpredictable illness, undermining patients' sense of certainty, impairing their self-image, and impacting on daily functioning.[8] Strategies to support patients with systemic sclerosis should focus on changes in appearance, promote self-esteem, and offer resilience training. Interventions should also emphasize practical tips to cope with fatigue and pain and improve workplace support.[8] Knowledge gained from this systematic review of qualitative studies in systemic sclerosis by Nakayama et al have been used to highlight current gaps around patient-centered care in the management of systemic sclerosis and as a reference point for the development of patient-centered care guidelines and recommendations for optimal management.[9,10]

2.3 | Osteoarthritis

Smith et al[5] undertook a systematic review of 33 studies, including 1314 patients with osteoarthritis, to describe the experience of the conservative management of osteoarthritis. They found that people with osteoarthritis are initially reluctant to seek a formal diagnosis due to a desire to maintain self-management. Once help is sought, people perceive healthcare providers to trivialize their symptoms.[5] The negative perception of interactions with healthcare staff is consistent across different healthcare settings (Canada, Taiwan, and the UK). Exercise as a form of treatment was considered important, but the difficulty in undertaking exercise due to symptoms of pain and concerns that exercise may further harm joints was a predominant concept. Many people felt conservative therapy was ineffective, a waste of time, and a mandatory "hoop" that needs to be jumped through before surgery. Primary studies linked this perception of ineffective management to the deleterious and trivializing attitude of healthcare professionals toward their care. The relationship between the patient and physiotherapist was a considered to be a key driver of exercise adherence by patients in promoting a sense of duty to the physiotherapist to continue exercising, providing moral support and practical guidance on how to exercise without exacerbating symptoms.[5]

Adherence to a regular exercise and a targeted physiotherapy program is the centerpiece of management in osteoarthritis to improve pain and restore joint function and is reflected in the most recent Osteoarthritis Research Society International (OARSI) treatment guidelines.[11] Systematic reviews of qualitative studies demonstrate that novel education programs are needed to foster positive exercise attitudes among people with osteoarthritis. Knowledge gained from the systematic reviews of qualitative studies in osteoarthritis by Smith et al have informed the development of a novel multidisciplinary interventional education program to improve self-management[12] and have also been used in the development of a computer-based clinical decision support tool to help support the conservative management of knee osteoarthritis.[13] Novel education approaches in osteoarthritis, for example the Good Life with osteoArthritis in Denmark (GLA:D) initiative, have led to positive behavior change in exercise adherence, reduction in pain and improvements in health-related quality of life.[14]

3 | FEATURES OF SYSTEMATIC REVIEWS AND SYNTHESIS OF QUALITATIVE RESEARCH

This section will discuss the features of systematic reviews of qualitative research generally but also refer to the examples above to explain the use of methodology and methods in rheumatology literature.

3.1 | Methodology

Methodology refers to the underlying theory that guides the research process in qualitative research. There are several methodologies used in systematic reviews of qualitative research. Thematic synthesis,[4] critical interpretive synthesis,[15] meta-ethnography,[16] and meta-study[17] are commonly used methodologies, which are summarized in Table 3. The methodology used provides a structure to inform the approach to literature search and selection, appraisal of primary studies, and synthesis of results.

3.2 | Literature search and selection

Systematic reviews of qualitative studies can use two types of search strategies. One approach is to conduct a comprehensive strategy to identify all studies relevant to a population, topic, and research question. The other is an iterative search strategy whereby researchers read and select papers that will aid in theory development. This latter approach is generally used in critical interpretive synthesis, and grounded theory synthesis.

Sourcing qualitative studies in a comprehensive manner is challenging as filters to identify qualitative research are not validated in standard electronic databases,[18-20] and sensitivity and specificity are unknown. Qualitative studies are also not well indexed in standard electronic databases, partly due to the range of methodological terms used to describe and report qualitative research.

Electronic databases such as MEDLINE, EMBASE, Cumulative Index for Nursing and Allied Health Literature (CINAHL), PsycINFO are commonly used in medical reviews.[21,22] Other specialist databases from other disciplines (eg, sociology, education, and nutrition) should be searched if they are relevant to the review topic. In the search strategy, terms relating to the population, the clinical or health topic, and qualitative methodology and social phenomena may be used. Table 2 shows examples of common MeSH and non-MeSH terms relevant to the methods, methodology and social phenomena. Hand searching reference lists of relevant articles and Google Scholar searches may also be conducted to identify additional studies.

3.3 | Appraisal of primary studies

The appraisal of primary qualitative studies is contentious,[23,24] with no consensus on a single framework applicable to the numerous methodologies used in qualitative research. There is little empiric evidence to support whether different approaches impact on the credibility and transferability of results. However, appraisal of the included primary studies can allow readers to assess the rigor of the overall review findings.[25,26]

Most systematic reviews of qualitative research published in rheumatology journals include a quality appraisal of included primary studies. There are 3 approaches to assessing primary qualitative studies: assessment of conduct and methodology, appraisal of transparency of reporting, and analysis of how well the findings contribute to theory development. The Critical Appraisal Skills Program (CASP) and The Consolidated Criteria for Reporting Qualitative Health Research (COREQ) have been used in systematic reviews to assess the quality of primary qualitative studies.[25,26]

The CASP qualitative checklist contains 10 questions to assess the methods, conduct, methodology, and rigor of primary qualitative studies. The last question of the CASP checklist asks the reader to consider the value of the study in relation to existing research. COREQ appraises the comprehensiveness of reporting in primary qualitative studies in 3 domains: research team and reflexivity (the extent to which the study recognizes the researchers' influence in the research), the study design (reporting of the theoretical framework, selection of participants, population, and data collection), and analysis and findings.

The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) working group have recently developed the Confidence in the Evidence from Reviews of Qualitative Research (GRADE-CERqual) framework. GRADE-CERqual grades the confidence of findings in systematic reviews of primary qualitative studies based on four principles of methodological limitation, coherence, adequacy, and relevance of data.[27] While GRADE-CERqual has not been widely adopted, it does allow readers to assess if the reviews finding is a reasonable representation of a phenomena, or if they have confidence of the acceptability of an intervention.[28]

TABLE 2 Methodology, methods, and social phenomena search terms

Methodology search terms	Methods search terms	Social phenomena search terms	
MeSH terms: Qualitative research Grounded theory Non-MeSH terms: Theme/thematic Thematic analysis Phenomenology Content analysis Ethnography	MeSH terms: Focus groups Non-MeSH term Interview Semi-structured interview	MeSH terms: Decision making Illness behavior Health knowledge, attitudes, practice Attitude to health Social psychologyInterpersonal relations Patient satisfaction Self-esteem/self-concept Quality of life Lifestyle Social support Social adjustment Psychological adaptation Communication Emotions	Non-MeSH terms Health belief Social belief

3.4 | Qualitative synthesis

Qualitative synthesis generates new findings that go beyond a summary of the findings of primary studies. Instead, analysis of concepts within and across studies can generate new concepts and theoretical constructs to provide comprehensive evidence across different populations and healthcare settings.[29] There are various methods of qualitative synthesis used in health literature including thematic synthesis, meta-ethnography, and critical interpretive synthesis.[29]

Thematic synthesis in a systematic review of qualitative studies involves coding the findings reported in the primary study. Coding of reported data will typically encompass analyzing the results, including quotations, or discussion sections of papers, identifying common concepts between studies, grouping these into themes, synthesizing themes in the context of how the studies relate to each other and generating new themes and displaying these concepts in a schematic model.

Returning to the example above, Kelly et al used a thematic synthesis approach. All text under the results, participant quotations and discussion section of included papers were reviewed. The first author inductively identified initial concepts and developed a coding framework after discussion with other authors. Themes and subthemes were developed and refined in an iterative process between authors. HyperResearch software was used to systematically apply the initial themes and subthemes to all included text and results, further refining the final themes. Conceptual links between themes were identified and used to develop a published thematic schema. The thematic schema explains the broader understanding of the data, framing the subthemes according to factors that lead people to either accept or be more resistant to accepting DMARDs.

Meta-ethnography is another common method of qualitative synthesis used in rheumatology literature. This method was developed by Noblit and Hare and involves seven stages.[30] The first three stages involve deciding on whether a qualitative synthesis on the proposed topic is required ("getting started"); deciding on the scope of the search, method of literature search and method of appraisal ("deciding what is relevant") and reading and re-reading the studies to identify first-order constructs and second-order constructs ("reading the studies").[16,31] The last four stages describe the synthesis of concepts identified in primary studies and involves identifying, describing and comparing the concepts used in each study to develop third-order constructs ("determining how the studies are related"); exploring similarities and differences between second-order constructs from each account to determine how they relate and interact with each other ("translating studies into each other"), and comparing these translations to develop third-order constructs, new interpretations or conceptual models ("synthesizing translations"). The final stage, "expressing the synthesis," describes communicating the results of the synthesis in a comprehensible and accessible way.[16,31]

The example above by Smith et al describing the experience of osteoarthritis used a meta-ethnography approach. After reading all studies and deciding on the relevance for inclusion, three reviewers independently identified all emerging themes and metaphors. Concepts were compared in a grid to determine how the concepts related to each other or were juxtaposed. Reciprocal translation occurred where similar themes were "matched" across studies, ensuring included themes accurately describe concepts from different papers.[16,32] Refutational analysis was also undertaken, where studies with competing explanations were compared and translations were developed to explain these differences.[16,32] For example, the paper describes contrasting attitudes toward taking non-steroidal anti-inflammatories including fear of gastrointestinal side effects but also concern about exacerbation of symptoms with medication discontinuation, leading to a reliance on a potentially dangerous medication. Finally, the authors developed a "line of argument" where the authors summarize the main three themes and display this is in a schematic figure to explain the relationship between concepts.

Dixon-Woods et al first described critical interpretive synthesis where searching, sampling, critique and analysis of primary studies occur simultaneously and in an iterative process toward theory generation.[33] Searching is intentionally purposive, where studies are selected if they add to the development of theory. As data is extracted, a new synthetic construct is developed by recursive and reflexive comparison of new themes to the existing concepts ensuring that theory generated is plausible, fair and critically informed. Critical interpretive synthesis is unique in the way the searching and purposive selection of studies and concepts form a part of the synthesis and theory generation.[33]

4 | REPORTING AND APPRAISING SYSTEMATIC REVIEWS OF QUALITATIVE STUDIES

The "Enhancing transparency in reporting the synthesis of qualitative research (ENTREQ) Statement" facilitates comprehensive reporting of systematic reviews and synthesis of qualitative research.[3] The ENTREQ framework includes 21 items across five domains: introduction, methods and methodology, literature search and selection, appraisal of primary studies, and synthesis of findings.

To guide the appraisal of systematic review of qualitative research, we suggest using the 4 constructs proposed by Guba and Lincoln to assess rigor: credibility (are the findings trustworthy?), confirmability (are the interpretations derived from the data?), dependability (is the process logical and auditable?) and transferability (are the findings relevant to other settings and contexts?).[34] While these values are traditionally used to guide the assessment of rigor of primary qualitative studies, they can also be applied to evaluate systematic reviews of qualitative studies.[35,36] In the next section, we outline strategies from ENTREQ under these four construct headings so that readers can make an evaluation when reading systematic reviews of qualitative studies.

4.1 | Credibility

Credibility informs the degree to which the findings can be trusted.[35] In primary qualitative studies, credibility refers to how

TABLE 3 Summary of common methodologies for the synthesis of qualitative health research

Methodology	Aim of review	Literature search	Quality appraisal	Analytical principles and techniques
Critical interpretive synthesis	New theoretical conceptualization – synthetic construct	Theoretical sampling	Examines the degree to which the primary study will inform the theory development	• Concurrent iteration of the research question • Extract data and summarize • Define and apply codes to create themes
Thematic synthesis	Generate themes that offer a new interpretation beyond the primary studies	Systematic and comprehensive	Assesses the aims, context, rationale, methods and findings, reliability, validity and methods for ensuring findings are grounded in participant perspectives	• Line by line coding of results • Organization into descriptive concepts • Iterative analysis into themes
Meta-ethnography	To develop higher-order interpretations based on primary studies	Not specified	Assesses the relevance of study: deciding on the scope of the search, method of literature search and method of appraisal	• Develop 1st and 2nd order constructs • Compare differences and similarities between studies (translation) • Synthesis of translations and comparisons to create theory and a line of argument
Meta-study	Describe differences in research findings and methods, to develop a new interpretation of the phenomena	Not specified	Focuses on the process of research used in the primary study, to appraise the rigor of research methods and theory	Combines 3 components of: • Analysis of findings (meta-analysis) • Analysis of methods (meta-methods) • Analysis of theory (meta-theory)

well the study reflects the experiences of participants.[36,37] In a systematic review, credibility refers to the degree to which the overall findings represent the data and results reported in the primary qualitative studies. The credibility of the synthesis can be increased by strategies to improve the breadth and scope of relevant data and reduce individual researcher bias. The systematic review should provide a description of the setting and population of included studies in detail, set clear definitions of the sections of the primary qualitative paper that will be included (eg, primary paper quotations, results and discussion sections). Investigator triangulation is a practice in which multiple investigators are involved in the independent analysis of data allowing for refinement and agreement of themes. Triangulation ensures that individual investigators can reflect on their own biases, which may have impacted on the study data (researcher reflexivity). The systematic review should provide a "thick description," including a comprehensive description of the concepts and themes so that readers can reflect on whether the results capture the relevant depth of data in the primary studies.

In Nakayama et al, the authors describe using investigator triangulation to ensure the analysis reflected the "full breadth and depth of data" analyzed. The first and second author undertook independent assessment of each study. Disagreements were resolved through discussion with the last author. Preliminary themes were discussed among all authors and adjustments were made to themes and subthemes as new concepts emerged. The authors also provide a comprehensive ("thick") description of themes and subthemes identified by including a rich and profound understandings of people's experiences rather than superficial descriptions of already understood experiences or clinical manifestations. For example, when describing patient physical symptoms associated with systemic sclerosis in the subtheme entitled "Palpable physical limitations", the authors do not just simply describe the clinical characteristics of skin thickening, fatigue, and pain. Rather, they provide a description on the psychological and functional impacts of these symptoms. People with systemic sclerosis do not just feel "tight" because of skin thickening, they feel exasperated by mismatches between their mental astuteness and physical incapability. Equally, social impairment in having to abandon leisure activities is explained as an intense feeling of guilt at "ruining everybody's time".

4.2 | Dependability

Dependability refers to the extent to which the process of interpretation of data is logical and can be audited. Dependable systematic reviews demonstrate that interpretative decisions made by the researchers are transparent. Dependability can be enhanced by detailing the explicit search strategies, including the databases searched and search strategy used. Studies improve their dependability by specifying the rationale for appraising studies (eg, CASP or COREQ) and by providing explicit details of the criteria used for inclusion and exclusion of studies. Using data analysis software assures that

decision making around thematic analysis was systematically recorded and can be audited.

Smith et al describe a clear search strategy including the electronic databases searched (AMED, CINAHL, EMBASE, PsychINFO, SportsDisc, MEDLINE, Cochrane Clinical Trials Registry) and outlines a search strategy used for the MEDLINE database. Studies were included if they reported perceptions of the non-operative management of knee osteoarthritis. Studies describing healthcare provider perceptions or patient experience toward surgery were excluded. The included and excluded papers are described in a PRISMA flowchart. No computer software is used but the authors describe using a "grid" to examine matching and juxtaposed concepts.

4.3 | Transferability

This principle is the extent to which the concepts generated in the thematic synthesis are relevant and applicable to other healthcare settings and populations.[35,37] Assessing the transferability of findings is improved if a systematic review gives a clear description of the study characteristics (eg, the healthcare setting and description of the population) and provides a thorough description of the findings of each study (thick description).

In Kelly et al, a study describing concepts around medication adherence in rheumatoid arthritis and spondylarthritis, the characteristics of included studies are outlined in a table. We can tell that most studies (73%) describe the experience of people with rheumatoid arthritis and all studies occur in North America, Europe or Australia. This may have implications for the transferability of these results to patients with other types of inflammatory arthritis or for patients in non-Western medical settings. This information also highlights the need for further qualitative research in other healthcare settings. A detailed description of each study including the country of publication, sample size, age and gender of participants, type of arthritis, disease duration, treatment, method of data collection (eg, focus groups), methodological framework (eg, thematic analysis) and topic is included as a table in the supplementary material. The contributions of each study referenced to each subtheme is also found in supplementary material Tables S1 and S2.

4.4 | Confirmability

Confirmability strives to establish that findings in the synthesis are derived from the data and have not been misinterpreted or distorted by the researcher.[35] Strategies to improve the substance and true interpretation of data in systematic reviews include investigator checking, where the primary studies are independently assessed by multiple investigators to ensure the concepts and themes generated incorporate the data and concepts presented in the primary studies. Adding quotations from primary studies links the concepts outlined in the systematic review and establishes that the conclusions of the systematic review are founded in the results of the primary studies. The concepts generated in the analysis can be further confirmed by referencing the contribution of each study to each theme, either in a table or in the description of the results.

Confirmability of the reviews findings is demonstrated in Nakayama et al by using investigator checking and referenced quotations from included primary studies. The authors report that 2 reviewers independently assessed each study. Referenced quotations from primary studies are also presented in Table 3 under each subtheme. Table 3 outlines the contributions of each study to the individual subthemes.

5 | CONCLUSION

Systematic reviews and synthesis of qualitative studies can provide deep and novel insights into people's experiences, values, and beliefs across multiple healthcare settings and populations. In rheumatology, they have been used to develop new conceptual models to understand patient experience, identify gaps in care, and provide evidence for patient-centered interventions. This review provides an overview of the methodology and methods of systematic reviews of qualitative studies with reference to recent examples in rheumatology literature. The principles of credibility, dependability, transferability, and confirmability provide a framework for the practical appraisal of the findings of reviews.

CONFLICT OF INTEREST

All authors declare no relevant conflicts of interest.

AUTHOR CONTRIBUTIONS

All listed authors have made substantial contributions to this paper including the conception, design and writing of the work, drafting, and revising the manuscript critically for intellectual content. All authors have approved the final version to be published.

ORCID

Daniel Sumpton https://orcid.org/0000-0002-2590-5426
Ayano Kelly https://orcid.org/0000-0003-3325-3840

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Effect of laying on of hands as a complementary therapy for pain and functioning in older women with knee osteoarthritis: A randomized controlled clinical trial

Katy Andrade Monteiro Zacaron[1,2] | Cláudia Soares dos Santos[1] | Cyntia Pace Schmitz Corrêa[1,3] | Yuri Cotta e Silva[1] | Isabel Cristina Fonseca Reis[1] | Maryana Sant'Ana Simões[1] | Giancarlo Lucchetti[1]

[1]School of Medicine, Federal University of Juiz de Fora, Juiz de Fora, Brazil

[2]Institute of Life Sciences, Department of Physiotherapy, Federal University of Juiz de Fora, Governador Valadares, Brazil

[3]Faculty of Physical Therapy, Federal University of Juiz de Fora, Juiz de Fora, Brazil

Correspondence
Giancarlo Lucchetti, School of Medicine, Federal University of Juiz de Fora, Av. Eugenio do Nascimento s/n – Bairro Dom Bosco, 36038-330, Juiz de Fora, Minas Gerais, Brazil.
Email: g.lucchetti@yahoo.com.br

Abstract

Aim: To assess the effects of laying on of hands (LooH) as a complementary therapy to kinesiotherapy, on pain, joint stiffness, and functional capacity of older women with knee osteoarthritis (KOA) compared to a control group.

Methods: In this randomized controlled clinical trial, participants were assigned into 3 groups: LooH with a spiritual component ("Spiritist passe" Group - SPG), LooH without a spiritual component (LooH Group - LHG), and a control group receiving no complementary intervention (Control Group - CG). Patients were assessed at baseline, 8 weeks, and 16 weeks. Primary outcomes were joint stiffness and functional capacity (Western Ontario and McMaster Universities Osteoarthritis Index [WOMAC]), and pain (WOMAC and visual analog scale). Secondary outcomes were anxiety, depression, mobility, and quality of life. Differences between groups were evaluated using an intention-to-treat approach.

Results: A total of 120 women (mean age = 69.2 ± 5.2 years) with KOA were randomized (40 participants per group). At 8 weeks, SPG differed significantly from the LHG for WOMAC Functional Status (between-group difference in the change = 0.97; 95% CI: 0.35 to 1.59, $P = .001$); Anxiety levels (between-group difference in the change = 1.38; 95% CI: 0.11 to 2.65, $P = .027$); and also from the CG for all outcomes with exception of WOMAC Stiffness. After 16 weeks, SPG differed significantly from the LHG only for WOMAC Functional Status (between-group difference in the change = 0.92; 95% CI: 0.32 to 1.52, $P = .001$]) and also from the CG for all outcomes with exception of WOMAC Stiffness and timed up-and-go.

Conclusion: Our results suggest that LooH with a "spiritual component" may promote better long-term functional outcomes than both LooH without a "spiritual component" and a control group without LooH.

Trial registration: ClinicalTrials.gov Identifier NCT02917356.

KEYWORDS
complementary therapies, holistic therapies, knee osteoarthritis, spiritual healing, spiritual therapies, therapeutic touch

1 | INTRODUCTION

Osteoarthritis (OA) is a multifactorial progressive disease that is more prevalent in older women, where the knee is the weight-bearing joint most affected by the condition.[1] Because knee OA (KOA) results in pain and disability which cause a decline in work and social functioning, the condition may contribute to mental health problems[2] and negatively impact quality of life (QoL).[3] There is a vast array of options for treating this condition, including kinesiotherapy, recognized as one of the most important non-pharmacological therapies for KOA by many organizations, such as the Osteoarthritis Research Society International (OARSI), The European League Against Rheumatism (EULAR), National Institute for Health and Care Excellence (NICE) and The American College of Rheumatology (ACR).[4-7]

Although kinesiotherapy and other physical therapy and medical approaches for OA can be beneficial, their efficacy remains limited. Due to these limitations, a large contingent of patients seeks complementary and alternative medicine (CAM) therapies for the treatment of OA.[8] CAMs are a group of medical and healthcare systems, practices and products, which are not considered to be part of conventional medicine.[9]

The focus of the present study is the laying on of hands (LooH) CAM therapy. LooH is defined as the use of hands on or near the body to help in the "healing" of diseases.[10] The mechanisms for such therapy are not well elucidated; however, some theories hypothesize that the human body is composed of "energy fields", and according to this theory, the patient could exchange energy with the healers, even without physical contact.[10] LooH is a CAM used throughout the world, among different cultures and religions and includes: Reiki, Johrei and external *Qigong*, bioenergy, contemporary metaphysical tradition and, Therapeutic Touch and Healing Touch, where the latter are used by Western healthcare professions, mainly in the nursing context.[11] Although LooH can be provided in several different ways, the technique is usually delivered as follows: the "healer" prepares him/herself to administer the technique (ie, centering to a calm, quiet and balanced condition), attunes to an energy, moves hands with the palms facing toward the patient and at a distance of about 1-2 inches over the body of the patient in a smooth movement with the intention to "heal".[12] Studies have investigated the effect of LooH in KOA patients, demonstrating positive effects of external *Qigong*, Healing Touch and Therapeutic Touch on pain, functioning, mobility, joint stiffness, muscle strength, depression, and mood.[13-17]

LooH is also used in the tradition of Spiritism, the third-largest religion in Brazil. LooH is referred to as "Spiritist Passe" (SP) in this tradition and is part of the therapies practiced free of charge under Spiritism.[18] The SP is defined as "energy transfusion, derived from the Spiritist healer and from good Spirits, or a combination of both, that changes the cell field".[19] Therefore, this tradition is supposed to have a "spiritual component", in which an alleged "spiritual energy" would act in the patient in addition to the energy provided by the healer. It is estimated that there are 1650 Spiritist centers offering SP in Brazil and these centers receive individuals from all religious backgrounds. A previous study investigating 55 Spiritist centers in São Paulo, Brazil found that these centers receive approximately 60 000 attendees per month and that SP is offered in all of these centers.[20]

Concerning the scientific background of SP, the first study published on this subject was an experiment demonstrating that SP was able to inhibit bacterial growth in vitro.[21] Subsequently, some clinical trials evaluated SP, finding that participants who received SP showed reduced anxiety and depressive symptoms, negative effects and muscle tension, as well as improvements in QoL, immunological response, peripheral oxyhemoglobin saturation and well-being.[22-27]

Despite this promising evidence, these studies have limitations that require caution when interpreting their results. Most trials have not evaluated SP provider characteristics, the control groups did not have the intention to "heal the patient", these studies involved only a small number of intervention sessions and short follow-up periods, and lacked an intention-to-treat (ITT) analysis. Finally, to our knowledge, there are no studies investigating differences in outcomes between LooH with a "spiritual component" and LooH using a more secular approach.

Therefore, the aim of this study was to assess the effects of LooH (with and without a spiritual component), as a complementary therapy to kinesiotherapy, on pain, joint stiffness, and functional capacity of older women (60 years old) with KOA compared to a control group. As a secondary objective, levels of QoL, and depression and anxiety symptoms, were also compared for the 3 intervention arms of the study.

2 | STUDY DESIGN AND METHODS

This is a triple-blind (ie, blinded assessor, patient and statistician), single-center, prospective, parallel and randomized controlled trial, registered on clinicaltrials.gov under number NCT02917356. A full detailed description of the methods for this trial can be found in a previous publication.[28] This study was approved by the Research Ethics Committee of the Federal University of Juiz de Fora, Brazil under registry CAAE 52 623 115.0.0000.5147.

2.1 | Randomization

Patients were randomly assigned into 3 groups at a 1:1:1 ratio. A researcher, not involved with data collection, randomized the patients

using block randomization procedures (block size = 6) and computer-generated random numbers - List randomizer of permutations (random.org). Participants were allocated into 3 groups: LooH with a spiritual component ("Spiritist Passe Group", SPG), LooH without a spiritual component (Laying on of Hands Group, LHG) and a group without LooH (Control Group, CG).

2.2 | Sample and setting

The sample comprised women with KOA. These participants were recruited using different strategies: advertisement of the study protocol through posters and lectures at the Primary Health Care Units; referrals by health professionals; and by spontaneous demand. The study was carried out in a public health setting in the city of Juiz de Fora, Brazil, where medical, physical therapy and nursing care are provided to patients aged 60 years and older.

2.3 | Eligibility criteria

For inclusion in the study, participants had to be: female; aged 60 years or older; have primary OA in both knees; OA grade II or III according to Kellgren and Lawrence criteria[29]; a Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score > 5[30]; use stable doses of analgesic or anti-inflammatory drugs during intervention (ie, in each session of the intervention, researchers asked if there was a change in the dose of analgesics in order to identify acute problems and to avoid its influence in the results of the trial. In order to be included, participants were required to maintain the same dosage of analgesics throughout the trial duration); not perform physical exercises, physical therapy or any kind of energy therapy different from those proposed in this study; and be able to read, understand, and speak Portuguese. Exclusion criteria included: use of oral, systemic injectable or intra-articular steroids in the 3 months leading up to study screening; use of intra-articular hyaluronate in the 3 months leading up to study screening; previous hip or knee arthroplasty; presence of neurological diseases or other rheumatic diseases; report other causes of pain in lower limbs; have medical contraindications for light-to-moderate physical activity and; have cognitive impairment, as assessed by the Mini-Mental State Examination.[31]

2.4 | Procedure

The patients, the researcher who enrolled the participants, the physical therapists who provided the kinesiotherapy, the outcome assessor, and the researcher conducting statistical analyses were all blinded to treatment assignment. The blinding of patients was done using swimming goggles (Master Beach Black®) painted black (Spray Mundial Prime®) and patients were wearing black goggles while receiving the CAM therapy (ie, "Spiritist Passe Group", LooH without a spiritual component or no LooH), but not while receiving physical therapy.

2.4.1 | Kinesiotherapy program

Patients enrolled in all groups participated in a 45-minute, light-to-moderate group kinesiotherapy program consisting of 5-minute warm-up and stretching, 37-minute exercise (strengthening of lower limbs and neuromotor exercise - motor skills and balance), and 3-minute relaxation (detailed description in Zacaron et al[28]). The intervention took place twice a week for 8 weeks and was supervised by 6 trained physical therapists blinded to treatment assignment. Perceived exertion during kinesiotherapy was measured using the revised Borg Scale (0-10) after each session.[32]

2.4.2 | Complementary interventions

During the sessions for all 3 groups, patients remained seated and received the following verbal command: "Relax and calm your mind". All groups received treatment simultaneously in different dimly lit rooms, which were rotated daily. The interventions were applied without touching the patients, for 5 minutes once a week (8 weeks of intervention). The SPG received SP, applied after kinesiotherapy, and SP providers were oriented to think about "healing the patient". The LHG received LooH (with no spiritual component), applied after the kinesiotherapy, and LooH providers were oriented to think about "healing the patient". The CG received only kinesiotherapy, but not LooH. To make control patients feel the presence of someone in the room, they were accompanied by volunteers who moved slowly and randomly to simulate the presence of someone performing LooH. However, there was no intention to heal the patient in this group.[28]

2.5 | Measures

Patients were assessed by a blinded researcher at baseline (0 week), post-intervention (8 weeks) and follow-up (16 weeks). During the follow-up period (8-16 weeks), patients were periodically contacted by phone to ensure they had not received additional KOA interventions, where doing so excluded them from the study. Patients were then invited for the final follow-up assessment (16 weeks). All primary and secondary measures were assessed at the same day.

2.5.1 | Sample characteristics

Sociodemographic, clinical, and anthropometric (Filizola® anthropometric scale) data; religiousness (Duke University Religion Index - DUREL),[33] spirituality (Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being Scale - FACIT-Sp 12),[34] optimism (Life Orientation Test-Revised version - LOT-R),[35] and credibility and expectancy about the effect of treatment (section I of Credibility/Expectancy Questionnaire - CEQ)[36] levels were collected to characterize the older women with OA (detailed description in Zacaron et al[28]).

2.5.2 | Primary outcomes

Pain intensity in the knees was assessed using the visual analog scale (VAS) and WOMAC Pain subscale.[30,37] Functional capacity was assessed by the WOMAC functional capacity subscale.[30,37] The WOMAC instrument, validated for use in Brazil, comprises 5 items related to pain, 2 items related to joint stiffness, and 17 items related to functional capacity. Each item was scored from 0 (none) to 10 (extreme). Higher scores represent worse health status.[37] The timeframe for the primary endpoint was defined as both 8 and 16 weeks.

2.5.3 | Secondary outcomes

Anxiety and depression symptoms were assessed using the Hospital Anxiety and Depression Scale (HADS).[38] Functional mobility was evaluated using the timed up-and-go (TUG) test[39] and QoL was determined using the World Health Organization Quality of Life-Bref (WHOQOL-Bref).[40] Perceived changes in symptoms resulting from treatment were recorded on a 5-point Likert scale: worsened, unchanged, slightly improved, much improved, and healed.[41] In order to collect patients' opinions about different aspects of the treatment and on the clinical application of LooH, 5 questions were applied at the 16th week evaluating which group participants believed they were enrolled in, the success of the therapy, and whether LooH should be used as a complementary therapy. The timeframe for the secondary endpoint was defined as both 8 and 16 weeks.

2.5.4 | Intervention staff characteristics

Sociodemographic, dietary and substance use/abuse data (assessment form developed by researchers) and well-being (Subjective Well-being - SWB)[42] information were collected to define the characteristics of SP and LooH providers.

In order to collect the state of physical and mental health of SP and LooH providers, prior to each session, they were asked the following question: "Have you had any bad experiences during the last week that have affected your physical or mental health? (yes or no)".

2.6 | Sample size

Sample size was calculated based on a previous study investigating the effect of therapeutic touch on KOA,[17] and previous studies using the total score for each subscale of the WOMAC instrument.[14,43] The sample size was based on multiple primary outcomes (ie, the trial is successful if there is a significant improvement in at least 1 primary outcome). The minimum required sample was 105 participants for this trial, adopting an alpha of 0.05 and a power (1-Beta) of 0.80. The complete sample size calculation is described in a previous publication.[28]

2.7 | Statistical analysis

Descriptive analysis was performed using frequency, percentage, mean, and standard deviation for all baseline characteristics.

The 3 groups were compared at baseline in terms of their sociodemographics, clinical conditions and instruments, using the Chi-square test for categorical variables and analysis of variance (ANOVA) for independent measurements, using Bonferroni as a post hoc test.

Then, a 3 × 3 repeated-measures multiple analysis of variance - MANOVA (group [LHG, CG, SPG] by time [baseline, 8th week and 16th week]) on the dependent variables WOMAC Pain, WOMAC Stiffness, WOMAC Functional Capacity, VAS, HAD Anxiety, HAD Depression, TUG and WHOQOL-Bref was carried out. If significant, the subsequent post hoc analyses were conducted through Bonferroni post hoc tests.

Finally, the differences between changes in scores for each scale (post – Pretests) between groups were analyzed using ANOVA for independent measurements and Bonferroni as a post hoc test. In case of imbalances, analysis of covariance (ANCOVA) was carried out considering baseline scores.

All statistical analyses were performed using the ITT and the per protocol (PP) approaches. For the PP analysis, patients having missed more than 4 sessions of kinesiotherapy or 2 sessions of CAM therapy were excluded.

A total of 13 patients (out of 120) had their data imputed. Missing data were handled under the assumption of "missing at random" (MAR). The method of Multiple Imputations was used (method "fully conditional specification" – an iterative Markov chain Monte Carlo method available in SPSS) with 10 iterations for every imputation. We imputed the missing values of outcomes at 8 and 16 weeks of follow-up (ie, WOMAC Pain, WOMAC Stiffness, WOMAC Functional Capacity, VAS, HAD Anxiety, HAD Depression, TUG and WHOQOL-Bref) and the following predictors were used in linear regression models in order to estimate the follow-up outcomes: baseline data of all outcomes, age and grade of KOA. At 16 weeks, outcomes assessed at 8 weeks were also used if available.

The Statistical Package for Social Sciences software version 17.0 (IBM Corp, Armonk, NY, USA) was used for the multiple imputation and for all statistical analyses.

3 | RESULTS

The detailed Consolidated Standards for Reporting Trials (CONSORT) diagram depicting the selection process and exclusion and inclusion criteria is given in Figure 1. Of 1082 patients initially invited to participate, 120 were included and randomly assigned into 3 groups (n = 40 per group). During the intervention period, there were withdrawals/ dropouts due to absences (3 in SPG, 4 in LHG and 1 in CG), other treatment (1 in SPG) and goggles intolerance (1 in CG). During the follow-up period, there were withdrawals due to loss of contact (1 in SPG) and other treatment (2 in CG).

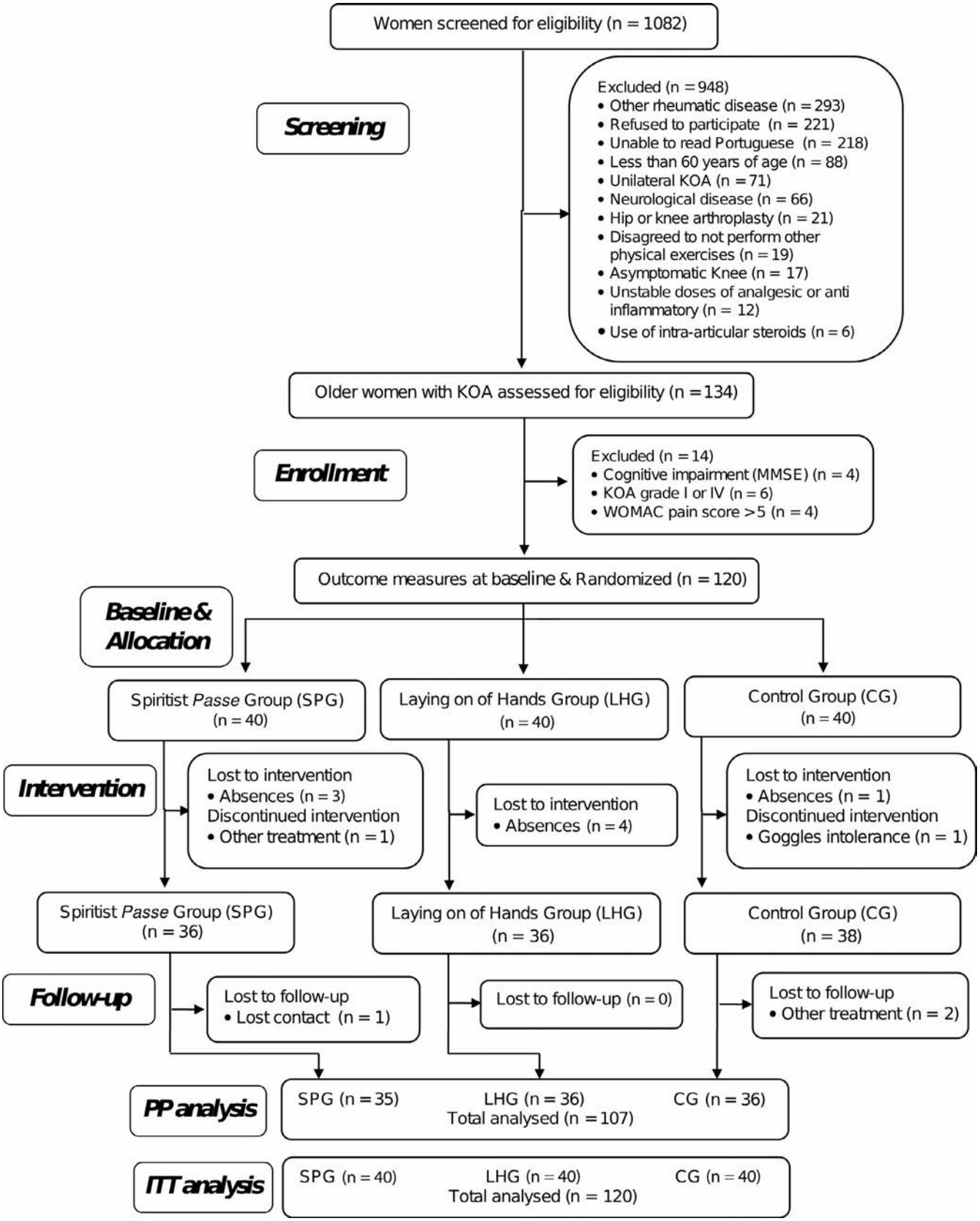

FIGURE 1 Consolidated Standards for Reporting Trials (CONSORT) flow chart. Bars represent 95% confidence intervals. ITT, intention-to-treat; KOA, knee osteoarthritis; MMSE, Mini-Mental State Examination; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index

Therefore, a total of 107 patients completed the study (35 in SPG, 36 in LHG and 36 in CG). However, all 120 randomized patients were included in ITT analysis.

3.1 | Baseline characteristics

3.1.1 | Participants

At baseline, there were no significant differences in relation to the sociodemographic characteristics and clinical conditions and instruments. The study sample comprised women who were elderly, of White ethnicity, had more than 4 years of formal education and were married (Table 1).

3.1.2 | Staff

There were no significant differences in sociodemographic data, dietary status, substance use/abuse or well-being among the SP, LooH providers and volunteers accompanying the control group. Likewise, there were no differences concerning the occurrence of bad experiences among the groups (Table S1).

3.2 | Analyses for the primary and secondary outcomes

Univariate analyses were conducted on each dependent variable as a follow-up test to MANOVA The comparison among groups showed differences for the scores of VAS, WOMAC Pain, WOMAC Functional capacity and Total WHOQOL-Bref.

The follow-up outcomes of the groups are shown in Figures 2 and 3. At 8 weeks, Bonferroni post hoc tests revealed the following differences: (a) VAS-Pain, differences between SPG and CG, and between LHG and CG, but not between SPG and LHG; (b) WOMAC Pain, differences between SPG and LHG, between SPG and CG, and between LHG and CG; and (c) WOMAC Functional Capacity, differences between SPG and CG and SPG and LHG, but not between LHG and CG. There were no differences for the scores of WOMAC Stiffness among groups. In relation to the secondary outcomes, there were significant differences in: (a) HAD Depression, differences between SPG and CG, but not between LHG and CG and LHG and SPG; (b) WHOQOL Total Score, differences between SPG and CG and LHG and CG, but not LHG and SPG. There were no differences for TUG and HAD Anxiety.

At 16 weeks, Bonferroni post hoc tests revealed the following differences: (a) VAS-Pain, differences between SPG and CG, and between LHG and CG, but not between SPG and LHG; (b) WOMAC Pain, differences between CG and LHG, and between SPG and CG, but not for LHG and SPG; and (c) WOMAC Functional Capacity, differences between SPG and CG, and between SPG and LHG, but not between LHG and CG. There were no differences for the scores of WOMAC Stiffness among groups. In relation to the secondary outcomes, there were significant differences in WHOQOL Total Score, differences between SPG and CG and for LHG and CG, but not for LHG and SPG. There were no differences for TUG, HAD Depression and HAD Anxiety.

While investigating the differences between changes in scores for each scale (Table 2) at 8 weeks, SPG differed significantly from the LHG for WOMAC Functional Status (adjusted mean = −2.99, 95% CI: −3.35 to −2.64 vs. −2.02, 95% CI: −2.38 to −1.66; between-group difference in the change = 0.97, 95% CI 0.35 to 1.59, $P = .001$); HAD Anxiety (−3.77, 95% CI:-4.74 to −2.80 vs. −2.39, 95% CI: −3.12 to −1.66; between-group difference in the change = 1.38, 95% CI: 0.11 to 2.65, $P = .027$); and also to the CG for all outcomes with exception of WOMAC Stiffness. After 16 weeks, the difference in change for SPG differed significantly from the LHG only for WOMAC Functional Status (adjusted mean = −2.85, 95% CI: −3.20 to −2.51 vs. −1.93, 95% CI: −2.27 to −1.58; between-group difference in the change = 0.92, 95% CI: 0.32 to 1.52, $P = .001$) and also to the CG for all outcomes with exception of WOMAC Stiffness and TUG.

In the PP analysis, results were maintained for the MANOVA procedure. However, while investigating the differences between changes in scores for each scale there were differences between SPG and LHG in the WOMAC Pain and WOMAC Function. These results can be visualized in the Supplementary material (PP).

3.3 | Other analyses

There was no difference between groups regarding the numbers of kinesiotherapy ($P = .713$) and LooH ($P = .717$) sessions received by each group after 8 weeks of protocol (Table S2). Based on pre- and post-intervention VAS-Pain measures for each CAM session, no adverse effects were observed for SP application and only 2 LHG patients reported a maximum 1-point increase in pain.

Concerning the perceptions and opinions of the participants, most believed LooH should be used to treat OA and depression/anxiety as a complementary therapy. Likewise, most participants believed that health professionals should consider using LooH in clinical settings. These differences were not significant among groups (Table S2).

Finally, most patients believed they had participated in the SPG, as opposed to the CG or LHG. Although there was no significant difference in this perception between groups, only 57.6% of the SPG believed they received Spiritist Passe as compared to 72.7% of the LHG and 71.9% of the CG ($P = .194$), and most felt the interventions had improved their condition (Table S2).

4 | DISCUSSION

The results of the present study suggest that LooH with a "spiritual component" may promote better outcomes than LooH without a "spiritual component" or a control group without LooH. Our results

TABLE 1 Baseline anthropometric, sociodemographic and clinical characteristics of studied groups

	LHG (n = 40)	CG (n = 40)	SPG (n = 40)
	Mean (SD)	Mean (SD)	Mean (SD)
Age, y	68.85 (5.37)	69.45 (4.84)	69.50 (5.68)
Weight, kg	77.18 (15.38)	75.11 (12.92)	75.28 (13.00)
BMI,(kg/m^2	30.40 (5.45)	29.98 (4.60)	30.75 (4.60)
MMSE	27.73 (2.29)	27.78 (2.08)	27.68 (2.48)
DUREL Organizational	2.90 (1.42)	2.28 (1.10)	2.35 (1.25)
DUREL Non-organizational	1.63 (0.80)	1.63 (1.07)	1.65 (1.07)
DUREL Intrinsic	5.13 (1.81)	4.38 (1.48)	4.90 (1.63)
FACIT-Sp12 Peace	10.88 (3.33)	10.80 (3.13)	10.73 (2.93)
FACIT-Sp12 Meaning of life	11.93 (2.24)	11.63 (2.22)	11.90 (2.47)
FACIT-Sp12 Faith	12.30 (2.13)	12.10 (1.99)	11.95 (2.08)
LOT-R Positive	9.13 (1.50)	9.30 (1.13)	8.93 (1.43)
LOT-R Negative	8.35 (1.67)	8.20 (1.57)	8.38 (1.51)
LOT-R Total	17.48 (2.76)	17.50 (2.25)	17.30 (2.56)
VAS	7.23 (1.30)	6.71 (1.34)	6.96 (1.39)
WOMAC Pain	6.77 (1.19)	6.26 (1.25)	6.41 (1.21)
WOMAC Stiffness	2.34 (2.10)	1.68 (2.22)	1.78 (2.26)
WOMAC Functional capacity	6.45 (1.27)	5.84 (1.32)	5.94 (1.40)
TUG, s	13.89 (2.30)	13.01 (2.85)	13.44 (2.40)
HAD Anxiety	8.23 (4.51)	7.78 (3.62)	8.43 (4.44)
HAD Depression	7.70 (4.27)	6.70 (3.42)	6.55 (3.63)
WHOQOL-Bref Total	13.10 (2.39)	13.20 (2.40)	13.30 (2.34)
Treatment Credibility	21.73 (3.35)	21.33 (3.20)	21.40 (3.15)
Treatment Expectancy	76.75 (14.56)	75.50 (12.18)	75.25 (13.77)
	n (%)	n (%)	n (%)
Ethnicity: White	23 (57.5%)	19 (47.5%)	21 (52.5%)
Marital status: married/cohabitating	17 (42.5%)	15 (37.5%)	16 (40.0%)
Religious affiliation			
Catholic	24 (60.0%)	22 (55.0%)	28 (70.0%)
Evangelical/Protestant	7 (17.5%)	9 (22.5%)	7 (17.5%)
Spiritist	2 (5.0%)	7 (17.5%)	2 (5.0%)
Messianic	1 (2.5%)	0 (0.0%)	0 (0.0%)
None, but believe in God	5 (12.5%)	2 (5.0%)	3 (7.5%)
None, and do not believe in God	1 (2.5%)	0 (0.0%)	0 (0.0%)
Schooling, 4 + y	27 (67.5%)	31 (77.5%)	30 (75.0%)
KOA level			
II	4 (10.0%)	7 (17.5%)	7 (17.5%)
III	36 (90.0%)	33 (82.5%)	33 (82.5%)

Abbreviations: LHG, laying on of hands (LooH) without a spiritual component; CG, control; SPG, LooH with a spiritual component (Spiritist Passe); BMI, body mass index; DUREL, Duke University Religion Index; FACIT-Sp12, Functional Assessment of Chronic Illness Therapy-Spiritual Well-Being Scale; LOT-R, Life Orientation Test-Revised; MMSE, Mini-Mental State Examination; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; TUG, timed up-and-go test; HAD, Hospital Anxiety and Depression Scale; WHOQOL-Bref, World Health Organization Quality of Life-Bref; KOA, knee osteoarthritis.

showed that the SPG (which applied SP once a week for 8 weeks) differed significantly from the LHG on 2 primary outcomes of the study (namely, WOMAC Pain and WOMAC functional capacity) and to the CG for virtually all outcomes. The results were maintained after the end of the intervention. These findings add to the current literature and will be discussed below.

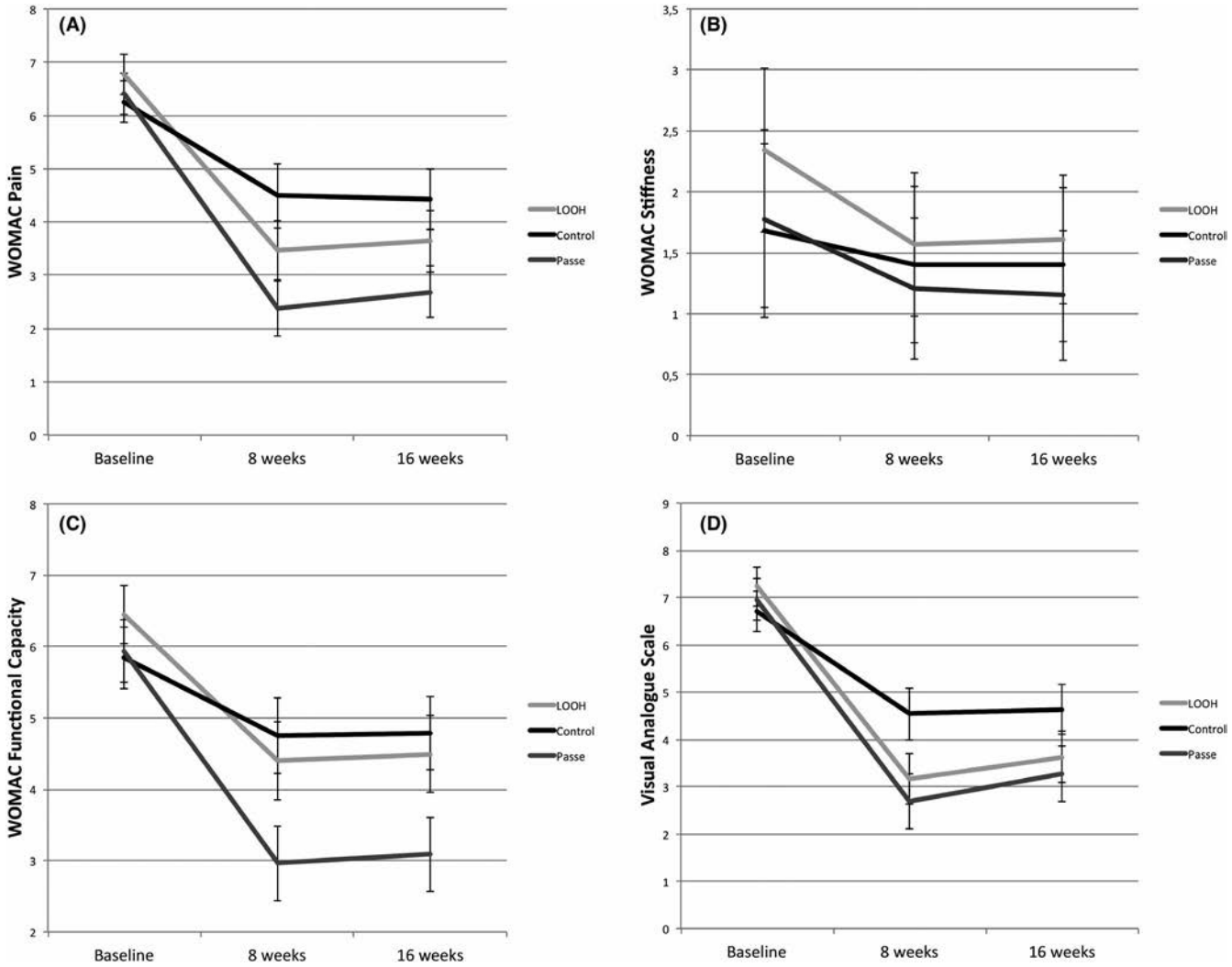

FIGURE 2 Differences among groups for the primary outcomes Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Pain (A), WOMAC Stiffness (B), WOMAC Functional Capacity (C) and visual analog scale pain (D). Bars represent 95% confidence intervals. LooH, laying on of hands without a spiritual component; Passe, laying on of hands with a spiritual component - "Spiritist Passe Group"; Control, group without laying on of hands (Control Group)

In agreement with our results, previous studies on KOA using other LooH approaches, such as therapeutic touch, healing touch or external *Qigong*, reported significant reduction in pain[13,44] and depression,[14] and a significant increase in functional capacity.[13,44] Although there are no studies specifically investigating SP in OA, clinical trials employing SP in patients with various types of health problems have shown promising evidence of reduction in depressive symptoms[22,45] and improvement in QoL.[22,45]

Primary and secondary outcomes were significantly improved between baseline and week 8 in all groups and remained significant between baseline and week 16. The changes in the WOMAC subscales revealed a minimal clinically important difference (MCID) for improvement. A previous study,[46] using WOMAC scale from 0 to 10 (the same used in our study) detected MCID changes of −0.75 for WOMAC Pain (in our study ranging from −1.82 in the CG to −3.71 in the SPG), −0.72 for WOMAC Stiffness (in our study ranging from −0.28 in the CG to −0.76 in the LHG) and 0.67 for WOMAC Function (in our study ranging from −1.05 in the CG to −2.84 in the SPG). Concerning VAS, changes of −1.2 points are considered MCID[47] and in our study this result ranged from −2.16 in the CG to −4.27 in the SPG. Despite the improvements, it is noteworthy that there was a significant difference between applying LooH with and without a "spiritual component" in the primary outcome functional capacity after 8 and 16 weeks. There are several factors that might explain these findings. Some authors suggest that patients submitted to "spiritual healing" may assimilate a "vital energy" passed by the healers and this could be a possible mechanism promoting salutary effects in the mental and physical health of subjects.[27] This has not been scientifically proven and should be interpreted with caution. Another explanation is that patients may have discovered which group they were assigned to and, thus, were more prone to indicate better changes in the group submitted to SP. This seems unlikely in our study, since patients were successfully blinded and the SPG had lowest perception of receiving "spiritual healing". It is also possible that the intention to heal the patient, and

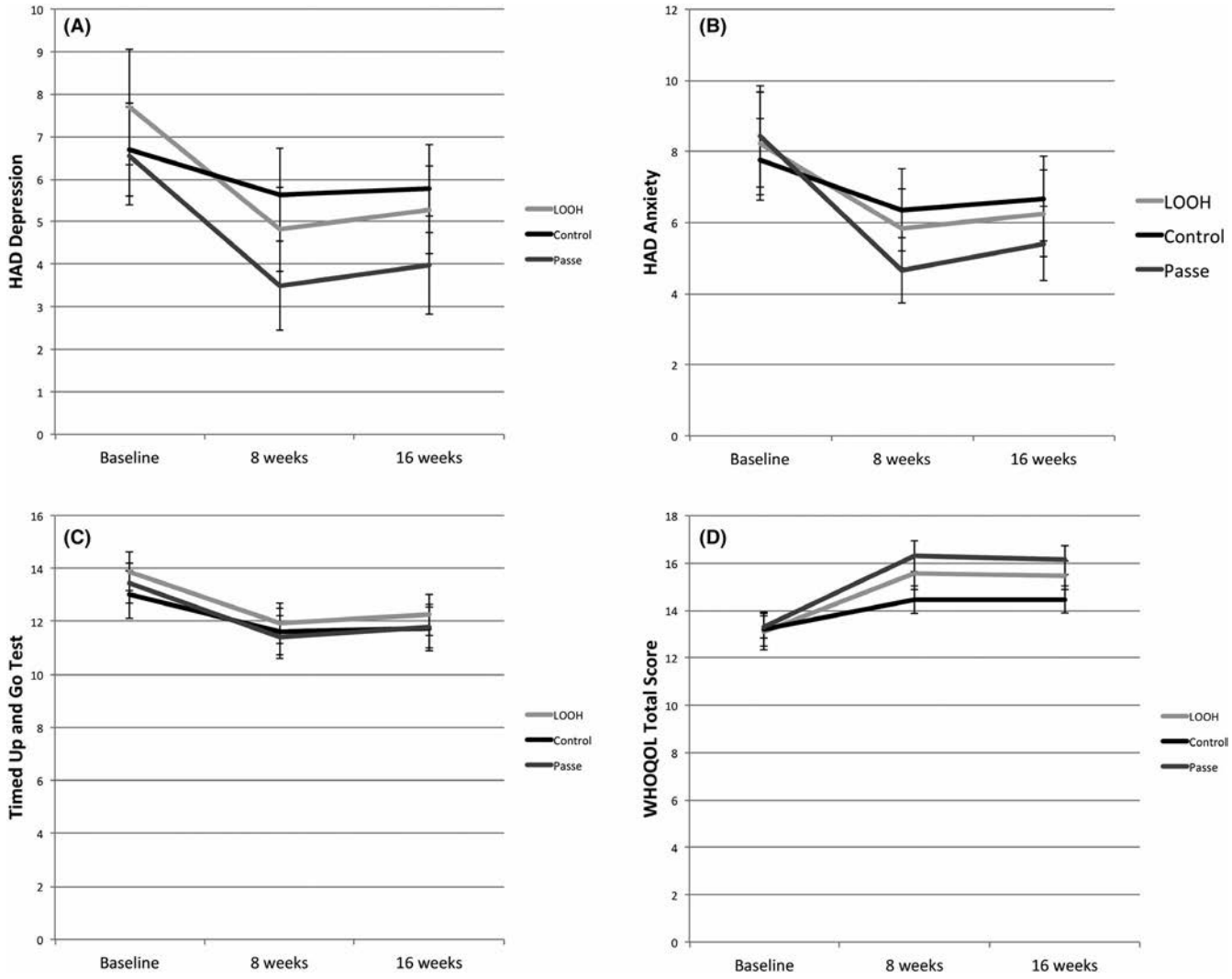

FIGURE 3 Differences among groups for the secondary outcomes Hospital Anxiety and Depression Scale (HAD) Depression (A), HAD Anxiety (B), timed up-and-go test (C) and World Health Organization Quality of Life-Bref (WHOQOL-Bref) (D). Bars representing 95% confidence intervals. LooH, laying on of hands without a spiritual component; Passe, laying on of hands with a spiritual component - "Spiritist Passe Group"; Control, group without laying on of hands (Control Group)

not a "spiritual power", was responsible for these outcomes. Although this is a controversial topic, a previous meta-analysis[48] showed that only having an intention to heal someone was associated with better health outcomes. Likewise, several studies have shown that secular types of LooH, such as therapeutic touch, might be associated with better outcomes relative to control groups.[13-17] In order to minimize this problem, the present study included a control group without "intention to heal", along with another group that had "intention to heal" without a "spiritual connection". Interestingly, even when the SPG and LHG had the same intention to heal the patient, the results were still different between groups. It is unclear whether the level of concentration or training among Spiritist healers was higher than laypersons and, in some way, this could have impacted our results. Since both groups had the same sociodemographic characteristics, other differences concerning age, gender and emotional status seem not to be responsible for these outcomes. Future studies should compare "spiritual healers" against therapeutic touch providers.

Despite the positive results, in the present trial, no evidence was found that the SPG was superior to other groups in relation to mobility (measured by the TUG) and knee stiffness. Our explanation for these negative findings is that the TUG may be not an appropriately responsive performance-based test to assess patients with moderate-to-severe pain due to the transition between sit-to-stand and the short distance used in this measure.[49,50] There also may have been a "floor effect" for the stiffness measure, since this symptom was low in most patients.

The present study has some limitations that should be considered when evaluating the results. First, the absence of patients who were male, younger, with secondary KOA and grades I and IV, limits the generalizability of these findings. Second, although the researchers asked the participants not to switch medication during the study period, it is not possible to guarantee that patients adhered to this recommendation, which may influence one group more than another. Third, although the randomization may

TABLE 2 Between-group differences in change in outcome measures over time

	Group				
	LHG (n = 40)	CG (n = 40)	SPG (n = 40)		Between-group differences in change
Variable	Mean (SD) [95% CI]	Mean (SD) [95% CI]	Mean (SD) [95% CI]	*P*	Mean [95% CI], (*P* value)
VAS[a] (8 weeks – Baseline)	-4.07 (1.28) [−4.48 to −3.65]	-2.16 (1.31) [−2.58 to −1.74]	-4.27 (1.81) [−4.85 to −3.69]	**<.001**	LHG × CG: −1.91 [−2.71 to −1.09] (*P* < .001), LHG × SPG: 0.20 [−0.60 to 1.00] (*P* > .999), CG × SPG: 2.11 [1.29 to 2.91] (*P*< .001)
VAS[a] (16 weeks – Baseline)	-3.60 (1.20) [−3.98 to −3.21]	-2.06 (1.36) [−2.50 to −1.62]	-3.69 (1.74) [−4.25 to −3.13]	**<.001**	LHG × CG: −1.53 [−2.33 to −0.74] (*P* < .001), LHG × SPG: 0.09 [−0.70 to 0.88] (*P* > .999), CG × SPG: 1.63 [0.83 to 2.42] (*P* < .001)
WOMAC Pain[a] (8 weeks – Baseline)	-3.30 (1.09) [−3.65 to −2.95]	-1.77 (1.28) [−2.18 to −1.35]	-4.02 (1.76) [−4.59 to −3.46]	**<.001**	LHG × CG: −1.53 [−2.29 to −0.76] (*P* < .001), LHG × SPG: 0.72 [−0.03 to 1.49] (*P* = .068), CG × SPG: 2.25 [1.49 to 3.02] (*P* < .001)
WOMAC Pain[a] (16 weeks – Baseline)	-3.13 (1.00) [−3.45 to −2.81]	-1.82 (1.19) [−2.20 to −1.44]	-3.71 (1.53) [−4.20 to −3.22]	**<.001**	LHG × CG: −1.31 [−1.99 to −0.62] (*P* < .001), LHG × SPG: 0.58 [−0.10 to 1.26] (*P* = .125), CG × SPG: 1.89 [1.20 to 2.57] (*P* < .001)
WOMAC Stiffness[b] (8 weeks – Baseline)	-0.67 (0.98) [−0.92 to −0.43]	-0.33 (0.67) [−0.58 to −0.09]	-0.60 (1.01) [−0.84 to −0.35]	.128	LHG × CG: −0.34 [−0.085 to 0.765] (*P* = .164), LHG × SPG: −0.07 [−0.49 to 0.35] (*P* > .999), CG × SPG: 0.27 [−0.157 to 0.687] (*P* = .389)
WOMAC Stiffness[b] (16 weeks – Baseline)	-0.74 (0.96) [−1.05 to −0.42]	-0.28 (0.77) [−0.59 to −0.03]	-0.62 (1.19) [−0.93 to −0.31]	.163	LHG × CG: −0.46 [−1.01 to 0.09] (*P* = .134), LHG × SPG: −0.12 [−0.664 to 0.433] (*P* > .999), CG × SPG: 0.34 [−0.88 to 0.19] (*P* = .374)
WOMAC Functional capacity[b] (8 weeks – Baseline)	-2.02 (0.93) [−2.38 to −1.66]	-1.10 (0.89) [−1.46 to −0.74]	-2.99 (1.47) [−3.35 to −2.64]	**<.001**	LHG × CG: −0.92 [−1.54 to −0.29] (*P* = .002), LHG × SPG: 0.97 [0.35 to 1.59] (*P* = .001), CG × SPG: −1.89 [−2.50 to −1.28] (*P* < .001)
WOMAC Functional capacity[b] (16 weeks – Baseline)	-1.93 (0.84) [−2.27 to −1.58]	-1.07 (0.86) [−1.41 to −0.73]	-2.85 (1.44) [−3.20 to −2.51]	**<.001**	LHG × CG: −0.86 [−1.45 to −0.254] (*P* = .002), LHG × SPG: 0.92 [0.32 to 1.52] (*P* = .001), CG × SPG: 1.78 [1.19 to 2.37] (*P* < .001)
TUG, s[a] (8 weeks – Baseline)	-1.95 (0.61) [−2.15 to −1.76]	-1.39 (0.60) [−1.59 to −1.20]	-2.03 (0.80) [−2.29 to −1.77]	**<.001**	LHG × CG: −0.56 [−0.92 to −0.18] (*P* = .001), LHG × SPG: 0.08 [−0.29 to 0.44] (*P* > .999), CG × SPG: 0.64 [0.26 to 1.00] (*P* < .001)
TUG, s[a] (16 weeks – Baseline)	-1.65 (0.73) [−1.88 to −1.41]	-1.25 (0.69) [−1;47 to −1.03]	-1.65 (0.79) [−1.91 to −1.40]	**.024**	LHG × CG: −0.40 [−0.79 to 0.01] (*P* = .056), LHG × SPG: 0.01 [−0.39 to 0.40] (*P* > .999), CG × SPG: 0.40 [−0.01 to 0.80] (*P* = .052)
HAD Anxiety[a] (8 weeks – Baseline)	-2.39 (2.28) [−3.12 to −1.66]	-1.42 (1.40) [−1.87 to −0.98]	-3.77 (3.03) [−4.74 to −2.80]	**<.001**	LHG × CG: −0.97 [−2.23 to 0.30] (*P* = .203), LHG × SPG: 1.38 [0.11 to 2.65] (*P* = .027), CG × SPG: 2.35 [1.08 to 3.61] (*P* < .001)
HAD Anxiety[a] (16 weeks – Baseline)	-1.96 (2.19) [−2.66 to −1.26]	-1.09 (1.51) [−1.58 to −0.60]	-3.01 (2.97) [−3.96 to −2.05]	**.001**	LHG × CG: −0.87 [−2.12 to 0.38] (*P* = .282), LHG × SPG: 1.05 [−0.21 to 2.29] (*P* = .137), CG × SPG: 1.92 [0.66 to 3.16] (*P* = .001)

(Continues)

TABLE 2 (Continued)

Variable	Group				
	LHG (n = 40)	CG (n = 40)	SPG (n = 40)		Between-group differences in change
	Mean (SD) [95% CI]	Mean (SD) [95% CI]	Mean (SD) [95% CI]	*P*	Mean [95% CI], (*P* value)
HAD Depression[a] (8 weeks – Baseline)	-2.88 (2.40) [−3.65 to −2.11]	-1.08 (1.32) [−1.50 to −0.65]	-3.05 (2.17) [−3.75 to −2.36]	**<.001**	LHG × CG: −1.80 [−2.89 to −0.69] (*P* = .001), LHG × SPG: 0.17 [−0.92 to 1.27] (*P* > .999), CG × SPG: 1.97 [0.87 to 3.07] (*P* < .001)
HAD Depression[a] (16 weeks – Baseline)	-2.41 (2.77) [−3.29 to −1.52]	-0.91 (1.24) [−1.31 to −0.51]	-2.57 (2.33) [−3.31 to −1.82]	**.002**	LHG × CG: −1.50 [−2.70 to −0.27] (*P* = .009), LHG × SPG: 0.16 [−1.04 to 1.36] (*P* > .999), CG × SPG: 1.66 [0.45 to 2.86] (*P* = .003)
WHOQOL-Bref Global[a] (8 weeks – Baseline)	2.47 (1.49) [1.99 to 2.94]	1.26 (1.33) [0.83 to 1.68]	2.69 (2.07) [2,03 to 3.35]	**<.001**	LHG × CG: 1.21 [0.30 to 2.17] (*P* = .004), LHG × SPG: −0.22 [−1.16 to 0.72] (*P* > .999), CG × SPG: −1.43 [−2.39 to −0.53] (*P* = .001)
WHOQOL-Bref Global[a] (16 weeks – Baseline)	2.38 (1.54) [1.89 to 2.87]	1.26 (1.41) [0.80 to 1.71]	2.52 (2.13) [1.83 to 3.20]	**.002**	LHG × CG: 1.12 [0.18 to 2.06] (*P* = .013), LHG × SPG:-0.14 [−1.07 to 0.79] (*P* > .999), CG × SPG: −1.26 [0.32 to 2.19] (*P* = .004)

Abbreviations: LHG, laying on of hands (LooH) without spiritual component; CG, control; SPG, LooH with spiritual component (Spiritist Passe); BMI, body mass index; CAM, complementary and alternative medicine; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; TUG, timed up-and-go test; HAD, Hospital Anxiety and Depression Scale; WHOQOL-Bref: World Health Organization Quality of Life-Bref.

[a]Analysis of variance

[b]Adjusted means using analysis of covariance with baseline scores of WOMAC Stiffness and Functional capacity as covariates.

Bold values are indicates p<0.05.

have minimized this problem, it was not possible to achieve a totally homogeneous group in terms of medical comorbidities and medications in use. Fourth, it was not possible to blind SP and LooH providers to the treatment they were giving. Fifth, the same kinesiotherapy program was offered to all patients, despite the fact that OA may present differently and individualized treatment is always desirable. Sixth, although the different effect on function scores between the SPG and LHG groups is supposed to be due to a "spiritual connection", no difference was observed for the majority of other primary outcomes. The fact that this finding was due to chance cannot be excluded. Finally, adverse effects were determined only by an increase in pain or complaints by participants during each kinesiotherapy and CAM session, where this may have led to underestimation of other adverse effects.

The strengths of this study include the randomized controlled design with attention to key methodological features, avoiding confounding factors and compliance with the CONSORT checklist. Moreover, the similarity of the groups at study baseline demonstrates the success of the randomization process. The assessment of quality of the blinding found no difference in guessing the treatment conditions among the 3 groups. The dropout/withdrawal rate was low and similar for all groups, indicating that subjects were committed to remaining in the study. Unlike other CAM and SP trials,[13-17,22-27] our study used an ITT analysis, providing more solid evidence. Finally, the present study added to the current scientific literature, comparing the so-called "spiritual healing" therapies with other interventions.

Although CAM therapies are often rejected because of a lack of belief in their theory, our positive findings could have implications for clinical practice in KOA, mainly due to the low risk of adverse effects compared to those caused by current pharmacological modalities (eg, nonsteroidal anti-inflammatory drugs) and given the low cost of and easy access to CAM therapies. In the case of LooH interventions, as reported previously, there are several ways to provide them to patients according to the type of LooH. For instance, Therapeutic Touch is a non-religious technique used by nurses and other healthcare professionals and is available through certificate programs around the world, ranging from a few hours to more than a year of training.[51] Other techniques, such as Johrei and SP, are related to religious practices and, for this reason, the training is available only to members of these religious traditions.

Future research should include different sample characteristics, combining other physical therapy modalities, such as the 6-minute walk test to assess functional mobility,[50] exploring the mechanisms and physiological basis of healing with LooH therapies (biological markers), with longer follow-ups, and determining an optimal dosage of LooH (frequency and duration).

5 | CONCLUSION

The present results suggest that kinesiotherapy, in combination with LooH with a spiritual component, was more effective for reducing knee pain and improving functioning than kinesiotherapy in combination with LooH without a spiritual component in older women with KOA; and more effective for reducing knee pain and improving functioning and QoL compared to kinesiotherapy alone. The mechanisms underlying the effect of CAM therapies in OA should be further explored in future studies.

ACKNOWLEDGEMENTS

None

CONFLICT OF INTEREST

The authors declare no competing interests.

ETHICAL APPROVAL

This study was approved by the Research Ethics Committee of the Federal University of Juiz de Fora, Brazil under registry CAAE 52 623 115.0.0000.5147.

CONSENT TO PARTICIPATE

All participants signed a consent term.

DATA AVAILABILITY STATEMENT

Data are available upon request to the corresponding author.

ORCID

Giancarlo Lucchetti https://orcid.org/0000-0002-5384-9476

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Interferon III-related *IL28RA* variant is associated with rheumatoid arthritis and systemic lupus erythematosus and specific disease sub-phenotypes

Manuela Nunes Drehmer[1] | Gabriel Vaisam Castro[1] | Ivanio Alves Pereira[2] | Ilíada Rainha de Souza[1] | Sara Emelie Löfgren[1,3]

[1]Department of Cell Biology, Embryology and Genetics, Federal University of Santa Catarina, Florianopolis, Brazil

[2]Rheumatology Division, University Hospital Polydoro Ernani de Sao Thiago, Federal University of Santa Catarina, Florianopolis, Brazil

[3]Biogenetika Individualized Medicine, Florianopolis, Brazil

Correspondence
Sara Emelie Löfgren, Department of Cell Biology, Embryology and Genetics, Lapoge - Federal University of Santa Catarina, Florianopolis – SC 88040-900, Brazil.
Email: sara.lofgren@ufsc.br

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Abstract

Background: The interferon pathways have been commonly implicated in autoimmune disease development but the identity of the genes involved has not yet been fully clarified. Variation in genes involved in interferon pathways is expected to have a role in the etiology of these diseases.

Methods: The potential association of a polymorphism in the *IL28RA* gene, involved in these pathways, with susceptibility to systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) and disease-related phenotypes was investigated in 603 Brazilian individuals (354 well-characterized SLE and RA patients, and 249 controls). *IL28RA* (rs4649203) variant was genotyped by TaqMan assay. Statistical analysis was performed including both diseases and a comprehensive list of patient clinical manifestations.

Results: The rs4649203-G (minor) allele was associated with SLE and RA occurrence and was shown to be a risk factor for serositis and anemia among SLE patients as well as a protective factor for rheumatoid vasculitis and rheumatoid nodules in RA patients, suggesting an association with a milder form of the disease.

Conclusions: The *IL28RA* gene may contribute to SLE and RA susceptibility and to specific clinical manifestations of the diseases.

KEYWORDS

autoimmune disease, genetic association, IL28RA, interferon pathway, rheumatoid arthritis, systemic lupus erythematosus

1 | INTRODUCTION

Autoimmune disorders (ADs) represent one of the most prevalent groups of non-infectious diseases in the western world, with alarming increase in incidence in recent years. The majority of ADs are chronic and very debilitating, and some are already within the top 10 leading causes of death among women over 65.[1] Despite this, the etiological mechanisms are still unclear and there are no effective genetic markers that can be used in diagnosis and treatment for the majority of ADs today.

Rheumatoid arthritis (RA) is one of the most common systemic ADs, characterized by a chronic inflammation that may affect many tissues, primarily synovial tissue. Common symptoms of RA consist of bone and joint destruction[2,3] and several patient subsets can be

distinguished based on the presence of extra-articular manifestations.[2] Because of the high heterogeneity of the disorder the diagnosis is complex and relies on clinical findings and complementary tests. Systemic lupus erythematosus (SLE) on the other hand has an even wider spectrum of clinical manifestations including several tissues and the presence of a panel of different autoantibody profiles.[4] The high heterogeneity of the symptoms combined with the varied degrees of severity and the alternating phases of remission and flares makes the diagnose of SLE a notable challenge.[4] And importantly, there are no reliable markers today that can help predict which of these varied clinical features the patient will develop in the course of the disease.

Both diseases are believed to have a strong genetic contribution, and it is known that many different ADs share a common set of susceptibility genes, probably reflecting basal immunological dysregulation and autoimmune reactions. These genetic markers would be important to understand and detect general susceptibility to autoimmunity. Others, on the other hand, seem to be more specific to one or a few ADs, reflecting specific pathways giving rise to specific diseases. In addition, some variants seem to be contributing to specific clinical feature within a disease. These genetic variants could be used as specific markers for diagnostic and prognostic purposes and the development of more specific therapies.

Several both shared and specific genetic risk loci have been identified in the last years, and, not surprisingly, many of these are related to inflammation. One group of key factors that have been in several ways related to ADs are the cytokines and their receptors, especially those involved in inflammatory pathways such as the interferon pathway. In one of these studies, performed in Europeans, a variant in the interleukin (IL)-28 receptor alpha (*IL28RA*) was primarily identified as a potential susceptibility loci for psoriasis.[6]

The human *IL28RA* gene codes for the interferon lambda receptor 1 (also known as IFNLR1), a transmembrane protein that heterodimerizes with another subunit, IL-10RB, to form a type II cytokine receptor. This receptor interacts with specific cytokines involved in several immune responses, such as IL-28A, IL-28B and IL-29, also known as class III or lambda interferons (IFN-λ).[7,8] The most studied biological role of type III interferons has been their antiviral activity, and their role in autoimmune and inflammatory diseases remains largely unknown. However, recent studies indicate a possibly important role in immune homeostasis and AD susceptibility. It has been shown that T helper (Th)2 cells produce IL-28, and that endogenous IL-28 can confer protection against murine experimental autoimmune encephalomyelitis.[9] In addition, treatment with IL-28A in mice reduces the numbers of proinflammatory Th17 and γδ T cells in the joints and inguinal lymph nodes.[10] On the other hand, the expression of IL-28 was shown to be upregulated in peripheral blood mononuclear cells from patients with SLE,[11,12] supporting the likelihood that IL-28 expression may be correlated with the pathogenesis of SLE and possibly of other ADs. Thus variants in genes involved in this pathway may lead to changes in the immune responses induced by IFN-λ ligands and may contribute to the development of autoimmunity, although the association is largely unexplored.[7,13]

A genome-wide association study performed in Chinese Han identified one single nucleotide polymorphism (SNP) in *IL28RA*, rs4649203, as a susceptibility variant shared by psoriasis and SLE.[14] However, in contrast to the European study, in Chinese the variant showed a protective effect against these diseases. The same variant was also tested for association with multiple sclerosis in Spanish and German patients with no consistent association found.[7] These results suggest that the variant may have distinct biological effects in different ADs and populations.[14]

The functional consequence of this SNP is not known. However, its location in the 3′ untranslated region suggests that it may influence gene expression and therefore affect the signaling of the receptor and, by consequence, the pathway.[15]

Of note, the very few studies so far with this gene and variant have been in Chinese or European populations. There are no studies evaluating the association of this SNP in other populations, or for other ADs. Thus, we performed a case-control study to investigate whether the variant rs4649203 in the *IL28RA* gene is associated with 2 autoimmune diseases, SLE and AR, as well as their clinical manifestations, in a Brazilian population.

2 | MATERIALS AND METHODS

The study enrolled a total of 603 individuals (178 well-characterized SLE patients, 176 well-characterized RA patients and 249 controls). The RA and SLE samples were obtained from the University Hospital of the Federal University of Santa Catarina (UFSC) and were diagnosed using the standard criteria of the American College of Rheumatology (ACR) and European League Against Rheumatism/ACR guidelines.[5,16] All clinical data were obtained from the medical records and laboratory results. The characterization of the patient groups is shown in Tables 1 and 2. The controls were blood donors of the same hospital, clinically classified as healthy individuals with no individual record of SLE, RA or any other autoimmune disorders and with no known family history of any AD. Cases and controls groups were matched on mean age and gender. All the enrolled patients and controls signed appropriate informed consent and the study was approved by the ethics committee of the University of Santa Catarina and the University Hospital (Case number 172/06).

Genomic DNA was extracted from peripheral blood lymphocytes by the well-established phenol-chloroform protocol and were diluted to concentrations of 20 ng/μL All DNA samples were stored at −20°C. The SNP rs4649203 (A/G) was genotyped using the pre-designed TaqMan genotyping assay (Thermo Fisher Scientific, cat. no. 4351379; C_27915464 10) and using the HT7900 real-time polymerase chain reaction system by Thermo Fisher Scientific (Waltham City, MA, USA). Twenty DNA samples with known genotypes were included as controls and random samples (10% of total) were sequenced by Sanger sequencing for validation.

Through χ^2 test, the variant was tested for significant deviation from Hardy–Weinberg equilibrium in the control group and satisfied

TABLE 1 Clinical characteristics of the 175 SLE patients analyzed in the study

Characteristics	n (%)	
Male	7	(4.0)
Female	168	(95.5)
Arthritis	130	(74.3)
Discoid rash	77	(44.0)
Malar rash	52	(29.7)
Photosensitivity	51	(29.1)
Serositis	17	(9.7)
Oral ulcers	39	(22.3)
Nephritis	44	(25.1)
Hematologic disorders		
Anemia	65	(37.1)
Leukopenia	33	(18.9)
Thrombocytopenia	26	(14.9)
Neurological disorder	55	(31.4)
Immunological indices		
Anti-ANA+	156	(89.1)
Anti-DNA+	43	(24.6)
Anti-cardiolipin+	29	(16.6)
Anti-La/SSB+	22	(12.6)
Anti-RNP+	54	(30.9)
Anti-Ro/SSA+	66	(37.7)
Anti-Sm+	21	(12.0)
Other ADs present[a]	35	(20.0)
SLE in family	37	(21.1)
Other ADs in family	56	(32.0)
Number of ACR criteria, mean (±SD)	5.54 (±1.65)	
Age at onset, mean (±SD)	29.9 (±10.8)	

Abbreviations: ACR, American College of Rheumatology; ADs, autoimmune diseases; anti-ANA, antinuclear antibody; anti-RNP, anti-ribonucleoprotein; SLE, systemic lupus erythematosus.

[a]29 patients with coexisting Sjögren's disease, 4 patients with coexisting psoriasis, 1 patient with coexisting autoimmune thyroid disease and 1 patient with coexisting type 1 diabetes.

TABLE 2 Clinical characterization of the 176 RA patients analyzed in the study

Characteristic	n (%)	
Male	27	(15.3)
Female	149	(84.7)
Synovitis	130	(73.86)
Rheumatoid nodules	29	(16.48)
Rheumatoid vasculitis	8	(4.55)
Cardiopathy	46	(26.14)
Hematological disorders		
Anemia	36	(20.5)
Leukopenia	4	(2.3)
Leukocytosis	10	(5.7)
Thrombocytopenia	7	(4.0)
Elevated ESR	127	(72.2)
Elevated CRP	86	(48.9)
Immunological indices		
Anti-ANA+	116	(65.9)
Anti-CCP+	88	(50.0)
Rheumatoid factor+	128	(72.7)
Anti-RNP+	98	(55.7)
Anti-HCV+	48	(27.3)
Anti-Sm+	25	(14.2)
Anti-Ro+	40	(22.7)
Anti-La+	24	(13.6)
Antilupic+	16	(9.09)
Elevated TGP	29	(16.5)
Other ADs present[a]	18	(10.2)
Other ADs in the family	14	(7.9)
DAS28, mean (±SD)	3.69 (±0.92)	
Age at onset, mean (±SD)	43.15 (±12.9)	

Abbreviations: Anti-CCP, anti-cyclic citrullinated peptide antibody; Anti-Sm, anti-Smith antibodies; CRP, C-reactive protein; DAS28, Disease Activity Score based on 28 joints; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis.

[a]11 patients with coexisting Sjögren's disease, 3 patients with coexisting psoriasis, 2 patients with coexisting antiphospholipid syndrome, 1 patient with coexisting type 1 diabetes and 1 patient with coexisting autoimmune thyroid disease.

this condition with *P* values of >.05. Statistical analysis was performed by logistic regression using SPSS software. Association was tested for the 2 diseases as well as for all main criteria for diagnosis of SLE and RA as well as other relevant manifestations and family history. The genetic association between the SNP and diseases susceptibility and specific clinical manifestations was measured using the odds ratios (ORs) and 95% confidence intervals (CIs). A *P* value of <.05 was considered statistically significant.

3 | RESULTS

The description of the clinical characteristics of the SLE and RA patients enrolled in the study is shown in Tables 1 and 2, respectively. The patients' data revealed a similar profile as in European studies. Approximately 95% and 85% of the SLE and RA patients were female, respectively. The autoantibodies play an essential role in the pathogenesis of many ADs and mediate both tissue injury and systemic inflammation. Our results showed that rheumatoid factor was present in about 89% and 73% of SLE and RA patients, respectively. Antinuclear antibodies (ANAs) were also frequent (about 66%) in RA patients. In RA, 72% of the patients presented elevated erythrocyte sedimentation rate (ESR). The high frequency of these autoantibodies and elevated ESR

suggest that most patients may be in the more advanced stages of RA.

When testing for diseases association, the SNP rs4649203 was found associated with the occurrence of SLE by genotypic association (rs4649203-GG) (OR = 1.71, *P* = .029) (Table 3). When stratifying by specific clinical manifestations of SLE, the minor allele rs4649203-G was associated with the presence of anemia (including any kind of anemia) (OR = 1.82, *P* = .032) and rs4649203-GG genotype with the presence of serositis (OR = 0.33, *P* = .025), showing a protective effect to this manifestation (Table 3).

For RA on the other hand, there was no association of the variant with the disease per se. However, when stratifying by gender, a relatively strong association in the dominant model (GG + GA vs AA) was identified analyzing only male individuals (OR = 4.67, *P* = .006) (Table 4); association was not found when evaluating only females. Interestingly, the same allele was also associated as a protective factor for the presence of rheumatoid vasculitis (OR = 0.21, *P* = .038) and rheumatoid nodules (OR = 0.32, *P* = .029) (Table 4).

4 | DISCUSSION

Despite the increasing evidence revealing the importance of specific cytokines and the type III interferons in autoimmune pathways, the pathological role and potential effects of these factors in the development and/or triggering of AD is still unclear.

Here we show that a variant in the IFN-λ signaling receptor *IL28RA* gene is associated with SLE in a Brazilian population and in addition we report for the first time an association with RA, as well as with some specific manifestations of both diseases.

Several studies have revealed that IFNs play a pivotal role in the pathogenesis of ADs.[17] Studies conducted in experimental models and observations in AD patients have revealed that they modulate innate and adaptive immunity and are key factors in regulation of inflammation.[18,19] The contribution of type I IFNs to development and manifestation of autoimmunity is well established.[20,21] Type I IFNs are commonly upregulated in systemic ADs such as SLE, Aicardi–Goutieres syndrome, Sjögren's syndrome, type I diabetes, and psoriasis.[21] The majority of adult patients and 90% of pediatric patients with SLE have elevated peripheral IFNα.[22] Interestingly, type III IFNs

TABLE 3 Multiple logistic regression association analysis of SLE susceptibility

	MAF				GG genotype			
Characteristic	+	−	**OR (CI)**	***P* value**	+	−	**OR (CI)**	***P* value**
SLE	0.50	0.47	0.83 (0.59-1.17)	.281	0.26	0.16	1.71 (1.06-2.85)	**.029**
Arthritis	0.45	0.50	0.95 (0.74-1.21)	.692	0.19	0.30	0.88 (0.53-1.45)	.628
Discoid rash	0.43	0.49	0.87 (0.63-1.20)	.392	0.17	0.26	0.73 (0.36-1.41)	.582
Malar rash	0.46	0.47	0.98 (0.74-1.30)	.887	0.17	0.28	0.90 (0.48-1.66)	.224
Photosensitivity	0.44	0.50	0.90 (0.69-1.19)	.470	0.20	0.24	0.83 (0.47-1.42)	.731
Serositis	0.49	0.37	0.69 (0.44-1.06)	.091	0.40	0.26	0.33 (0.13-0.87)	**.024**
Oral ulcers	0.49	0.45	1.09 (0.79-1.51)	.590	0.26	0.19	1.20 (0.64-2.23)	.768
Nephritis	0.45	0.47	0.96 (0.69-1.31)	.783	0.22	0.21	0.94 (0.49-1.74)	.784
Hematologic disorders								
Anemia	0.45	0.33	1.82 (1.05-3.17)	**.032**	0.25	0.21	0.96 (0.49-1.82)	.386
Thrombocytopenia	0.38	0.48	0.72 (0.40-1.25)	.241	0.20	0.22	0.60 (0.19-1.65)	.331
Neurological disorder	0.51	0.44	1.21 (0.83-1.76)	.319	0.25	0.20	1.44 (0.67-3.11)	.546
Immunological indices								
Anti-ANA	0.46	0.53	0.97 (0.78-1.21)	.805	0.22	0.25	0.95 (0.61-1.47)	.947
Anti-DNA	0.49	0.46	1.04 (0.68-1.59)	.848	0.21	0.23	1.08 (0.42-2.69)	.744
Anti-cardiolipin	0.47	0.47	0.98 (0.58-1.64)	.932	0.21	0.23	0.94 (0.31-2.72)	.951
Anti-RNP	0.44	0.48	0.83 (0.56-1.21)	.328	0.19	0.24	0.67 (0.29-1.46)	.605
Anti-Ro/SSA	0.53	0.43	1.30 (0.93-1.84)	.295	0.29	0.18	1.68 (0.86-3.37)	.319
	AA		**AG**		**GG**			***P* value**
Number of ACR criteria (mean ± SD)	6.10 ± 1.80		5.87 ± 1.60		5.82 ± 1.57			.517
Age at onset (mean ± SD)	31.9 ± 11.9		28.9 ± 9.43		29.4 ± 12.0			.942

Note: Distribution of the allele and genotype frequencies of epidemiological and clinical data for the case group (+) (SLE patients who present the manifestation) and control group (−) (patients who do not have the manifestation), measure of association odds ratio (OR), confidence intervals (CI 95%) and *P* values. Significant values are noted in bold.

Abbreviations: ACR, American College of Rheumatology; anti-ANA, antinuclear antibodies; anti-RNP, anti-ribonucleoprotein antibodies; MAF, minor allele frequency; SSA, Sjögren's syndrome A.

TABLE 4 Multiple logistic regression association analysis of RA susceptibility

	MAF				GG genotype			
Characteristic	+	−	**OR (CI)**	***P* value**	+	−	**OR (CI)**	***P* value**
RA	0.46	0.44	0.84 (0.61-1.14)	.261	0.19	0.17	1.48 (0.73-2.63)	.274
RA - Male	0.47	0.34	1.70 (0.73-3.72)	.055	0.14	0.10	4.67 (1.56-13.92)	**.006**
RA - Female	0.49	0.48	0.94 (0.67-1.32)	.715	0.21	0.18	0.86 (0.40-1.41)	.682
Rheumatoid nodules	0.36	0.48	0.71 (0.40-1.20)	.204	0.10	0.22	0.32 (0.15-0.74)	**.029**
Rheumatoid vasculitis	0.25	0.47	0.47 (0.13-1.37)	.172	0.12	0.20	0.21 (0.05-0.92)	**.038**
Cardiopathy	0.48	0.45	1.08 (0.71-1.65)	.701	0.24	0.18	1.23 (0.53-2.80)	.428
Hematologic disorders								
Anemia	0.43	0.47	0.85 (0.53-1.36)	.502	0.20	0.19	0.79 (0.29-1.95)	.306
Leukocytosis	0.40	0.46	0.70 (0.24-1.90)	.489	0.20	0.19	2.16 (0.27-26.6)	.756
Thrombocytopenia	0.50	0.45	1.33 (0.45-3.98)	.601	0.23	0.18	1.64 (0.29-9.61)	.136
Elevated ALT	0.48	0.45	1.02 (0.61-1.72)	.927	0.10	0.21	0.94 (0.18-4.30)	.116
Immunological indices								
Anti-CCP+	0.44	0.50	0.89 (0.66-1.21)	.469	0.15	0.23	0.75 (0.36-1.48)	.713
Rheumatoid factor+	0.46	0.46	0.97 (0.76-1.25)	.829	0.19	0.20	0.94 (0.54-1.60)	.972
	AA		**AG**		**GG**			***P* value**
Number of ACR criteria (mean ± SD)	4.72 ± 1.66		4.18 ± 1.60		3.97 ± 1.73			.089
DAS28 (mean ± SD)	3.58 ± 0.91		3.77 ± 0.86		3.60 ± 1.08			.473
Age of onset (mean ± SD)	42.0 ± 12.5		44.6 ± 12.7		40.4 ± 13.6			.239

Note: Distribution of the allele and genotype frequencies of epidemiological and clinical data for the case group (+) (RA patients who present the clinical manifestation) and control group (–) (patients who do not have the clinical manifestation), measure of association odds ratio (OR), confidence intervals (CI 95%) and *P* values. Significant values are noted in bold.

Abbreviations: ACR, American College of Rheumatology; ALT, alanine transaminase; anti-CCP, anti-cyclic citrullinated peptide; CRP, C-reactive protein; DAS28, Disease Activity Score of 28 joints; ESR, erythrocyte sedimentation rate; MAF, minor allele frequency.

do not seem to be linked to ADs in the same way. In fact, type III IFN has been shown to alleviate symptoms in a mouse model of arthritis.[10] Further, in a murine model of colitis, a disease that can be autoimmune in humans, IFNλ signaling specifically in neutrophils leads to a reduction of intestinal pathology.[23] In addition, mice lacking the IFNλ receptor (IFNλR1) have exacerbated disease in a model of asthma.[24] The presence of genetic variants in IFN receptor genes can result in imbalances in inflammatory pathways and potentially influence a person's susceptibility to develop ADs. This potentially protective role of IFNλ in these and other ADs has only begun to be explored in humans.

In this study a gender-specific association of the rs4649203 variant was observed. For RA the AA genotype was identified as a risk factor for the male subjects and for SLE the association was found in women. RA is a gender dimorphic AD that occurs approximately 3 times more frequently in women than in men. While it is more prevalent in women, men who develop the disorder often experience a more severe form of the disease.[25,26] For SLE, studies have revealed that men need to inherit a higher number of risk alleles to develop the disease than women, probably because of the lack of other risk factors, such as female hormones, and/or the presence of protective factors, such as male hormones.[27] These results could suggest a genotype–gender interaction effect of *IL28RA* on SLE and RA pathogenesis, although the mechanisms for these effects would still need to be addressed. However, it has to be recognized that the bias could also be due to differences in samples sizes; in both cases the numbers of male patients were low.

Exploring individual clinical manifestations, the G allele was correlated with the presence of anemia in SLE. Anemia affected about 37% of the patients in this study and it is listed as one of the most common manifestations of SLE by the ACR.[28] On the other hand, the GG genotype conferred a protective effect for serositis in SLE patients. Serositis is also a fairly common manifestation, present in 17% of our patients, slightly higher than described in Chinese, Canadian and European patients (12%-16%).[29-31] This association is interesting since cardiovascular disease is the leading cause of morbidity and mortality in patients with SLE.[32]

For RA the G allele was a protective factor for rheumatoid vasculitis and rheumatoid nodules. In this study, about 4.5% of patients with RA presented with rheumatoid vasculitis. This vascular involvement is the most serious extra-articular complication of RA, with high morbidity and mortality.[32] The presence of rheumatoid nodules is a rather specific and common manifestation of RA, especially in later phases. Our findings suggest that the G allele is associated with a milder form of disease manifestation.

We could not find association with the occurrence of other ADs in the same patient or presence of relatives with RA, SLE or other AD in the family, which had not been investigated before for this gene.

Of note, none of the associations remained significant after Bonferroni correction by multiple testing. However, true associations that do not withstand multiple testing corrections may be neglected. It might represent a possible true correlation that deserves more studies.

While the effect of this variant is not known, it suggests that the changes in expression pattern or activity of IFN-λ receptor, may alter IFN-λ expression and signaling, influencing the susceptibility to ADs. IFN-λ activates monocytes and macrophages to produce proinflammatory cytokines that in AD patients is believed to contribute to the inflammation processes and associated organ damage.[33]

Determining functional consequences of the *IL28RA* polymorphisms reported herein might improve our understanding of the pathogenesis of AD, determining a more personalized diagnostics and provide new targets for therapeutic interventions.

The association of genetic markers with particular clinical manifestations within the diseases is of great importance in the understanding of the different disease pathways and may represent important tools in the characterization, diagnostics, prognostics and treatment of AD patients with more specific means.

In this study only one SNP was analyzed and we acknowledge the small sample size and the fact that the patients had coexisting ADs which might influence the results. Thus, our results are suggestive. However, to the best of our knowledge this is the first study presenting an association of this polymorphism with RA and the first with SLE in South Americans, and might contribute to the understanding of the mechanism behind the effect of genetic polymorphisms in genes in the type III IFN pathway and development of RA, SLE as well as other AD. Further studies might help to clarify the potential impact of this genetic variant in this pathway and in ADs.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Sara Emelie Löfgren https://orcid.org/0000-0002-1680-7349

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ORIGINAL ARTICLE

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Neutrophil to lymphocyte ratio predicts glucocorticoid resistance in polymyalgia rheumatica

Claire E. Owen[1,2] | Christopher McMaster[1] | David F. L. Liew[1,2] | Jessica L. Leung[1,2] | Andrew M. Scott[2,3,4] | Russell R. C. Buchanan[1,2]

[1]Department of Rheumatology, Austin Health, Heidelberg, VIC, Australia

[2]Department of Medicine, University of Melbourne, Parkville, VIC, Australia

[3]Department of Molecular Imaging and Therapy, Austin Health, Heidelberg, VIC, Australia

[4]Olivia Newton-John Cancer Research Institute, and School of Cancer Medicine, La Trobe University, Heidelberg, VIC, Australia

Correspondence
Claire E. Owen, FRACP, Rheumatology Department, Austin Health – Repatriation Campus, Level 1, North Wing, 300 Waterdale Road, Heidelberg West VIC 3081, Australia.
Email: claire.owen@austin.org.au

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Abstract

Aim: Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) correlate with disease activity in several rheumatic diseases; however, their utility in polymyalgia rheumatica (PMR) remains unclear. This study evaluated their relationship with disease activity and glucocorticoid resistance in PMR.

Method: Data for disease activity (PMR-AS) and full blood examination was obtained from a prospective observational cohort comprising newly diagnosed, steroid-naïve PMR patients treated with low-dose glucocorticoid therapy. Glucocorticoid resistance was defined as non-response to prednisolone 15 mg/d or initial response followed by flare (PMR-AS ≥ 9.35 or Δ ≥6.6) upon weaning to 5 mg/d. Univariable Bayesian linear regression analysis of the relationship between PMR-AS (baseline and mean) and NLR and PLR was performed. Predictors of glucocorticoid resistance were identified using a multivariable outcome model, with variables derived from Bayesian model selection.

Results: Of the 32 included patients, 16 (50%) fulfilled the primary outcome measure of glucocorticoid resistance. These participants were older, typically female, and had higher baseline C-reactive protein than their glucocorticoid-responsive counterparts. A statistically significant relationship was identified between PMR-AS and both NLR (odds ratio [OR] 28.1; 95% CI 1.6-54.7) and PLR (OR 40.6; 95% CI 10.1-71.4) at baseline, with PLR also found to correlate with disease activity during follow-up (OR 15.6; 95% CI 2.7-28.2). Baseline NLR proved a statistically significant predictor of glucocorticoid-resistant PMR (OR 14.01; 95% CI 1.49-278.06).

Conclusion: Baseline NLR can predict glucocorticoid resistance in newly diagnosed PMR patients. Both NLR and PLR may be reliable biomarkers of disease activity in PMR.

KEYWORDS

glucocorticoids, lymphocytes, neutrophils, polymyalgia rheumatica, prognosis

1 | INTRODUCTION

Polymyalgia rheumatica (PMR) is an inflammatory condition characterized by subacute-onset shoulder and hip pain, and stiffness. Oral glucocorticoids represent the treatment mainstay and while cessation of therapy is the ultimate goal, up to 50% of patients still require prednisolone 2-3 years after diagnosis.[1] A higher baseline erythrocyte sedimentation rate (ESR) has been associated with disease relapse and lower probability of glucocorticoid discontinuation; however, these findings have not been consistently reproduced.[2] To date, a reliable and readily accessible biomarker to measure disease activity and predict resistance to standardized low-dose glucocorticoid therapy has not been identified in PMR.

Neutrophil, platelet and lymphocyte counts undergo temporary changes in an active inflammatory state. Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) can be calculated from a routine full blood examination (FBE) using the neutrophil count or platelet count divided by the lymphocyte count. In oncology, high NLR and PLR are associated with poor prognostic outcomes across a range of solid organ malignancies.[3,4] These indices have similarly been found to correlate with disease activity in several rheumatic diseases including most recently PMR, but their role in predicting treatment response in this condition is less well-defined.[5-8]

In this study, we sought to prospectively characterize the relationship between NLR and PLR, and disease activity in patients with newly diagnosed PMR. The ability of NLR and PLR to predict resistance to standardized low-dose glucocorticoid therapy was also evaluated.

2 | METHOD

2.1 | Patients

Patients with newly diagnosed, steroid-naïve PMR according to the 2012 European League Against Rheumatism/American College of Rheumatology classification criteria[9] were prospectively recruited from primary care, community and hospital rheumatology practices. Participants with symptoms suggestive of giant cell arteritis (GCA) including headache, jaw claudication, scalp tenderness or visual disturbance were excluded, along with cases of cancer within the past 5 years, neuromuscular disease, active infection, other inflammatory conditions (eg, rheumatoid arthritis [RA]) and chronic pain syndromes. The study was approved by the Austin Health Human Research Ethics Committee (HREC/14/Austin/158) prior to commencement and registered with the Australian New Zealand Clinical Trials Registry (trial identification ACTRN1261400696695).

Following written consent, demographic and clinical data were collected and the Health Assessment Questionnaire–Disability Index (HAQ-DI) completed. A standard physical examination was undertaken including measurement of the shoulder range of motion (elevation of upper limb score). In order to measure disease activity using the PMR-activity score (PMR-AS),[10] participants marked a visual analog scale indicating pain severity and the investigator provided a physician global assessment. Relevant differential diagnoses were excluded by testing creatine kinase, thyroid function, rheumatoid factor, anti-citrullinated peptide antibodies (ACPA) and anti-neutrophil cytoplasmic antibodies. FBE, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were performed at baseline and each subsequent visit. A whole-body ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography/computed tomography (PET/CT) scan (Gemini-TF64 or Ingenuity-TF128 PET/CT system; Phillips, Cleveland, OH, USA) from skull vertex to toes was also undertaken in all participants within 7 days of study enrolment.

2.2 | Treatment protocol

Participants were treated with a standardized schedule of low-dose glucocorticoid therapy as outlined in the British Society of Rheumatology (BSR) guideline.[11] Follow-up visits were scheduled at weeks 4, 8, 16, 24, 32 and 46 (the minimum duration of this treatment protocol) and disease activity was re-calculated. A PMR-AS score of ≥ 9.35 in a patient who had achieved clinical remission (PMR-AS < 9.35) or increase in PMR-AS by ≥ 6.6 if the participant had been previously responding to treatment (PMR-AS ≥ 9.35 but falling between successive visits) constituted the definition of disease relapse. When this occurred, the patient's prednisolone dose was increased to the previous higher dose for 4 weeks and an additional study visit performed to determine if the weaning schedule could be reinstated. If relapse occurred on more than 2 occasions, methotrexate was commenced as a steroid-sparing agent, and the participant was withdrawn from the study.

2.3 | Glucocorticoid resistance

Glucocorticoid resistance has been previously defined by Mori et al in PMR as non-response to an initial prednisolone dose of 15 mg/d or initial response followed by disease relapse upon weaning prednisolone to 5 mg/d.[12] This definition was accordingly utilized as the primary outcome measure for glucocorticoid-resistant disease in our study.

2.4 | Statistical analyses

All statistical analyses were conducted using R version 3.5.2 and the package *rstanarm*.[13,14] Results for continuous variables are reported as mean ± standard deviation or median (interquartile range). NLR and PLR, both right-skewed distributions, were log-transformed prior to analysis. Univariable Bayesian linear regression was used to analyze the relationship between NLR and PLR, and PMR-AS (baseline and mean). Several multivariable, Bayesian logistic regression models were compared using leave-one-out cross-validation – from the *loo* package[15] – to select predictors of resistance to low-dose glucocorticoid

therapy. All outcomes are reported as odds ratios (OR) with 95% credible intervals (CI).[16] Weakly informative prior distributions were applied using Student's *t* distribution with 7 degrees of freedom.[17]
[Correction added on 07 December 2020, after first online publication: typographical error has been amended under statistical analysis section]

3 | RESULTS

Thirty-two of the 35 patients (91.4%) recruited at baseline were suitable for inclusion in the final analysis (Table 1): 1 patient was excluded due to large vessel vasculitis on whole-body PET/CT consistent with concomitant GCA; another patient's final diagnosis was revised to Parkinson's disease; and the third patient required a modified schedule of glucocorticoid therapy due to a history of prednisolone-induced central serous retinopathy.

Twenty-five out of 32 participants (78.1%) relapsed during the 46-week follow-up period. Relapse most commonly occurred at week 24, at which point in time patients were taking a median prednisolone dose of 7 mg. Three participants required the initiation of methotrexate as dictated by the study protocol.

Sixteen patients (50%) fulfilled the primary outcome measure of resistance to low-dose glucocorticoid therapy; their demographic details compared with those participants with glucocorticoid-responsive PMR are presented in Table 2.

Glucocorticoid-resistant patients were older and typically female compared with their glucocorticoid-responsive counterparts. Baseline CRP, but not ESR, and disease activity (PMR-AS) were also higher. In terms of hematologic parameters, median white cell count (WCC), neutrophil and platelet counts were similar between the 2 groups, but the median lymphocyte count was lower in glucocorticoid-resistant patients (1.7, 1.6-1.8 cf. 2.1, 1.9-2.3). Consequently, values for mean log-transformed NLR (1.20 ± 0.48) and PLR (5.37 ± 0.37) were higher in this group than the glucocorticoid-responsive cohort (NLR 0.87 ± 0.38; PLR 5.12 ± 0.38).

A statistically significant relationship was identified between disease activity (PMR-AS) and both NLR (OR 28.1; 95% CI 1.6-54.7) and PLR (OR 40.6; 95% CI 10.1-71.4) at baseline. During follow-up, PLR correlated with mean PMR-AS (OR 15.6; 95% CI 2.7-28.2), with a trend toward significance observed between NLR and mean PMR-AS (OR 10.1; 95% CI −0.9 - 21.3).

Using Bayesian outcome modeling, baseline NLR was found to be a statistically significant predictor of glucocorticoid-resistant PMR during follow-up (OR 14.01; 95% CI 1.49-278.06). A trend toward significance was also noted for baseline PLR (OR 5.02; 0.49-71.54), along with age (OR 1.14; 95% CI 1.00-1.34) and female gender (OR 2.98; 0.54-18.39). There was no correlation between baseline CRP and glucocorticoid resistance after adjusting for demographic factors (OR 1.02; 95% CI 0.99-1.05). Figure 1 illustrates the relationship between baseline laboratory values for CRP, ESR and NLR, and glucocorticoid-resistant PMR.

TABLE 1 Baseline demographic information

Demographic	Patients (N = 32)
Age, y	69.0 ± 7.2
Male	17 (53.1%)
Caucasian	31 (96.9%)
BMI, kg/m^2	27.7 ± 5.1
Shoulder pain	32 (100%)
Hip pain	28 (87.5%)
Median EMS, min	120 (67.5-195)
EULAR/ACR Clinical Algorithm Score	5.2 ± 0.6
Median CRP, mg/L	42.9 (21.8-65.7)
Median ESR, mm	47.5 (30.5-68)
PMR-AS	74.2 ± 35.7
Median HAQ-DI	1.7 (1.1-2.1)

Note: Values are means ± standard deviation, unless otherwise stated; median values are reported with 25% and 75% quartiles.

Abreviations: BMI, body mass index; EMS, early morning stiffness; EULAR, European League Against Rheumatism; ACR, American College of Rheumatology; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PMR-AS, polymyalgia rheumatica-activity score; HAQ-DI, Health Assessment Questionnaire–Disability Index.

4 | DISCUSSION

In a cohort of 32 patients with newly diagnosed PMR, 16 (50%) had a disease course characterized by resistance to low-dose glucocorticoid therapy, with baseline NLR proving a distinct predictor of this outcome. A trend toward significance was also observed between NLR and disease activity, with a statistically significant correlation found between PLR and PMR-AS both at baseline and during follow-up. All participants were steroid-naïve at study enrolment, glucocorticoid therapy thus having no bearing upon baseline hematologic parameters.

An increasing body of evidence now refutes historical opinion that PMR is a self-limiting, perpetually steroid-responsive entity. In a recent population-based study, around 25% of patients required more than 4 years of continuous prednisolone.[18] Significant morbidity is known to arise from this treatment paradigm whereby glucocorticoid monotherapy is advocated in the first instance and steroid-sparing agents are only initiated following recurrent relapse.[11,19] However, on whole-body magnetic resonance imaging (MRI) a complete patient-reported response to prednisolone has been shown to correlate with an extracapsular pattern of inflammation, thereby confirming the existence of discrete phenotypic differences between glucocorticoid-responsive and -resistant PMR cases.[20] Identification of baseline NLR as a predictor of later glucocorticoid resistance in this study therefore has certain clinical relevance. Not only does it add to the paucity of recognized adverse prognostic indicators in this condition (such as female gender, peripheral joint involvement and high ESR)[2]

TABLE 2 Demographic comparison of glucocorticoid-resistant and -responsive patients

Demographic	Glucocorticoid-resistant (n = 16)	Glucocorticoid-responsive (n = 16)
Age, y	71.9 ± 7.0	66.1 ± 6.4
Gender		
Female	10 (62.5%)	5 (31.25%)
Male	6 (37.50%)	11 (68.75%)
CRP, mg/L	59.3 ± 46.0	36.4 ± 27.6
ESR, mm	50.7 ± 28.6	49.4 ± 30.1
PMR-AS	85.3 ± 35.3	63.0 ± 33.7
Median WCC, $\times 10^9$/L	8.55 (7.4-9.25)	7.95 (7.0-9.23)
Median neutrophils, $\times 10^9$/L	5.5 (4.9-6.73)	5.1 (3.8-5.9)
Median lymphocyte, $\times 10^9$/L	1.7 (1.6-1.8)	2.1 (1.9-2.3)
Median platelets, $\times 10^9$/L	362 (297-409)	332 (264-448)
NLR, log-transformed	1.2 ± 0.4	0.8 ± 0.4
PLR, log-transformed	5.37 ± 0.37	5.12 ± 0.38

Note: Values are means ± standard deviation, unless otherwise stated; median values are reported with 25% and 75% quartiles.

Abreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PMR-AS, polymyalgia rheumatica-activity score; WCC, white cell count; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio.

but it also further characterizes the subset of patients with relapsing PMR who may benefit from early disease-modifying anti-rheumatic drug (DMARD) initiation.

Relying upon systemic markers of inflammation to assess disease activity in PMR can be problematic. Normal CRP and ESR results are observed in 14% and 27% of relapses respectively.[21] There is consequently an unmet need for a novel biomarker that accurately reflects the underlying inflammatory state in PMR. In research settings, the PMR-AS is considered a valid and reliable measure of disease activity; a value ≥ 9.35 is associated with a sensitivity of 96.6% and specificity of 90.7% for flare diagnosis.[22] However, the composite nature of this scoring system is less conducive to use in everyday clinical practice. In this study, a statistically significant relationship existed between PMR-AS and both NLR and PLR at baseline, with PLR also being found to correlate with disease activity during follow-up. We therefore propose that these indices, which can be simply calculated from an FBE, represent readily accessible and inexpensive biomarkers with the potential to measure PMR disease activity more reliably than conventional inflammatory markers like CRP and ESR.

Several other studies have recently investigated trends in hematologic parameters in PMR. In their retrospective analysis of 94 PMR patients contrasted with 242 RA patients, Jung et al found NLR and PLR to be significantly higher among PMR cases.[8] These levels subsequently diminished with treatment and were found to correlate with other measures of disease activity including CRP; however, an association between NLR or PLR and a relapsing disease course was not identified in this instance. However, a Japanese study has reported thrombocytosis as an adverse prognostic factor in PMR.[23] Using hierarchical cluster analysis, participants with a platelet count > 450 × 10^9/L were found to be less likely to exhibit a response to glucocorticoid therapy at 1 month. Finally, a change in leukocyte dynamics among patients with PMR and GCA compared with healthy and infection controls has been newly documented by van Sleen et al, with a shift toward the production of myeloid-lineage leukocytes noted prior to the commencement of prednisolone.[24] This bias ultimately persisted during treatment with a weaning schedule of glucocorticoid therapy and was even maintained in patients achieving drug-free remission.

Taken together, these results suggest that the inflammatory milieu which characterizes PMR directly impacts neutrophil and platelet production within the bone marrow. As other authors have previously hypothesized, this likely occurs secondary to high levels of the key cytokine interleukin-6 (IL-6), given this pro-inflammatory mediator is additionally an independent regulator of granulopoiesis and stimulates thrombopoiesis through thrombopoietin.[25,26] It is therefore entirely conceivable that hematologic parameters reported on an FBE may provide an accurate representation of underlying disease activity in PMR.

While the precise mechanism behind the association of high NLR with adverse survival in cancer patients is not known, neutrophilia as an inflammatory response is understood to suppress other immune cells including lymphocytes, activated T cells and natural killer cells, in which their infiltration of tumors is otherwise associated with positive cytotoxic treatment outcomes.[4] In RA, where a recent meta-analysis has demonstrated, a consistent relationship between NLR and PLR, and the presence of active disease, these indices are similarly thought to reflect the pathogenic mechanisms at play rather than being surrogate measures of the body's overall inflammatory state.[27]

The pathogenesis of PMR remains comparatively unclear, although it is hypothesized to arise from an aberrant immune response that follows an interaction between environmental factors,

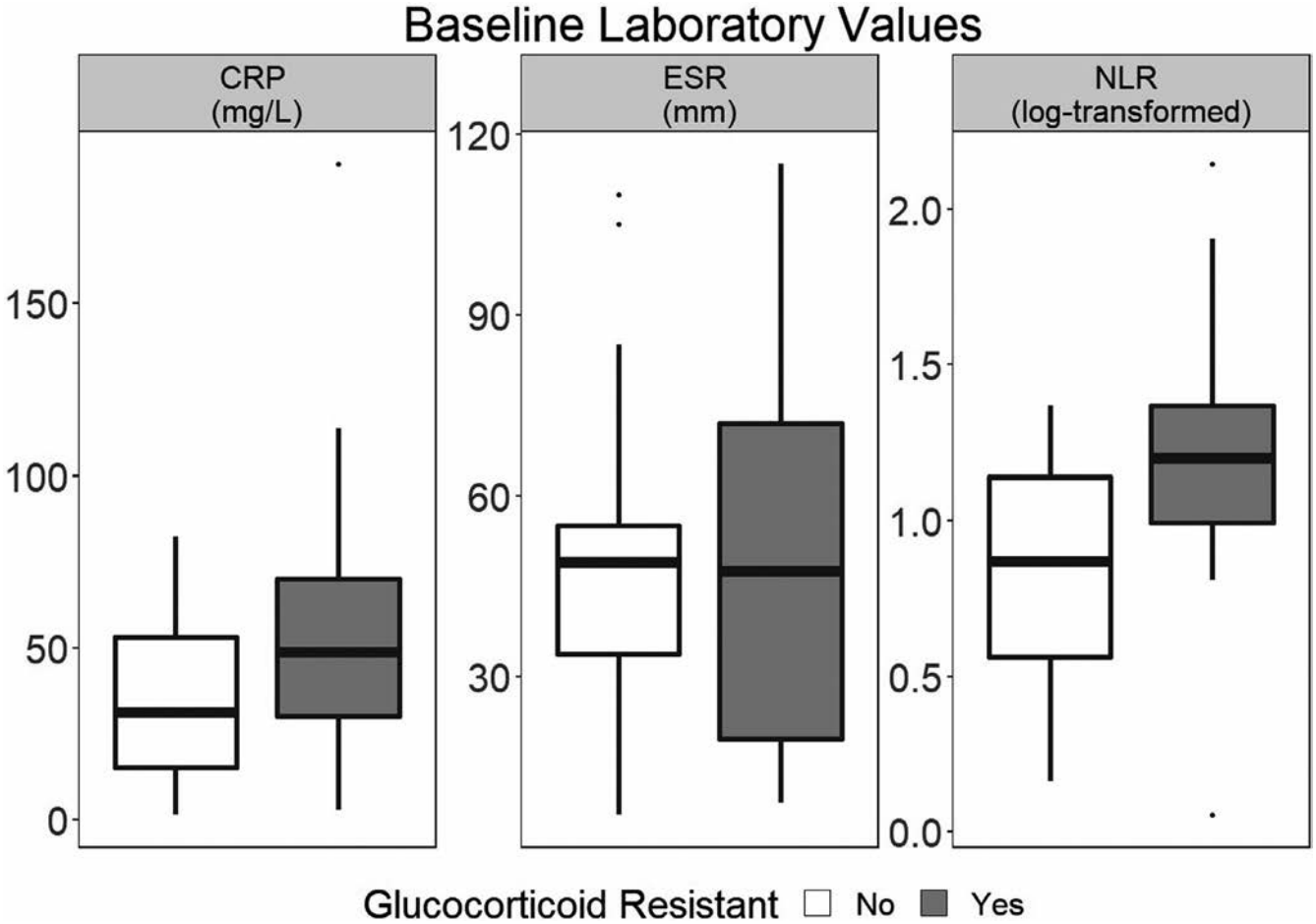

FIGURE 1 Box plots. The relationship between baseline CRP, ESR and NLR values, and glucocorticoid-resistant disease. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NLR, neutrophil to lymphocyte ratio

possibly viral, and the innate immune system in genetically predisposed individuals.[28] Activated macrophages predominate in both synovial and arterial samples taken from PMR patients. However, in the related condition GCA, neutrophils have been implicated as the promotors of an escaped pro-inflammatory disease phenotype.[29] More specifically, a subset of neutrophil granulocytes capable of suppressing T cell activity following the institution of high-dose prednisolone therapy have been demonstrated to lose this ability upon glucocorticoid weaning in the context of rising IL-17 and IL-6 levels. Loss of this neutrophil suppressor function is thought to result in unchecked T cell proliferation within the vessel wall and eventual disease relapse.

While this mechanism provides a biologically plausible explanation for a predominance of neutrophils enabling an adverse prognostic outcome in PMR, baseline neutrophil counts for glucocorticoid-resistant patients in our study were not substantially different from their glucocorticoid-responsive counterparts. Rather, lymphocyte counts were lower in this subset thereby producing higher NLR values. In general, no difference in total lymphocyte numbers is observed when PMR cases are compared with healthy controls.[30] Circulating B cell lymphopenia secondary to redistribution or intravascular marginalization of predominantly pro-inflammatory B-effector cells has been established to exist in newly diagnosed, steroid-naïve PMR.[31] This disturbance in B cell homeostasis corrects with glucocorticoid therapy and is sustained in clinical remission but reoccurs upon disease relapse. Unfortunately, we did not undertake lymphocyte subset testing as part of this study, hence it is unclear whether lower lymphocyte counts at baseline in glucocorticoid-resistant patients represented an exaggeration of this previously described B cell phenomenon. Further investigation of lymphocyte subsets and their association with glucocorticoid resistance in PMR is now planned.

There are several other limitations to this study. It involves a relatively small number of participants and consequently the results may not be generalizable to a larger, more heterogenous PMR cohort. As glucocorticoids have the potential to impact upon measured neutrophil and platelet counts, it is similarly not known if NLR can predict glucocorticoid-resistant disease in PMR if calculated from an FBE obtained once the patient has commenced prednisolone. Finally, this study provides proof-of-concept for the utility of NLR and PLR in measuring PMR disease activity and predicting treatment response. However, for use in everyday practice, cut-off values to aid clinical decision-making would be ideal. Unfortunately, this was beyond the scope of the current pilot and hence further validation of these biomarkers in a large, multi-center trial is necessary.

5 | CONCLUSION

Baseline NLR represents a readily accessible and inexpensive biomarker that can predict glucocorticoid resistance in newly diagnosed PMR. This finding further characterizes a distinct subgroup of patients with relapsing disease in whom early initiation of DMARD therapy may be appropriate. In addition, both NLR and PLR appear to be reliable measures of PMR disease activity, outperforming conventional inflammatory markers like CRP and ESR. Further studies are now needed to validate these biomarkers for implementation in routine clinical practice.

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CONFLICT OF INTEREST

CO has received speaking honoraria from Roche, Janssen, Novartis and Pfizer, and meeting sponsorship from Roche. CM declares no conflict of interest. DL declares no conflict of interest. JL has received a speaking honorarium from Abbvie and meeting sponsorship from Gilead. AS declares no conflict of interest. RB declares no conflict of interest.

AUTHOR CONTRIBUTIONS

CO designed the study, collected the patient data and was chiefly responsible for writing the manuscript. CM analyzed and interpreted all data presented. DL proposed further study of NLR and PLR in PMR and together with JL, AS and RB improved several iterations of the manuscript. All authors read and approved the final product.

TRIAL REGISTRATION

Australian New Zealand Clinical Trials Registry (http://www.anzctr.org.au) – trial identification ACTRN1261400696695, registered 2/7/2014.

ORCID

Claire E. Owen https://orcid.org/0000-0002-2694-5411
David F. L. Liew https://orcid.org/0000-0001-8451-8883
Jessica L. Leung https://orcid.org/0000-0002-3834-2706

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ORIGINAL ARTICLE

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Incidence of giant cell arteritis after bisphosphonate exposure: A retrospective cohort study

Salman Bin Mahmood[1] | Yuanyuan Ji[2] | Yi Peng[2] | Zaki Abou Zahr[3]

[1]Department of Internal Medicine, Hennepin Healthcare, Minneapolis, MN, USA

[2]Chronic Disease Research Group, Hennepin Healthcare Research Institute, Minneapolis, MN, USA

[3]Division of Rheumatology, Minneapolis, MN, USA

Correspondence
Zaki Abou Zahr, Division of Rheumatology, Hennepin Healthcare, 701 Park Ave S, G5 Minneapolis, MN 55415, USA.
Email: zaki.abouzahr@hcmed.org

Abstract

Objectives: Bisphosphonates may cause autoimmune reactions via a cytokine-mediated acute phase response. A case of giant cell arteritis (GCA) after zoledronate injection was recently reported. We aimed to evaluate this association by reviewing the incidence of GCA after bisphosphonate administration.

Methods: This was a retrospective study using the medical claims data of elderly patients in the 20% Medicare random sample from 2008-2014 who had received zoledronate or ibandronate. Patients who had a diagnosis claim of GCA within the past year before receiving either bisphosphonate were excluded. The development of GCA was assessed in 2 ways: GCA diagnosis claim within 28 days of bisphosphonate injection and another claim within 90 days of initial claim; and temporal artery biopsy claim within 28 days of bisphosphonate injection and GCA diagnosis claim within 90 days of biopsy. Due to the Centers for Medicare & Medicaid Services reporting requirements we excluded numbers less than 11 from analysis.

Results: The incidence of GCA was 0.010% and 0.013% after zoledronate and ibandronate injection respectively. In the zoledronate group incidence was highest in patients aged 75-85 years (0.011%), in Whites (0.011%), in the northeast census region (0.013%) and higher in females (0.011% vs 0.009%). All GCA cases noted in the ibandronate group involved White females. We are unable to report incidences by age and region due to the paucity of data.

Conclusion: The incidence of GCA after bisphosphonate injection was not increased compared to the generally reported incidence in the USA.

KEYWORDS

bisphosphonate, GCA, giant cell arteritis, ibandronate, zoledronate

1 | INTRODUCTION

Giant cell arteritis (GCA) is a vasculitis causing granulomatous inflammation involving medium to large sized vessels and can lead to blindness.[1] Bisphosphonates, particularly amino-bisphosphonates such as zoledronate, have been reported to cause an acute phase response (APR) that can rarely elicit autoimmune reactions such as uveitis and scleritis.[2] A recent case report highlighted the development of GCA after administration of zoledronate. We sought to further evaluate this association via a retrospective study of patients who had received either zoledronate or ibandronate.

2 | METHODS

2.1 | Data source

We used the 20% Medicare random sample data (including Part A and Part B) from 2008 to 2014.

The Medicare data include enrollment information, demographic characteristics, and medical claims from Part A and Part B. The Medicare claims files contain information collected by Medicare to allow payment for healthcare services provided to Medicare beneficiaries in the United States and its territories. Data are available for each institutional and non-institutional claim type, with each record being a claim. Standard analytic files (SAFs) generated by the Centers for Medicare & Medicaid Services (CMS) were used. The SAFs are created by processing the National Claims History File's raw claims through final action algorithms that match the original claim with adjusted claims to resolve any discrepancies. SAFs are available for each institutional claim type (inpatient, outpatient, skilled nursing facility, home health agency, carrier, or hospice). Non-institutional Part B physician/supplier SAFs include final action claims for physician services, laboratory services, and durable medical equipment.

2.2 | Study population and patient selection

The study population consisted of elderly patients in the Medicare 20% sample who had received zoledronate or ibandronate injection between 2008 and 2014.

Patients defined from the 20% Medicare data were included if they met the following criteria:

- Both Medicare Parts A and B coverage, not in a health maintenance organization.
- Received at least 1 zoledronate or ibandronate injection.
- Patients who had a diagnosis claim of GCA within the past year before having received either bisphosphonate were excluded.

This exclusion was done to avoid patients who have active GCA as treatment often lasts 6-12 months to complete. This way nearly all cases can be considered for incidence calculation. Ethics board approval along with a waiver of consent for the retrospective review of these patients was obtained through the Office for Human Subjects Research (HSR #16-4235).

2.3 | Study design

This is a retrospective cohort study. The aim is using Medicare claims data to determine whether bisphosphonate injection would cause an increased incidence of GCA compared to the generally reported incidence for the US population.

The presence of GCA is defined in 2 ways with 2 follow-up periods each.

Giant cell arteritis was defined by at least 2 GCA diagnoses in 2 follow-up periods (Figure 1):

- Index date: the first date of zoledronate/ibandronate injection
- First follow-up period: index date + 28 days. Follow-up was censored at death or at end of Part A and B coverage for Medicare patients.
- Second index date: the earliest GCA diagnosis date within first 28 days follow-up period.
- Second follow-up period: second index date + 90 days. Follow-up was censored at death or at end of Part A and B coverage for Medicare patients.

GCA was defined by biopsy and GCA diagnosis in 2 follow-up periods (Figure 2):

- Index date: the first date of zoledronate/ibandronate injection.
- First follow-up period: index date + 28 days. Follow-up was censored at death or at end of Part A and B coverage for Medicare patients.
- Second index date: the earliest biopsy date within first 28 follow-up period.
- Second follow-up period: second index date + 90 days. Follow-up was censored at death or at end of Part A and B coverage for Medicare patients.

The rationale behind this design is due to the nature of GCA presentation with the first claim in 28 days often being submitted by first responders in the emergency room, primary care or by the providers obtaining the biopsy. However, this is preliminary and needs confirmation by a subspecialist. By 90 days, patients are almost certainly seen by a subspecialist who confirms or drops the diagnosis claim.

3 | DEFINITIONS

3.1 | Definition of giant cell arteritis cases

Cases of GCA were defined in 2 ways for each medication:

- The baseline index date, that is, the date of zoledronate/ibandronate injection was defined by International Classification of Disease 9th edition Medical Coding (ICD-9-CM) diagnosis code (see Appendix A).
- One GCA diagnosis claim defined by ICD-9-CM diagnosis code (see Appendix A) during the first 28 days follow-up period and another GCA was diagnosed during the second 90 days follow-up period. If there are more than one GCA diagnosis claims within the first 28 days period, we followed the earliest claim of the GCA diagnosis. All GCA claims were captured regardless of the providers' specialty who submitted the claim. This is due to the fact that more than one specialty could be included for the first or second

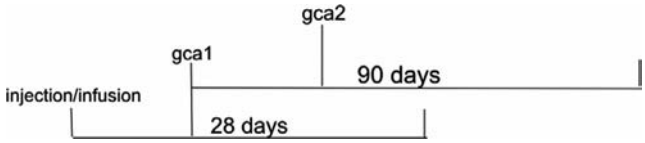

FIGURE 1 Algorithm for giant cell arteritis (gca) diagnosis based on diagnosis claims

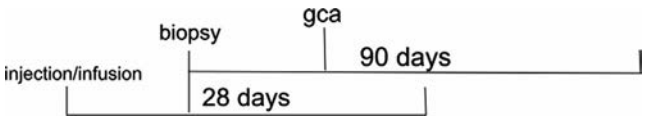

FIGURE 2 Algorithm for giant cell arteritis (gca) diagnosis based on temporal artery biopsy claim followed by diagnosis claim

claim. Additionally, the specific specialty that submitted the claim is not attainable via the Medicare database.

- One biopsy claim defined by Healthcare Common Procedure Coding System (HCPCS) code (see Appendix B) during the first 28 days follow-up period and at least one GCA diagnosed during the second 90 days follow-up period. If there are more than one biopsy within the first 28 days period, we followed the earliest claim of the biopsy. The biopsy claim is regardless of the final result of the biopsy as results cannot be captured via the database. Additionally, GCA continues to be the diagnosis if clinically appropriate even if the biopsy is negative.

3.2 | Definition of covariates

Demographic factors included in this study were gender, age, race and census region.

3.3 | Analysis

Descriptive analyses were performed:

- Baseline patient characteristics for each medication were obtained and stratified by age, gender, race and region.
- Incidence of GCA was calculated as a percentage and reported overall and separately for the different medications.

In compliance with CMS' reporting rules, values 1 to 10 cannot be reported and were excluded from the analyses.

We performed Chi-squared testing to assess statistical significance and *P* values less than .05 were considered significant.

4 | RESULTS

Patient selection and final study sample size for each cohort are presented in Table 1.

We identified 674 444 claims for zoledronate and 86 430 for ibandronate, out of which 70 and 11 respectively met our criteria for a new diagnosis of GCA. Hence, the incidence of GCA was 0.010% and 0.013% after zoledronate and ibandronate administration respectively (Table 2). The highest number of GCA cases were present in the 75-85 years age group with an incidence of 0.011% for the zoledronate group. We were not able to compute incidences stratified by age for the ibandronate group due to the small number of cases captured. We found the incidence to be higher in females compared to males for zoledronate (0.011% vs 0.009%) and all cases of GCA noted in the ibandronate group involved females. Racially, the highest number of GCA cases were observed in Whites for the zoledronate group with an incidence of 0.011%. All GCA cases in the ibandronate group were observed in Whites. Looking at the regional level, the highest incidence was in the northeast for zoledronate (0.013%) followed by the west (0.012%). Due to the paucity of cases captured, we could not compute incidences according to region for the ibandronate group.

5 | DISCUSSION

Giant cell arteritis is an autoimmune condition characterized by granulomatous inflammation of medium to large sized arteries that usually affects individuals aged 50 years and older. Common symptoms include headache, scalp tenderness and jaw claudication. Inflammation of the ophthalmic artery may lead to rapid and devastatingly permanent vision loss.[1]

Pro-inflammatory cytokines, particularly interleukin (IL)-6, are known to play a significant role in the pathogenesis of GCA. IL-6 promotes differentiation of $CD4^+$ positive naive T cells into IL-17, producing helper T cells which are implicated in the induction of autoimmune injury, while inhibiting differentiation of regulatory T cells.[3] Persistent elevations in IL-6 levels and the resultant imbalance between these T cell populations leads to promotion of autoimmune disease.

Bisphosphonates have been used to treat disorders of bone metabolism for decades. Their frequently described adverse effects include osteonecrosis of the jaw, gastrointestinal intolerance and esophagitis and atypical femoral fracture.[4] Another common adverse reaction described constitutes a transient American college of rheumatology which can affect up to 40% of patients receiving these drugs and is characterized by a wide range of symptoms including fever, musculoskeletal pain, joint swelling, and varying degrees of eye or orbit inflammation.[2,5] The mechanism behind this appears to be related to increased cytokine release including IL-1, tumor necrosis factor-α, and IL-6 from stimulation of mononuclear cells.[6,7]

Metyas et al[8] recently published the case of an elderly Caucasian female who suffered acute vision loss presumably due to developing GCA 3 days after receiving zoledronate; first raising the question of whether bisphosphonates could lead to the development of GCA. The presentation of this case was somewhat atypical for GCA and the temporal artery biopsy was negative. Despite that, given the seriousness of GCA leading to irreversible blindness, we sought to

TABLE 1 Patient selection from the Medicare 2008-2014 20% random sample

Study cohort	Zoledronate	Ibandronate
Total claims with infusion/injection	699 886	89 991
Total claims with infusion/injection covered by Medicare Part A and Part B	677 901	87 643
Exclude claims with GCA diagnosis 1 y before infusion/injection	674 444	86 430
Claims diagnosed with GCA after infusion/injection	3536	1407
Claims diagnosed with at least 1 GCA within 28 d after infusion/injection	145	39
Claims diagnosed with at least 2 GCA within 90 d after infusion/injection	56	11
Total claims of biopsy covered by Medicare Part A and Part B	23 858	23 858
Claims of biopsy after infusion/injection	38	[a]
Claims with at least 1 biopsy 28 d and diagnosed with GCA within 90 d	33	[a]
Final cohort	70	11

Abbreviation: GCA, giant cell arteritis.

[a]Number is <11 and cannot be reported.

TABLE 2 Incidence of GCA by age, gender, race and region

	Zoledronate				Ibandronate		
	Total claims	Claims with GCA	%	*P* value*	Total claims	Claims with GCA	%
Overall	674 444	70	0.010		86 430	11	0.013
Age group							
<65 y	70 451	[a]	[a]	<.0001	7085	[a]	[a]
65-75 y	294 016	25	0.009		29 972	0	0
75-85 y	238 091	26	0.011		35 399	[a]	[a]
>85 y	71 886	[a]	[a]		13 974	[a]	[a]
Gender							
Male	225 067	20	0.009	.834	5564	0	0
Female	449 377	50	0.011		80 866	11	0.014
Race							
White	594 903	63	0.011		81 071	11	0.014
Black	51 645	[a]	[a]		1827	0	0
Asian	7500	0	0		1330	0	0
Hispanic	8060	[a]	[a]		976	0	0
Other race	12 336	[a]	[a]		1226	0	0
Census region							
Northeast	119 627	15	0.013	.615	15 918	[a]	[a]
Midwest	165 621	17	0.010		20 613	[a]	[a]
South	272 236	24	0.009		32 368	[a]	[a]
West	115 706	14	0.012		17 037	[a]	[a]
Missing	1254	0	0		494	0	0

[a]Number is <11 and cannot be reported.

**P* values are calculated from Chi-squared test.

further evaluate this association. The findings of our study do not support this association as we did not observe higher rates of incidence after the administration of either zoledronate or ibandronate.

The incidence of GCA varies widely across the world with numbers ranging from about 2 to 22/100 000 and in the USA is estimated to be up to 20/100 000 (0.020%). The highest numbers are

noted in Olmstead County, Minnesota in the Midwest region of the country with the area's population being of overwhelmingly northern European descent that likely accounts for this finding.[9] The disease is also known to predominantly affect women and the incidence increases with advancing age.

The incidences of GCA we observed in our study for both zoledronate and ibandronate were comparable to nationwide data. We also noted trends similar to those previously observed with a significantly higher incidence noted in the 75-85 years age group and a higher incidence trend among women and Caucasians in the zoledronate group. Interestingly, our data showed a trend toward higher incidences in the northeast and west regions of the USA whereas the highest incidences have been previously reported in the midwest, but these findings did not reach statistical significance. Due to the small sample size we were not able to compute *P* values for the ibandronate cohort.

5.1 | Limitations

Limitations of our study include the retrospective design and the inclusion of patients with only Medicare as the primary payer. Patients with other plans and insurance coverage were not captured. Despite the overall large sample size, we were able to capture relatively few cases of GCA in each group. Furthermore, due to the reporting requirements of CMS we could not report numbers less than 11 and were not able to assess if any differences existed in the incidence of GCA among the different racial groups included in our study.

We were not able to include a control sample from within the Medicare database to directly contrast the incidence of GCA between those who had and had not received bisphosphonates and rather report a comparison between our sample and previously reported nationwide data. However, the incidences we observed for our cohort are comparable to those reported in the general population and we believe this is a valid method of comparison as using a control group would be expected to yield the same result.

6 | CONCLUSION

The findings of our study do not support an association between bisphosphonates and the subsequent development of GCA. However, clinicians should continue to be cognizant of the rare development of auto-inflammatory reactions after bisphosphonate administration.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

AUTHORS' DISCLAIMER

The views expressed in this article are the authors' own and do not reflect an official position of Hennepin Healthcare.

ORCID

Salman Bin Mahmood https://orcid.org/0000-0001-7023-7060

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APPENDIX A

ICD-9-CM diagnosis codes used to identify GCA

Disease	ICD-9-CM diagnosis codes
Giant cell arteritis	4465

APPENDIX B

ICD-9-CM HCPCS codes used to identify medications and procedures

	ICD-9-CM diagnosis codes
Biopsy	37 609
Zoledronic acid infusion	J3487, J3488, J3489
Prolia injection	C9272, J0897
Ibandronate injection	J1740, C9229

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Survival and causes of death for Takayasu's arteritis in Korea: A retrospective population-based study

Shin Yi Jang | Taek Kyu Park | Duk-Kyung Kim

Division of Cardiology, Department of Medicine, Heart Vascular Stroke Institute, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea

Correspondence
Duk-Kyung Kim, Division of Cardiology, Department of Medicine, Heart Vascular Stroke Institute, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro Gangnam-gu, 06351 Seoul, South Korea.
Email: dukkyung11.kim@samsung.com

Abstract

Objective: Few studies have evaluated survival of Takayasu's arteritis (TAK; M31.4) in Korea. The purpose of this study was to assess the survival rate (SR) and causes of death for TAK.

Methods: Newly diagnosed TAK data (N = 2731) were collected from the National Health Insurance Service in Korea from 2006 through 2017. The Kaplan-Meier method was used. Korean death data was used from 2006 through 2018.

Results: The mean age was 48.1 (±16.9) years. The proportion of female patients was 74.4%. The most common cause of death in TAK was diseases of the circulatory system. The 1-, 3-, 5-, and 10-year SRs were 97.5%, 94.7%, 91.7%, and 84.7%, respectively. The 1-, 3-, 5-, and 10-year SRs by gender were 97.8%, 95.6%, 92.9%, and 86.3%, respectively, among females and 96.8%, 92.2%, 88.4%s and 79.7%, respectively, among males ($P < .001$).

Conclusions: The overall 10-year SR was about 85%. The 10-year SR in males was lower than that in females. The most common cause of death in TAK was diseases of the circulatory system.

KEYWORDS

cause of death, survival rates, Takayasu's arteritis

1 | INTRODUCTION

Takayasu's arteritis (TAK) is a chronic inflammatory vascular disease of unknown origin. The condition is also known as aortic arch syndrome, non-specific aortoarteritis, or pulseless disease, and is associated with an inter-arm blood pressure difference. It mainly affects the aorta and its branches, as well as the coronary or pulmonary arteries. The disease usually presents with an initial inflammatory phase characterized by signs and symptoms of systemic illness such as malaise, weight loss, loss of appetite, and joint pain. Because the symptoms may be vague, patients may attribute them to the common cold. If the disease is allowed to progress, a patient's arteries are narrowed by fibrosis. The secondary pulseless phase is characterized by vascular insufficiency from intimal narrowing of the vessels manifesting as arm or leg claudication, renal artery stenosis causing hypertension, and neurological manifestations due to decreased blood flow to the brain.[1] The acute phase of TAK is not easy to detect because there are no specific diagnostic methods. Therefore, some patients with TAK may present with late vascular changes without a preceding systemic illness.[2] Vascular stenosis can be seen using vascular ultrasonography, magnetic resonance imaging, computed tomography, or angiography. However, few studies have evaluated the TAK survival in Korea, where it is a rare disease. Therefore, in this paper, we describe the survival and cause of death of TAK in Korea between 2006 and 2017 using Korean National Health Insurance benefit data.

2 | METHODS

2.1 | Study population

The data of TAK patients who were newly diagnosed was collected from Korean National Health Insurance Service (KNHIS) records from 2006 through 2017 (N = 2731; female-male ratio = 3:1). The data consisted of primary and secondary diagnoses related to TAK (M31.4), according to the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10).

2.2 | KNHIS database

The universal coverage health insurance system in Korea for all citizens was initiated in 1963, based on the National Medical Insurance Act. And universal healthcare coverage was achieved in 1989.[3] The KNHIS database for health insurance subscribers and Medicare recipients excluding foreigners consists of the following 4 databases: (a) qualification database including age, gender, type of subscription, income rank, and death; (b) medical check-up database includes the health examination data and lifetime transition period medical check-ups at 40 years old and 66 years old; (c) medical institution database; and (d) treatment database including the type of disease, disease code using the ICD-10, and prescriptions. The treatment database has 4 categories of medicine, dentist, oriental medicine, and pharmacy. Among those 4 categories of the treatment database, we only used medicine. Further, we used variables from the qualifications database in conjunction with the treatment database.[4]

2.3 | Definition of variables

The age was defined as the first TAK diagnosed age. Age groups were defined as 0-9 years, 10-19 years, 20-29 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years, 70-79 years, and 80 years or older.

2.4 | Death data

In this study, we used the death data of Korean people from 2006 through 2018 from the Korea Statistics Promotion Institute. When Koreans die, Korean citizens must submit (a) a death declaration and (b) a death certificate or a corpse optometry report by a medical doctor to the town office of the deceased's place of residence or an area prescribed by law within 1 month of death, and its submission must be done by a direct family member or a person prescribed by law. The death declaration must include the following information: (a) the deceased's name, gender, identification number, address, death date and time, place of death, cause of death, death type such as death due to disease or accident, and if accident, type of accident, accident date, place of accident, nationality, and marriage status; (b) the reporter's name, identification number, relationship with the dead, and contact information; and (c) submitter's name and identification number. Among all the aforementioned information, we received only the cause of death and the date of death from the Korea Statistics Promotion Institute in accordance with the official procedure.

2.5 | Cause of death

We evaluated the primary cause of death as 1 of the following: certain infections and parasitic diseases (ICD-10: A00-B99); malignant neoplasms (ICD-10: C00-C97); endocrine, nutritional, and metabolic diseases (ICD-10: E00-E90); mental and behavioral disorders (ICD-10: F01-F99); diseases of the nervous system (ICD-10: G00-G98); diseases of the circulatory system (ICD-10: I00-I99); diseases of the respiratory system (ICD-10: J00-J98); diseases of the digestive system (ICD-10: K00-K92); diseases of the musculoskeletal system and connective tissue (ICD-10: M00-M99); diseases of the genitourinary system (ICD-10: N00-N99); congenital malformations, deformations and chromosomal abnormalities (ICD-10: Q00-Q99); symptoms, signs, and abnormal clinical and laboratory findings, not elsewhere classified (ICD-10: R00-R99); injury, poisoning, and certain other consequences of external causes (ICD-10: S00-T98); and not provided.

2.6 | Statistical methods

The differences in age and causes of death, by gender, were analyzed using Student's *t* test for continuous variables and the χ^2 test for categorical variables. The Kaplan-Meier method was also used to compare survival among patients with TAK overall, by gender, and by age group using log-rank tests.

2.7 | Ethics statement

Evaluation of the study protocol was exempted by the Institutional Review Board of Samsung Medical Center (IRB number 2017-02-032).

3 | RESULTS

The distribution by gender of the patients in Korea with newly diagnosed TAK (N = 2731) between 2006 and 2017 is presented in Table 1. The overall mean (±SD) age of TAK patients was 48.1 (±17.0) years, 48.0 (±19.7) years in males and 48.1 (±15.9) years in females (*P*= not significant). The male-to-female ratio was 1:3. The distribution of newly diagnosed TAK patients by age group was as follow: 1.14% in the under-10s, 5.31% in the 10s, 8.92% in the 20s, 13.3% in the 30s, 21.4% in the 40s, 23.7% in the 50s, 16.6% in the 60s, 7.47% in the 70s, and 2.16% in the 80 years and older group. The highest

TABLE 1 The distribution of general characteristics and causes of death in Takayasu's arteritis (TAK) by gender from 2006 through 2017

Variables	TAK total (N = 2731)	Male (n = 699)	Female (n = 2032)	*P* value*
	Mean ± SD, median [IQR], or number (%)			
Age (y) mean ± SD	48.1 ± 17.0	48.0 ± 19.7	48.1 ± 15.9	.922
Age (y) median [IQR]	49 [37, 60]	49 [35, 63]	49 [38, 59]	.922
0-9	31 (1.14)	15 (2.23)	16 (0.79)	<.001
10-19	145 (5.31)	59 (8.44)	86 (4.23)	
20-29	244 (8.92)	62 (8.87)	182 (8.96)	
30-39	362 (13.3)	89 (12.7)	273 (13.5)	
40-49	585 (21.4)	125 (17.9)	460 (22.6)	
50-59	648 (23.7)	128 (18.3)	520 (25.6)	
60-69	453 (16.6)	117 (16.7)	336 (16.5)	
70-79	204 (7.47)	77 (11.0)	127 (6.25)	
80+	59 (2.16)	27 (3.86)	32 (1.57)	
Gender, male	699 (25.6)	699 (100)	0 (0.00)	
Death	298 (10.9)	101 (14.5)	197 (9.69)	<.001
Causes of death, n = 298				.004
Certain infections and parasitic diseases (A00-B99)	7 (2.35)	4 (3.96)	3 (1.52)	
Malignant neoplasms (C00-C97)	34 (11.4)	19 (18.8)	15 (7.61)	
Endocrine, nutritional, and metabolic diseases (E00-E90)	14 (4.70)	7 (6.93)	7 (3.55)	
Mental and behavioral disorders (F01-F99)	2 (0.67)	0 (0.00)	2 (1.02)	
Diseases of the nervous system (G00-G98)	2 (0.67)	0 (0.00)	2 (1.02)	
Diseases of the circulatory system (I00-I99)	113 (37.9)	31 (30.7)	82 (41.6)	
Diseases of the respiratory system (J00-J98)	13 (4.36)	8 (7.92)	5 (2.54)	
Diseases of the digestive system (K00-K92)	5 (1.68)	1 (0.99)	4 (2.05)	
Diseases of the musculoskeletal system and connective tissue (M00-M99)	29 (9.73)	3 (2.97)	26 (13.2)	
Disease of the genitourinary system (N00-N99)	10 (3.36)	3 (2.97)	7 (3.55)	
Congenital malformations, deformations and chromosomal abnormalities (Q00-Q99)	1 (0.34)	0 (0.00)	1 (0.51)	
Symptoms, signs, and abnormal clinical and laboratory findings, not elsewhere classified (R00-R99)	11 (3.69)	4 (3.96)	7 (3.55)	
Injury, poisoning, and certain other consequences of external causes (S00-T98)	21 (7.05)	11 (10.9)	10 (5.08)	
Not provided	36 (12.1)	10 (9.90)	26 (13.2)	

Abbreviation: IQR, interquartile range.

*Student's *t* test or χ^2 test.

proportion of TAK patients occurred in the 50s age group for both genders ($P < .001$). The overall proportion of death with TAK was 10.9%. The 3 highest proportions among the causes of death in patients with TAK were diseases of the circulatory system, malignant neoplasms, and disease of the musculoskeletal system and connective tissue. We also show the distribution of specific cause of death in Table S1.

The 1-, 3-, 5-, and 10-year survival rates (SRs) of TAK were 97.5%, 94.7%, 91.7%, and 84.7%, respectively. The 1-, 3-, 5-, and 10-year SRs by gender were 97.8%, 95.6%, 92.9%, and 86.3%, respectively, among females and 96.8%, 92.2%, 88.4%, and 79.7%, respectively, among males ($P < .001$). The 1-, 3-, 5-, and 10-year SRs were 97.3%, 93.8%, 88.9%, and 71.5%, respectively, in the 60-69 age group; 90.6%, 81.4%, 71.1%, and 44.5%, respectively, in the 70-79 age group; and 88.1%, 70.9%, 55.2%, and 14.9%, respectively, in the 80 years and older age group ($P < .001$) (Figure 1 and Table S2).

4 | DISCUSSION

In this study, the 5- and 10-year SRs in TAK were 91.7% and 84.7%, respectively. Park et al showed an almost similar result: in a study

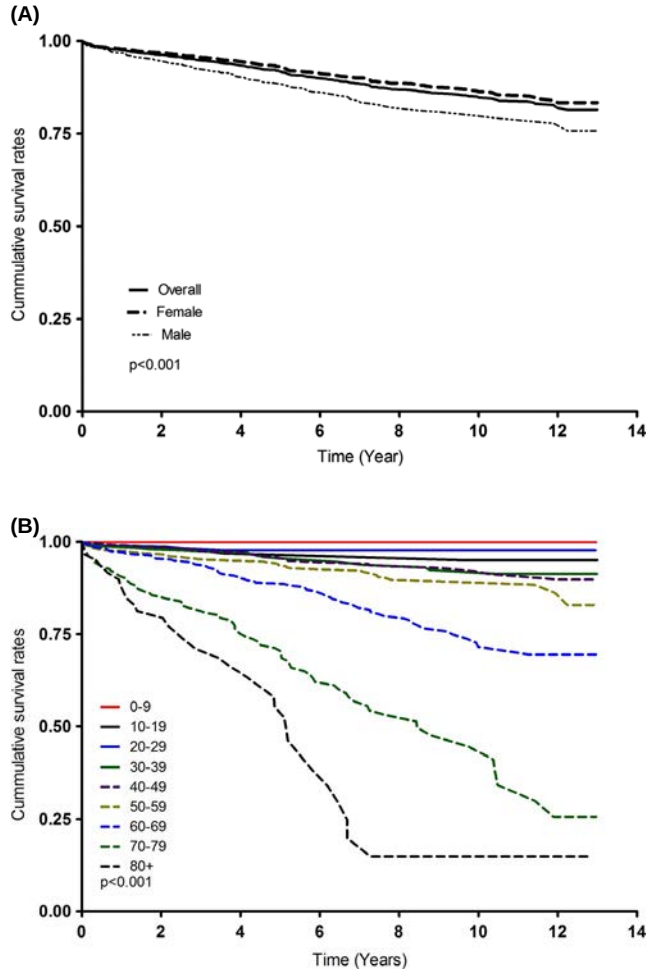

FIGURE 1 A, Takayasu's arteritis survival curve overall and by gender. B, Takayasu's arteritis survival curve by age group

with 612 TAK patients from 2008 to 2012, the 5-year SR was about 90%.[5] Furthermore, a French study on the 10-year SR among White and Black TAK patients showed that the SR of North African patients was lower than that of White patients.[6] The variations in SR difference might be due to ethnic differences. Male TAK patients were fewer in this study, because Korean males overall have a higher 10-year mortality rate and lower life expectancy than Korean females.[7,8]

Diseases of the circulatory system were found to be the most common cause of death among TAK patients in this study. They were also the most common cause of death among South African TAK patients from 1952 to 2002[9] and Chinese TAK patients from 1983 to 2014.[10] In addition, the proportions for the major causes of death among the codes starting with ICD-I are 33.6% for stroke death (code: I6x.x), and 54.8% for cardiac death including coronary artery disease (code: I2x.x and I4x.x), cardiac valve disease (code: I3x.x), and heart failure (code I5x.x) in this study (Table S1). Interestingly, in this study, the second and third most common causes of death among male TAK patients were malignant neoplasms, followed by injury, poisoning, and certain other consequences of external causes, whereas the second and third most common causes of death among females were diseases of the musculoskeletal system and connective tissue, followed by malignant neoplasms. Since there are insufficient data to explain why the second and third causes of death differ by gender, further study is required to investigate this difference.

The age group with the highest proportion of TAK patients in this study was the 50s group, a result that was similar to the distribution of the 82 French TAK patients.[6] In our study, the median age at diagnosis is 48 years and is relatively high. A previous large Korean cohort study showed mean age of TAK was 46 years,[5] which was compatible with our data. The disease usually presents with initial non-specific symptoms, which delay diagnosis. Actually, the diagnosed means age of TAK in a Unites States cohort of 126 patients was 31.5 years.[11] While the female-to-male ratio was 3:1 in this study, the gender ratio in previous TAK studies has varied from 29:1 to 2:1.[5,12,13]

4.1 | Limitations

There were several limitations to our study. First, National Health Insurance benefit records may have missed potential patients with TAK who did not use medical services or who paid for their own medical expenses. Also, it is possible that TAK patients who were not properly diagnosed were excluded. For these reasons, the SRs of TAK in this study might be under- or overestimated. Second, our data regarding cause of death is not based on hospital records but Korea Statistics Promotion Institute Database, which simply reflect TAK as cause of death when not specified. Third, we could not show clinical features such as vascular involvement in TAK due to limitations in the data. Therefore, because of the trend toward an increased prevalence of TAK,[14] a well-designed hospital-based TAK registry is needed in Korea.

5 | CONCLUSIONS

The overall 10-year SR was about 85%. The 10-year SR was lower in males and decreased with age. The most common cause of death among TAK patients was diseases of the circulatory system. Therefore, further effort is required to improve treatment for TAK patients worldwide.

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CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Conceptualization: Jang SY. Methodology: Jang SY, Kim DK. Formal analysis: Jang SY. Data curation: Kim DK, Park TK. Software: Jang SY.

Validation: Park TK. Investigation: Park TK. Writing - original draft preparation: Jang SY. Writing - review and editing: Jang SY, Park TK, Kim DK. Approval of final manuscript: Jang SY, Park TK, Kim DK.

ORCID

Shin Yi Jang https://orcid.org/0000-0003-4319-7029
Taek Kyu Park https://orcid.org/0000-0003-1440-3583
Duk-Kyung Kim https://orcid.org/0000-0001-9227-4196

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SUPPORTING INFORMATION

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

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Development of a Bangla version of the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and the Bath Ankylosing Spondylitis Functional Index (BASFI)

Syed Jamil Abdal[1] | Sabrina Yesmin[2] | Md. Nahiduzzamane Shazzad[3] | Mohammad Abul Kalam Azad[3] | Md. Abu Shahin[3] | Minhaj Rahim Choudhury[3] | Md. Nazrul Islam[3] | Syed Atiqul Haq[3]

[1]Green Life Center for Rheumatic Care & Research, Dhaka, Bangladesh

[2]Department of Medicine, Dr. Sirajul Islam Medical College, Dhaka, Bangladesh

[3]Department of Rheumatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

Correspondence
Syed Jamil Abdal, Medicine Specialist & Consultant Rheumatologist, Room-207, Green Life Center for Rheumatic Care & Research, 32 Green Road, Dhaka-1205, Bangladesh
Email: sjabdal@gmail.com

Abstract

Aim: Development of a Bangla version of the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and the Bath Ankylosing Spondylitis Functional Index (BASFI).

Methods: This biphasic observational study performed the translation and adaptation of the questionnaires carried out in 5 steps with pre-testing in 30 AS patients followed by the psychometric validation of the pre-final Bangla version utilizing content and construct validity in 115 AS patients. The reliability was examined through internal consistency and test–retest reliability involving 23 AS patients.

Results: After pre-testing of the pre-final Bangla version of both indices, the psychometric validation found that the convergent validity of Bangla version of BASDAI showed strong correlation with C-reactive protein (r = .75) and the Maastricht Ankylosing Spondylitis Enthesitis (r = .64), and moderate correlation with erythrocyte sedimentation rate (r = .49). Again, the Bangla BASFI showed significant correlation with occiput-to-wall distance (OWD) (r = .50), mentum-to-sternum distance (MSD) (r = .50), chest expansion (CE) (r = −.40), finger-to-floor (FFD) (r = .55), number of swollen joints (r = .69), and number of enthesitis (r = .68). The divergent validity demonstrated weak correlations between BASDAI and OWD (r = .43), MSD (r = .34), CE (r = −.44), FFD (r = .47). The divergent validity of BASFI could not be assessed due to lack of a suitable comparing parameter. The instruments revealed acceptable internal consistency as Cronbach's alpha was 0.86 for BASDAI and 0.93 for BASFI. A 7-day test–retest reliability measured by the intraclass correlation coefficient were 0.80 (CI at 95% = 0.58-0.90) for BASDAI and 0.83 (CI at 95% = 95% 0.64-0.92) for BASFI respectively.

Conclusions: Bangla version of BASDAI and BASFI may be useful in disease activity and functional ability assessment in AS patients.

KEYWORDS

BASDAI, BASFI, psychometrics, reliability, validity

1 | INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory disorder of unknown etiology mainly affecting the spine and sacroiliac joints. The major clinical manifestations of AS include inflammatory back pain, morning stiffness, and limited spinal activity and/or concurrent peripheral arthritis and enthesitis and even spinal deformity or ankylosis at the advanced stage. Physical disability occurs in about one-third of AS patients within 15-20 years after the diagnosis.[1,2]

The Bath Ankylosing Disease Activity Index (BASDAI)[3] measures disease activity while the Bath Ankylosing Spondylitis Functional Index (BASFI) measures functional aspects of the patients suffering from AS. [4]

The quantitative measurement of either physical or functional aspect of the impact of a disease is vital for assessing the outcome interventions aimed at reducing disease activity. A disease-specific quality of life (QoL) instrument is superior to a general one.[5] For response accuracy, a questionnaire in the mother tongue helps to obtain correct disease assessment and treatment outcomes. Bangla is one of the seventh most widely spoken languages in the world, with nearly 265 million using the language.[6]

The BASDAI and BASFI both were translated and culturally adapted in different languages such as Hindi,[7] Chinese,[8] Spanish,[9] Moroccan,[10] Iranian,[11] Russian,[12] Finnish,[13] Ukrainian,[14] Italian,[15] Swedish,[16] Turkish,[17] Taiwanese,[18] Tunisian[19] and many more.

1.1 | Aim of the study

The aim was to develop a validated Bangla version of BASDAI and BASFI for Bangla speaking AS patients to assess their disease activities and functional status, respectively.

2 | MATERIALS AND METHODS

2.1 | Study population

The study was conducted in the outpatient wing of the Department of Rheumatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh in 2 phases. In phase-I, the translation and adaptation of the Bangla version of the questionnaires was carried out. Then, in phase-II, validation of the translated and adapted questionnaires was achieved. The inclusion criteria were: adult (≥18 years) males/females suffering from AS (modified New York criteria) willing to participate in the pre-test, construct validity and test–retest reliability of both the BASDAI and BASFI indices. As per Beaton et al,[20] 30 in number participated in the pre-test. Utilizing Study Size 3.0 version,[21] the sample size was calculated as 115 in number for completing content validity and construct validity and 23 in number for test–retest reliability.

2.2 | Original BASDAI and BASFI

The original BASDAI[3] is a 6-point scale which measures level of disease activity while the BASFI[4] is a 10-point scale measuring functional aspects of patients suffering from AS.

2.3 | Translation and cultural adaptation of BASDAI and BASFI indices

The translation and cross-cultural adaptation of questionnaires had passed through 5-staged forward-backward procedure.[20] The first stage in adaptation was the forward translation that was carried out by 2 translators with Bangla as mother tongue. One of the translators (the investigator himself) was aware and the other (a Bangla teacher of a reputed college) was neither aware nor informed of the concepts of measurements. A synthesized Bangla version (Ts) produced through 2 initial translated versions based on local customs, habits, and usage of words. The Ts was back-translated into English by 2 translators (1 was a senior teacher of Dhaka University, and other is the teacher of a renowned English medium school) with good command in English. An Expert Committee composed of 1 methodologist, 3 rheumatologists, a language professional as well as all translators played a crucial role toward the achievement of cross-cultural equivalence including semantic, idiomatic, experiential, conceptual equivalences. Items, instructions and response options considered. Consensus was reached on the items, and when necessary, the translation and back-translation process were repeated to clarify how another wording of an item could work. The committee reviewed and compared all the translations with the original BASDAI and BASFI. Eventually, following the process, the pre-final Bangla version was developed.

2.4 | Pre-test (field testing)

The pre-test (field test) of the pre-final version questionnaires was carried out in the outpatient clinic of the Department of Rheumatology, BSMMU.

Each subject first completed the questionnaire and was then interviewed by the investigator to probe what he/she thought would be meant by each questionnaire item and by the relevant responses maintaining their privacy. The participant was encouraged to describe his/her way of expressing the items and any suggestions regarding them. The questionnaires were modified as per feedback from the probe.

2.5 | Psychometric evaluation of the Bangla BASDAI and BASFI indices

2.5.1 | Validity

For psychometric analysis, the validation of the pre-final Bengali version accomplished through 2 types of validity tests, that is content

validity and construct validity utilizing AS patients (where 2-sided Cronbach's alpha revealed significance level of 0.05 with a power of 0.8 with a H0: Cronbach's alpha is 0.7 and H1: Cronbach's alpha is 0.8 respectively).

The content validity assessed by the index of content validity (ICV) utilized 3 rheumatologists; each expert rated each item as 1 (not relevant at all), 2 (relatively relevant), 3 (good relevance) and 4 (fully relevant). The ICV of each item calculated using the summation of scores from each expert was divided by 3.

2.5.2 | Test–retest assessment

The reliability was examined in 2 ways: internal consistency and test–retest reliability. Internal consistency tested by Cronbach's alpha was an expected value, that is, 0.70. For test–retest reliability of the pre-final Bangla version of BASFI and BASDAI, patients were interviewed twice using the Bangla BASDAI and BASFI with an interval of 1 week without any intervention.

2.5.3 | Scoring of the BASDAI and BASFI

The BASDAI consists of a 0-10 scale measuring discomfort, pain, and fatigue (0 being no problem and 10 being the worst problem) in response to 6 questions related to the 6 major symptoms of AS such as fatigue, spinal pain, joint pain or swelling, enthesitis, duration and severity of morning stiffness. The average of the 2 scores relating to morning stiffness gave each symptom equal weighting. The measured scales of the first 4 questions were summated. The measured scales of the last 2 questions were summated and divided by 2. The summated results of all measured scales were then divided by 5. The scores of 4 or higher suggest suboptimal control of the disease.

Similarly, the 10 questions that comprise the BASFI,[4] a 10-point scale, of which the first 8 questions evaluate activities related to functional limitations and the final 2 questions evaluate the patients' ability to cope with activities of daily living. The mean of the 10 scales gives the BASFI score – a value between 0 and 10.

2.6 | Statistical analysis

2.6.1 | Validity

The convergent validity was measured by correlation between BASDAI and the Maastricht Ankylosing Spondylitis Enthesitis (MASES), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) and by correlation between BASFI and occiput-to-wall distance (OWD), chest expansion (CE), mentum-to-sternum distance (MSD), finger-to-floor (FFD), number of swollen joints (NSJ), and number of enthesitis (NE). The divergent validity was assessed by demonstrating weak or no correlations between metrological measurements and BASDAI. The correlation coefficients of >0.50, 0.35-0.50, and <0.35 were considered strong, moderate, and weak correlations, respectively. All these correlations were assessed using Spearman's rank correlation coefficient.

2.6.2 | Reliability

The reliability of pre-final Bangla versions of the BASDAI and BASFI were examined in 2 ways: internal consistency and test–retest reliability. Internal consistency was tested by Cronbach's alpha which was considered acceptable if the Cronbach's alpha was found to be either equal to or exceeded 0.70. The test–retest reliability was assessed using intraclass correlation coefficient (ICC) and Spearman's rank correlation coefficient. The ICC was considered acceptable at ≥0.70, and if so was considered as acceptable for test–retest reliability. The internal consistency was considered acceptable if the Cronbach's alpha was ≥0.70. A Spearman's rho was also expected to be at ≥0.40 to be considered as acceptable.

2.7 | Ethics

The study was approved by the institutional review board of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. Following the Declaration of Helsinki principles, informed consent was obtained from all participants before enrolment.

3 | RESULTS

3.1 | Patient characteristics

The socio-demographic characteristics of participating AS patients is shown in (Table 1).

3.2 | Acceptability and response distribution of the final Bangla BASDAI and BASFI

The acceptability and response distribution of the final Bangla BASDAI and BASFI was assessed by elaborate probing of each item and results are as follows.

3.2.1 | BASDAI probing

Although the expression "Overall level" was well-understood by most of the AS patients, it was better expressed by a group of Bangla words. The majority of AS patients understood both "fatigue" and "tiredness" with a single word. In the probing of the second question of BASDAI, "hip" was understood by a group of patients with a Bangla word although many of the patients understood with another word to mean "hip". The Expert Committee had kept both the words

for expressing “hip”. To mean “back”, out of 2 Bangla words chosen were understood by most of the patients. All patients understood “discomfort” expressed by a single word.

The most challenging translation was to express “morning stiffness” in the BASDAI questionnaire which most patients narrated as "feeling of jamming stiffness" or others' views were "feeling of jamming". The problem was solved by adapting the "feeling of jamming stiffness” in Bangla made it understandable by most of the patients. The percentage of understanding is shown in Table 2.

3.2.2 | BASFI probing

On probing of the first question, the translation of "tights" was a difficult task since this "clothing" is not worn in the vast majority of the population in Bangladesh, although a few females wore them in urban areas. All patients with rural background did not understand the meaning of the word "tights" as "clothes" even after detailed explanation by the investigator. However, the word "tight" was well-understood by all patients as "anything" which is not loose or some meaning like this. The Expert Committee found a word that is not indeed meaning "tights". Ultimately, "tights" was translated and adapted as "tight clothes those worn for the legs" to mean the word “tights” since nearly all patients know both the words. To mean “bending forward from the waist” 2 phrases were picked up; these were understood by most of the patients. Regarding the third questionnaire, the word “shelf” was understood by few patients. And so, a Bangla word was chosen and understood by the majority of the patients. While translating the 10th questionnaire, another phrase was chosen for the meaning of "physically demanding activities” understood by all of the patients which carried the same meaning and was adopted accordingly. For the meaning of “full day”, out of 2 words, one was chosen as being understood by all patients. The overall comprehensibility was good. The percentage of understanding is shown in Table 3.

TABLE 1 Characteristic of the participants

Parameter	Participants (N = 115)		
Age in y, mean, range	10.03, 18-59		
Gender, male, female	108, 7		
Mean disease duration in y	10.03		
Literacy levels, male, female	Primary school	44	50.6
	Secondary school	24	27.6
	Higher secondary	19	21.85
	Bachelor	16	18.4
	Masters	12	13.8

3.3 | Construct validity

The validity of both the indices (BASDAI and BASFI) was analyzed with correlation matrix such as Spearman's correlation coefficient calculations. The convergent validity of our Bangla version of BASDAI was showed by significant correlation with the CRP ($r = .75$) and the MASES ($r = .64$) and moderate correlation with ESR ($r = .49$).

TABLE 2 Probing of Bath Ankylosing Spondylitis Disease Activity Index

Items	Representative words/ group of words/phrase	Understood	Not understood	Percentage of understanding
Please	1st group of words	27	3	90
	2nd word	3	27	10
Overall	1st group of words	15	15	50
	2nd group of words	23	7	76.67
Fatigue/tiredness	1st group of words	25	5	83.33
	2nd word	29	1	96.67
Hip	1st word	17	13	56.67
	2nd group of words	12	18	40
Discomfort	1st word	27	3	90
	2nd group of words	3	27	10
	3rd group of words	30	0	100
Back	1st word	2	28	6.67
	2nd word	27	3	90
Morning stiffness	1st group of words	22	8	73.33
	2nd group of words	3	27	10
	3rd group of words	27	3	90
	4th word	1	29	3.33
	5th word	15	15	50

TABLE 3 Probing of Bath Ankylosing Spondylitis Functional Index

Items	Representative words/ group of words/phrase	Understood	Not understood	Percentage of understanding
Please	1st group of words	27	3	90
	2nd word	3	27	10
Tights	Tights	0	30[a]	0[a]
	Tights	27[b]	3[b]	90[b]
	1st word	13	17	43.33
	2nd group of words	30	0	100
Bending forward	1st group of words	25	5	83.33
	2nd group of words	21	9	70
Shelf	Shelf	4	26	13.33
	1st word	28	2	93.33
Physically demanding activities	1st group of words	15	15	50
	2nd group of words	30	0	100
Full day	1st group of words	30	0	100
	2nd word	25	5	83.33

[a]Patients with rural background

[b]Patients with urban background

On the other hand, the Bangla BASFI showed significant correlation with OWD ($r = .50$), MSD ($r = .50$), CE ($r = -.41$), FFD ($r = .55$), NSJ ($r = .67$), and NE ($r = .68$). The negative correlation of BASFI with CE was because of increased disease activity of the AS patients resulting in higher BASFI and lower CE, showing an inverse relationship between the 2 variables.

The divergent validity was assessed by demonstrating weak correlations between BASDAI and metrological measurements like OWD (0.430), MSD (0.339), CE (−0.441), FFD (0.472). The divergent validity of BASFI could not be assessed due to lack of a suitable comparing parameter.

All these correlations were assessed by using Spearman's rank correlation coefficient. Internal consistency was tested by Cronbach's alpha, which was found to be 0.863 and 0.934 for BASDAI and BASFI, respectively, which were more than our expected value (ie, 0.70).

3.4 | Reliability

The test–retest reliability was assessed using ICC of both the questionnaires and was acceptable (Table 4).

4 | DISCUSSION

In this study, we investigated the validity and reliability of the Bangla version of BASDAI and BASFI questionnaires in a Bangla speaking population of patients with AS. In determining the construct validity, the BASDAI was compared with total enthesis count ($r = .34$); general well-being in the last week ($r = .7$); spinal pain ($r = .53$) duration of morning stiffness ($r = .64$) in the Spanish[9] version while the Moroccan[10] version compared spinal pain ($r = .53$), number of nocturnal awakenings ($r = .57$), morning stiffness ($r = .65$), the enthesis index ($r = .47$), Bath Ankylosing Spondylitis Global Score (BAS-G; $r = .53$), BASFI ($r = .54$), ESR ($r = .41$; for all $P < .001$) with BASDAI. Similarly, in the Hindi[7] version, for construct validity, BASDAI was compared with ESR (0.31, $P = .05$), CRP (0.48, $P < .001$), enthesitis score (0.32, $P = .045$) OWD, CE. Likewise, the Iranian[11] version revealed comparison of BASDAI with patient global disease activity index, nocturnal back pain, total back pain, NSJ, NE, morning stiffness and BASFI for the construct validity of BASDAI. For the construct, the Chinese[8] version showed a comparison between BASDAI and disease duration, occipital wall distance, modified Schöber's test, CE, ESR, and CRP.

On the other hand, for construct validity BASFI was compared with Schöber's test ($r = -.4$); OWD ($r = .38$) and thoracic expansion ($r = -.3$) in the Spanish version.[6] The Moroccan[10] version compared Schöber's test ($r = -.56$), OWD ($r = .46$), CE ($r = -.46$), BASDAI ($r = .54$), Bath Ankylosing Spondylitis Metrology Index ($r = .70$), BAS-G) $r = .58$), BASRI ($r = .61$), radiological changes in sacroiliac joints ($r = .54$) Hindi[7] ESR (0.55, $P < .001$), CRP ($P = .60$, $P < .001$), OWD, CE in determining the construct validity of BASFI. In the Iranian[11] version, for the same, BASFI is compared with OWD, MSD, CE, FFD, NSJ, NE, nocturnal back pain, total back pain and BASDAI. Similarly, drug (infliximab) response study in the Chinese[8] version utilized the modified Schöber test, ESR, and CRP to compare with BASFI in the formulation of construct validity. In comparison, the Russian[12] version used tests of Schöber, Ott, Thomayer, and Forestier, chest wall extension, the duration of the disease, and the presence of hip joint involvement, ESR, CRP ($r = .73$, $P < .001$) in making the construct validity of BASFI. Similarly, the Finnish[13] version compared BASFI with

TABLE 4 BASDAI and BASFI ICC

	ICC	
	95% CI	
	BASDAI	BASFI
Single measures	0.798*	0.828*
Average measures	0.888	0.906

Abbreviations: BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; ICC, intraclass correlation coefficient.

*All correlations were statistically significant at $P < .001$

BASDAI, ESR, spinal movement measures of CE, Schöber S1 test and OWD, and radiological changes in the lumbar spine and sacroiliac joints while the Ukrainian[14] version compared Schöber's test, OWD, and CE measure.

In the above-mentioned various versions from different languages, the internal consistency of both the indices, the given Cronbach's alpha ranges from 0.86 to 0.90 for BASDAI and 0.81-0.9950 for BASFI respectively.

The reliability and responsiveness test–retest was carried out in a varying number of patients suffering from AS. For example, the number of patients was found to be 23 in an Italian[15] version while it was 153 in a Chinese[8] version. The interval timing of test–retest was 24 hours in most of the study versions, for example Swedish,[16] Turkish,[17] Spanish,[9] Moroccan[10] and Chinese[8] versions. Two weeks was taken in a Hindi[7] version while the interval timing in an Italian version was 1 day and in that of an Iranian version was 48 hours.

In this 2-phased study, English BASDAI and BASFI were translated and culturally adapted in Bangla, and then psychometric properties of these indices were evaluated in the clinical setting. There were no unfamiliar parameters in the questionnaire, but minor changes were made maintaining the expression but making it more understandable in Bangla. Considering the refined distinction of Bangla language and population literacy rate, people's response parameters were converted to a questionnaire format using the most straightforward, easily understandable words or phrases.

The average time to complete BASDAI and BASFI questionnaires required 4.25 minutes (range 5-7 minutes). All the parameters and findings of English BASDAI and BASFI were kept in the Bangla version. The Bangla versions of BASDAI and BASFI were well understood and accepted by a Bangla speaking Bangladeshi population. They agreed that the parameters and findings were necessary for their disease evaluation, and there was no unpleasant question and no missing item.

The reliability of both the indices as determined by Cronbach's alpha coefficient found a value close to the reliability value of similar studies. For example, internal consistency (Cronbach's alpha) of Bangla BASDAI (0.86) was similar to those of Moroccan[10] (0.86) and Taiwanian[18] (0.87) versions, although the Iranian[11] (0.95), Chinese[8] (0.95), Tunisian[19] (0.91) versions were a little more than that of the Bangla version.

Similarly, internal consistency (Cronbach's alpha) of Bangla BASFI (0.93) was very similar to those of Moroccan[10] (0.90), Iranian[11] (0.96), Taiwan[18] (0.94), Chinese[8] (0.96), Tunisian[19] (0.90), Italian[15] (0.90) and Finnish[13] (0.94) versions.

The test–retest reliability measured by the ICC found that Bangla BASDAI ICC of 0.80 was of similar to those of Spanish[9] (0.74), Finnish[13] (0.74), and Hindi[7] (0.87) but less than those of Moroccan[10] (0.93), Iranian,[11] (0.93), Tunisian[19] (0.96) versions. The Bangla BASFI ICC was 0.83 while those of Spanish[9] (0.68), Moroccan[10] (0.96), Hindi[7] (0.90), Iranian[11] (0.96), Tunisian[19] (0.93), Finnish[13] (0.99), and Italian[15] (0.91) were nearly similar to that of the Bangla version.

5 | STRENGTHS

The strengths of our developed Bangla versions of both BASDAI and BASFI were robust since we carried out the study in 115 participants and the figure is higher than any other similar study in the subcontinent. For example, the corresponding sample size in an Indian study was 41 which conferred a higher statistical power to our study.

6 | LIMITATIONS

Although our study showed acceptable validity and good reliability of the Bangla version of BASDAI and BASFI, there are a few limitations. As the study was carried out in a tertiary level hospital, it was not fully representative of the whole population of Bangladesh. The divergent validity of BASFI could not be assessed due to lack of a suitable comparing parameter. Moreover, colloquialism and illiteracy were also limitations.

The results of our work revealed that the Bangla version of both BASDAI and BASFI questionnaires had maintained all the properties from in the original English-language instruments. The Bangla questionnaires were found to be valid, reliable for assessment of disease activity and functional status of AS patients and can be applied in both clinical practice and for research purposes.

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CONFLICT OF INTEREST

The authors have no competing interest in this study.

ORCID

Syed Jamil Abdal https://orcid.org/0000-0002-4414-823X
Sabrina Yesmin https://orcid.org/0000-0002-7403-6845
Md. Nahiduzzamane Shazzad https://orcid.org/0000-0002-8535-4259
Syed Atiqul Haq https://orcid.org/0000-0003-4154-7283

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Evaluation of cognitive function in adult patients with juvenile idiopathic arthritis

Natalia Mena-Vázquez[1,2] | Pablo Cabezudo-García[1,3] | Fernando Ortiz-Márquez[4] | Gisela Díaz-Cordovés Rego[1,2] | Luis Muñoz-Becerra[1,3] | Sara Manrique-Arija[1,2] | Antonio Fernández-Nebro[1,2,4]

[1]Instituto de Investigación Biomédica de Málaga (IBIMA), Málaga, Spain

[2]UGC de Reumatología, Hospital Regional Universitario de Málaga, Málaga, Spain

[3]Servicio de Neurología, UGC de Neurociencias, Hospital Regional Universitario de Málaga, Málaga, Spain

[4]Departamento de Medicina, Universidad de Málaga, Málaga, Spain

Correspondence
Pablo Cabezudo-García, MD, Instituto de Investigación Biomédica de Málaga (IBIMA), Servicio de Neurología, UGC de Neurociencias, Hospital Regional Universitario de Málaga, Málaga . CP:29010, Spain.
Email: pablocabezudo@gmail.com

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Abstract

Objective: To evaluate cognitive function in adult patients with juvenile idiopathic arthritis (JIA) and associated factors.

Patients and methods: We performed a cross-sectional observational study of adult patients with JIA and a healthy control group (no inflammatory diseases) matched for age, gender, and educational level. Cognitive function was assessed using Wechsler Adult Intelligence Scale-III. The cognitive domains measured were attention/concentration, verbal function, visuospatial organization, working memory, and problem solving (Similarities). Other measures included clinical-epidemiological characteristics, comorbid conditions, and treatment. We performed a descriptive bivariate analysis and logistic regression to identify factors associated with visuospatial involvement.

Results: The study population comprised 104 subjects (52 with JIA and 52 healthy controls). Patients with JIA had poorer results for visuospatial function, with a lower median scaled score on the Block Design test (5.0 [4.0-8.0] vs 8.0 [5.0-10.0]; $P = .014$). The number of patients with scaled scores below the average range (<8) in visuospatial organization was significantly greater in the JIA group (67.3% vs 40.4%; $P = .006$). The multivariate analysis revealed time since diagnosis (odds ratio [95% CI], 1.03 [1.01-1.06]), inflammatory activity according to Juvenile Arthritis Disease Activity Score 27-joint count (1.94 [1.01-3.75]), and educational level (0.28 [0.08-0.94]) to be factors associated with visuospatial function.

Conclusion: Cognitive function in adult patients with JIA is poorer than in healthy controls at the expense of visuospatial function. Visuospatial function in JIA patients was inversely associated with disease duration, inflammatory activity, and lower educational level.

KEYWORDS

cognition, cognitive function, cognitive impairment, inflammatory arthritis, juvenile idiopathic arthritis

1 | INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease in children.[1] It encompasses a heterogeneous group of diseases that have in common arthritis of unknown origin, which persists for more than 6 weeks and first manifests before age 16 years. According to the 2001 consensus conference of the International League of Associations for Rheumatology (ILAR), there are 7 types of JIA,[2] the oligoarticular type being the most common. Peripheral joint inflammation is the main finding, although extra-articular involvement in the form of systemic manifestations and uveitis may also be recorded. Furthermore, associated comorbid conditions are not uncommon.[3] Much of our knowledge of JIA stems from previous studies in rheumatoid arthritis (RA),[4] where polyarthritis subtypes of JIA are considered to be similar to early-onset RA.

Involvement of cognitive function is one of the most common comorbidities in chronic inflammatory arthritis, for example, in RA, affecting from 38% to 71% of patients.[5] There is increasing evidence of the influence of the immune system on the brain. The proinflammatory state in patients with RA characterized by high blood levels of cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6 have been associated with poorer cognitive performance.[6] In fact, it was recently reported that cumulative inflammatory burden throughout the course of RA is associated with cognitive impairment in affected patients.[7] Other studies have reported that commonly used drugs for RA such as corticosteroids[8] and methotrexate,[9] as well as accelerated atherosclerosis associated with chronic systemic inflammation and autoantibodies, could lead to cognitive abnormalities.[10] Some case reports have attributed central nervous system involvement in JIA to vasculitis.[11] However, the relationship between JIA and cognitive function is not well established, and the literature assessing this relationship is scarce. One study attempted to identify an association between systemic JIA and poorer cognitive performance in patients aged 6 to 24 years and reported normal test scores for patients and for control subjects.[12] No studies have yet examined adults diagnosed with JIA during infancy and early adolescence, who have a longer disease history and have been exposed to a proinflammatory state for longer, with higher potential exposure of the central nervous system.

The objective of the present study was to evaluate and compare cognitive function between adult patients with JIA and healthy controls. We also aimed to identify those factors that were independently associated with poorer cognitive function in JIA.

2 | METHODS

2.1 | Design

We performed a cross-sectional observational study in which we compared a series of patients with JIA with a healthy control group matched for age, gender, and educational level. Recruitment was between April and December 2019. The study was approved by the Research Ethics Committee of Hospital Regional Universitario de Málaga (HRUM), Malaga, Spain. All of the subjects gave their written informed consent before participating.

2.2 | Study population

Cases were recruited consecutively from among patients aged ≥ 16 years with JIA classified according to the criteria of the ILAR 2001[13] and followed at the transition clinic of HRUM. We excluded patients with inflammatory or rheumatic diseases other than JIA and previous neurological diseases not associated with the course of JIA. We also excluded patients with pain and inflammation affecting the hands at the protocol date and patients with a history of hand erosions. The control group were recruited consecutively from the catchment area of our hospital and did not have any inflammatory or rheumatic diseases or previous neurological disease. We excluded cases and controls who were taking drugs that affect the central nervous system (antiepileptic drugs, antidepressants, benzodiazepines, and barbiturates).

2.3 | Protocol

Patients with JIA were followed up in a mixed unit covering pediatrics and rheumatology. They were seen in the pediatric clinic until age 14-16 years before passing to the transition clinic in the adult unit and followed every 3 to 6 months depending on their clinical situation. After signing the informed consent document, all participants were interviewed and examined for recording of clinical variables and completed a predesigned questionnaire for data collection. We evaluated the different cognitive domains using the Wechsler Adult Intelligence Scale (WAIS-III, 2nd revised edition in Spanish, TEA Ediciones), following the guidelines of the corresponding manual. The battery of subtests was administered by the same rheumatologist trained by an experienced neurologist and then scored by the same experienced neurologist. In addition, study subjects and controls self-completed the Beck Depression Inventory II (BDI-II).

2.4 | WAIS-III

WAIS-III is the 3rd version of the WAIS.[14] It is composed of 14 subtests divided into 2 scales. The verbal scale comprises the following subtests: Vocabulary, Similarities, Arithmetic, Digit Span, Information, Comprehension, and Letter-Number Sequencing. The performance scale comprises the following subtests: Picture Completion, Digit Symbol, Block Design, Matrix Reasoning, Picture Arrangements, and Symbol Search.[15]

The following cognitive domains and corresponding tests were selected based on previous literature[5,12]: Attention/Concentration using WAIS (Digit Span),[16,17] verbal function using WAIS (Vocabulary),[16] visuospatial organization using WAIS (Block Design),[16]

working memory using WAIS (Letter-Number Sequencing),[18] and reasoning/problem solving using WAIS (Similarities).[16,19] The subtests selected were administered and scored following the guidelines of the corresponding manual for administration and scoring.

2.5 | BDI-II

The BDI is a 21-item instrument for evaluating depressive symptoms. According to the scoring system, depression can be classified as minimal (0-13), mild (14-19), moderate (20-28), and severe (29-63). It has proven useful for identifying depression in various medical specialties, including rheumatology.[20] We used the 1996 version (BDI-II).[21]

2.6 | Operational definitions and variables

The main variable was cognitive function according to the scaled score obtained on the corresponding subtests for each cognitive domain.

We recorded demographic data, clinical data, laboratory data, and treatment. The protocol date was the date subjects were included in the study and evaluated. The demographic and clinical data included age (in years), gender, educational level (basic education, non-university higher education, university education), body mass index (BMI: weight in kg divided by height in m^2), and smoking (active smoker and nonsmoker). We also recorded traditional cardiovascular risk factors (diabetes mellitus, arterial hypertension, dyslipidemia, and obesity [BMI ≥ 30]) and the comorbidities included in the Charlson index.[22] The BDI-II score was recorded. Additional data recorded for patients with JIA included the date of onset of the disease, duration of the disease (time from diagnosis to the cut-off), diagnostic delay (months between onset of symptoms and diagnosis of JIA), uveitis, and the following laboratory data: rheumatoid factor (RF), positive if >20 IU/mL; anticitrullinated peptide antibodies (ACPA), positive if >10 IU/mL; positive human leukocyte antigen-B27 titer; and positive antinuclear antibody (ANA) titer at any point during the disease. Disease activity was assessed cross-sectionally using the following: Disease Activity Score of 28 joints – erythrocyte sedimentation rate (DAS28-ESR)[23]; Juvenile Arthritis Disease Activity Score (JADAS27) (based on a 27-joint count)[24]; and physical function by means of the Health Assessment Questionnaire (HAQ).[25] We recorded all current medication, synthetic disease-modifying antirheumatic drugs (DMARDs), (methotrexate, leflunomide, and sulfasalazine), biologic DMARDs (anti-TNF inhibitors, tocilizumab, abatacept, rituximab, and ustekinumab), and Janus-activated kinase inhibitors (tofacitinib and baricitinib). We also recorded previous therapy with DMARDs.

2.7 | Statistical analysis

We performed a descriptive analysis of the main study variables. Qualitative variables were expressed as a number and percentage. Quantitative variables were expressed as mean (± standard deviation [SD]) if they were normally distributed and as median (interquartile range [IQR]) if they were not normally distributed. The normality of the distribution of continuous variables was verified using the Kolmogorov-Smirnov test. The main cognitive performance variables were compared between subjects with JIA and the healthy controls. The Pearson χ^2 test was used to assess qualitative variables; the *t* test was used to assess quantitative variables. Finally, we performed a multivariate logistic regression analysis to identify factors associated with the function involved, namely, Visuospatial Organization. Sample size was calculated based on data from patients with RA owing to the lack of data on JIA in adults. With an alpha risk of 0.10 and a beta risk of 0.2 in a 2-sided contrast, we required 52 patients and 52 controls in order to detect a difference of ≥ 3 units on the WAIS-III. We assumed a common SD of 6.[26] Data entry and the statistical analysis were performed using the statistical program Rcommander.

3 | RESULTS

3.1 | Baseline characteristics

The study population comprised 104 subjects (52 patients, with JIA, 52 healthy controls). All selected patients and controls fulfilled the inclusion criteria. Three JIA patients were excluded due to the presence of pain and/or inflammation of the hands or history of hand erosion. Table 1 shows the baseline characteristics of both groups. Most subjects were women (67%), with a mean age of around 23 years and a non-university higher educational level in half of the cases. As for comorbidities, there were no significant differences in cardiovascular risk factors or in the other comorbid conditions in the Charlson index.

With respect to the clinical and laboratory characteristics of patients with JIA, more than half had oligoarticular disease, with a long time since diagnosis, and 40% had positive ANA titers. At the protocol date, most patients were in remission or with low inflammatory activity according to the DAS28 and had a JADAS27 score of around 3.9. As for treatment, 10 patients (19.2%) were not taking DMARDs at the protocol date and 42 (80.8%) were taking a DMARD, as follows: monotherapy with a synthetic DMARD, 16 patients (30.8%); monotherapy with a biological DMARD, 19 patients (36.5%); and combination therapy, 7 patients (13.5%).

3.2 | Cognitive function in patients and controls

Table 2 shows the differences in cognitive function between patients and controls. Scaled scores lower than 8 were considered below average.[27] The direct scores in each subtest were also evaluated. There were significant differences in the scaled scores for the Block Design test. The controls had a higher median scaled score than the patients (8.0 [5.0-10.0] vs 5.0 [4.0-8.0]; $P = .014$). As for

TABLE 1 Baseline characteristics of patients with JIA and healthy controls

Variable	JIA N = 52	Healthy controls N = 52	*P* value
Epidemiological characteristics			
Age in y, mean (±SD)	22.8 (5.1)	23.0 (5.7)	.901
Female, n (%)	35 (67.3)	35 (67.3)	1.000
Caucasian race, n (%)	50 (96.2)	52 (100)	.153
Smoking status			
Nonsmoker, n (%)	46 (88.5)	43 (82.7)	.402
Smoker, n (%)	6 (11.5)	9 (17.3)	
Educational level			
Basic education, n (%)	12 (23.1)	12 (23.1)	.986
Non-university higher education, n (%)	29 (55.8)	30 (57.7)	
University education, n (%)	11 (21.2)	10 (19.2)	
Comorbidities			
AHT, n (%)	1 (1.9)	0 (0.0)	.315
DM, n (%)	1 (1.9)	0 (0.0)	.315
Dyslipidemia, n (%)	2 (3.8)	0 (0.0)	.153
BMI, mean (SD)	22.3 (3.4)	22.5 (3.2)	.769
Normal, 18.5-24.9, n (%)	45 (86.5)	43 (82.7)	.585
Overweight, ≥25-≤30, n (%)	5 (9.6)	8 (15.4)	
Obesity, >30, n (%)	2 (3.8)	1 (1.9)	
Clinical-laboratory characteristics			
Time since diagnosis, mo, median (IQR)	134.1 (95.6-214.2)	-	-
Diagnostic delay, mo, median (IQR)	3.0 (2.3-3.0)	-	-
Type of JIA			
Systemic-onset, n (%)	3 (5.8)	-	-
Oligoarticular, n (%)	30 (57.7)	-	-
Polyarticular RF+, n (%)	1 (1.9)	-	-
Polyarticular RF−, n (%)	8 (15.4)	-	-
Psoriatic, n (%)	3 (5.8)	-	-
Enthesitis-related, n (%)	7 (13.5)	-	-
Nonspecific, n (%)	0 (0.0)		
RF > 10 IU/mL, n (%)	2 (3.8)	-	-
Anticitrullinated peptide antibody > 20 U/mL, n (%)	2 (3.8)	-	-
HLA-B27, n (%)	9 (17.3)	-	-
ANA, n (%)	21 (40.4)	-	-
Uveitis, n (%)	10 (19.2)	-	-
JADAS27, n (%)	3.9 (2.9-10.0)		
DAS28 at protocol, mean (±SD)	1.5 (0.9-2.3)	-	-
Remission-low activity, n (%)			
Moderate-high activity, n (%)			
HAQ, median (IQR)	0.0 (0.0-0.15)		
Treatment			
Synthetic DMARDs, n (%)	24 (46.2)	-	-
Biologic DMARDs, n (%)	27 (51.9)	-	-
Corticosteroids, n (%)	8 (15.3)		

Abbreviations: AHT, arterial hypertension; ANA, antinuclear antibody; BMI, body mass index; DAS28, Disease Activity Score of 28 joints – erythrocyte sedimentation rate; DM, diabetes mellitus; DMARD, disease-modifying antirheumatic drug; HAQ: Health Assessment Questionnaire; HLA, human leukocyte antigen; IQR, interquartile range; JADAS, Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis.

direct scores, differences were also recorded for the median Block Design test score, which was higher for the controls (37.0 [27.0-46.0] vs 28.0 [24.0-38.0]; $P = .008$). Differences were also recorded in the median direct score on the Letter-Number Sequencing test, which was higher in the controls (9.0 [8.0-10.0] vs 8.0 [6.2-9.7]; $P = .014$).

3.3 | Visuospatial organization in patients with JIA

Table 3 shows the characteristics of patients with JIA in terms of visuospatial organization measured according to the Block Design test. The scaled score for visuospatial organization was below average (<8) in the Block Design test in 35 patients. The mean age of patients in the below average range in visuospatial organization was lower than that of patients with a normal function (mean [SD], 19.5 [±4.5] vs 23.6 [±8.1] years; $P = .025$). Significant differences were found in educational level ($P = .018$). More patients had an average scaled score than below average scaled score, owing to their higher educational level, as follows: basic education (1 [5.9%] vs 11 [31.4%]), non-university higher-level education (9 [52.9%] vs 20 [57.1%]), and university education (4 [11.4%] vs 7 [41.2%]). Time since diagnosis of JIA was longer in patients with a below average scaled score than in those with an average scaled score (140.5 [85.4-216.0] vs 98.2 [49.7-147.0] months; $P = .037$). ANA were detected in more patients with below average scaled scores than in those with an average scaled score (17 [48.6%] vs 4 [23.9%]; $P = .044$). The median JADAS27 was higher in patients with a below average scaled score than in those with an average score (7.0 [2.9-15.9] vs 2.9 [2.4-4.2]; $P = .006$). No significant differences were found for the remaining epidemiological characteristics, clinical-laboratory characteristics, comorbidities, or treatments.

3.4 | Multivariate analysis

Table 4 shows the results of the multivariate model. Obtaining a below average scaled score in visuospatial organization in patients with JIA was associated with time since diagnosis in months and more pronounced inflammatory activity as measured by the JADAS27. Similarly, patients educated to university level were less likely to have poorer visuospatial organization as per the scaled score in the Block Design test.

4 | DISCUSSION

We performed a cross-sectional evaluation of cognitive function in patients with JIA and compared the results with those of a healthy control group matched for gender, age, and educational level. Our objective was to evaluate possible associations between cognitive function and clinical-laboratory and therapy-related factors in patients with a long history of JIA followed in a transition clinic. The cognitive domains selected for the evaluation of cognitive function were attention, working memory, problem solving, vocabulary, and visuospatial function. For that purpose, we used the subscales of the WAIS-III according to previous studies performed in patients with RA,[5] given that no studies to date have evaluated cognitive function exclusively in adults with JIA.

Our results show involvement of visuospatial organization in patients with JIA in the form of a larger percentage of subjects with below average scaled scores than the controls (67.3% vs. 40.4%). Feldmann et al[12] evaluated patients with JIA aged 6-24 years and found no differences in cognitive function compared to controls. One of the tests used by the authors was the Zahlen-Verbindungs test, which assesses visuomotor follow-up skills and can evaluate motor and sequencing speed (information processing).[28] However, the abovementioned studies do not specifically evaluate visuospatial organization using approaches such as the Block Design test. Various publications on patients with RA have specifically evaluated visuospatial organization. Many used the Block Design test[16,29,30] and reported contradictory results. In the uncontrolled study by Bartolini et al,[29] the percentage of patients in whom visuospatial organization was affected (71%) was similar to the percentage we reported. The authors attributed this association to a disconnection between the frontal-parietal lobes and the subcortical white matter resulting from microangiopathy after observing hypoperfusion in the cerebral single-photon emission computed tomography tests and the presence of white matter images indicating microangiopathy in magnetic resonance images. It is worth mentioning that they excluded patients with poor hand function due to arthritis, and this may have biased the results of the Block Design test. In order to avoid this possible bias in our study, patients with hand limitations at the time of the evaluation were excluded from the study. Furthermore, the mean HAQ of the patients included in our study was low, and there was no correlation between HAQ and scores on the cube test. Nevertheless, although we excluded patients with overt hand involvement, a discrete impact on motor performance due to a subtle motor impairment cannot be totally ruled out. Future studies should evaluate visuospatial function using tests that require the least possible manual dexterity or excluding patients with low scores in specific tests for hand dexterity and fine motor skills.

We found no differences between patients and controls for the remaining cognitive domains in the scaled scores evaluated in our study. In a systematic review on cognitive function in patients with RA published in 2018 by Meade et al,[5] the authors did in fact record poorer performance in all of these functions in patients with RA compared with healthy controls. The differences with our results could be explained by the fact that some of the mechanisms postulated as possible causes of cognitive impairment in RA, such as immune senescence[31] or accelerated atherosclerosis,[7] had not yet developed in the patients we assessed, who had JIA and a younger mean age. While there was no difference in the scaled scores in these tests, patients with JIA did have a lower direct score in the Letter-Number Sequencing test (working memory) than the healthy controls. One possible explanation for the fact that differences were

TABLE 2 Cognitive function in patients with JIA and healthy controls

Variable	JIA N = 52	Healthy controls N = 5 2	*P* value
Verbal function			
Vocabulary, direct score, median (IQR)	20.5 (13.0-27.0)	18.5 (10.0-25.0)	.551
Vocabulary, scaled score, median (IQR)	3.0 (3.0-5.0)	3.0 (3.0-5.0)	.662
Below average scaled score (<8), n (%)	32 (61.5)	30 (57.6)	.750
Problem solving function			
Similarity, direct score, median (IQR)	15.0 (12.0-18.0)	16.0 (13.0-18.0)	.200
Similarity, scale score, median (IQR)	7.0 (6.0-9.0)	8.0 (7.0-9.0)	.297
Below average scaled score (<8), n (%)	20 (38.5)	12 (23.1)	.120
Attention function			
Digit span, direct score, median (IQR)	14.0 (12.0-16.0)	14.0 (12.0-16.0)	.681
Digit span, scale score, median (IQR)	8.0 (6.0-10.0)	8.0 (6.0-10.0)	.783
Below average scaled score (<8), n (%)	18 (34.6)	19 (36.5)	.277
Working memory function			
Letter-Number Sequencing function, direct score, median (IQR)	8.0 (6.2-9.7)	9.0 (8.0-10.0)	.014*
Letter-Number Sequencing function, scaled score, median (IQR)	4.0 (6.5-9.7)	7.5 (6.0-10.0)	.154
Below average scaled score (<8), n (%)	26 (50.0)	17 (32.7)	.088
Visuospatial organization			
Block Design, direct score, median (IQR)	28.0 (24.0-38.)	37.0 (27.0-46.0)	.008**
Block Design, scaled score, median (IQR)	5.0 (4.0-8.0)	8.0 (5.0-10.0)	.014*
Below average scaled score (<8), n (%)	35 (67.3)	21 (40.4)	.006**
BDI number, mean (SD)	5.0 (2.0-8.0)	7.0 (3.0-12.0)	.150
Minimal depression (0-13)	44 (84.6)	40 (76.9)	.677
Mild depression (14-19)	4 (7.7)	4 (7.7)	
Moderate depression (20-28)	3 (5.8)	6 (11.5)	
Severe depression (≥29)	1 (1.9)	2 (3.8)	

Abbreviations: BDI, Beck Depression Inventory; IQR, interquartile range; JIA, juvenile idiopathic arthritis.

*$P < .05$

**$P < .01$

observed in working memory but not in attention (digit span test), when both are executive functions, is that the other functions, for example, visuospatial organization, also intervene in the Letter-Number Sequencing test.[32]

Our multivariate analysis revealed that the risk factors for poorer visuospatial organization in JIA patients were time since diagnosis and activity measured according to the JADAS27. This association has not been evaluated to date in JIA, although in patients with RA, Bartolini et al,[29] Katchamart et al[7] and Lee et al[33] found an association between impairment of visuospatial organization and greater disease activity and severity. The authors considered that this association was due to chronic inflammation over time, the effect of specific treatments such as corticosteroids, and accelerated atherosclerosis secondary to inflammation and to the presence of antibodies. Increased levels of anti-TNF alpha, IL-2, and IL-6 have a negative effect on cognitive functions in RA.[34] Increased levels of these inflammatory cytokines have also been reported in JIA.[35] Measurement of inflammatory activity in JIA is

TABLE 3 Characteristics of patients with JIA according to visuospatial organization

Variable	Inferior visuospatial organization N = 35	Noninferior visuospatial organization N = 17	*P* value
Epidemiological characteristics			
Age in y, mean (±SD)	19.5 (4.5)	23.6 (8.1)	.025*
Female, n (%)	25 (71.4)	10 (58.8)	.363
Male, n (%)	10 (28.6)	7 (41.2)	.363
Race, Caucasian, n (%)	34 (97.1)	16 (94.1)	.595
Smoking			
Nonsmoker, n (%)	30 (85.7)	16 (94.1)	.374
Smoker, n (%)	5 (14.3)	1 (5.9)	.374
Educational level			
Basic education, n (%)	11 (31.4)	1 (5.9)	.018*
Non-university higher education, n (%)	20 (57.1)	9 (52.9)	
University education, n (%)	4 (11.4)	7 (41.2)	
Comorbidities			
AHT, n (%)	0 (0.0)	1 (5.9)	.147
DM, n (%)	0 (0.0)	1 (5.9)	.147
Dyslipidemia, n (%)	1 (2.9)	1 (5.9)	.595
BMI, mean (±SD)	22.3 (3.1)	22.2 (4.2)	.969
Normal, 18.5-24.9, n (%)	31 (88.6)	14 (82.4)	.801
Overweight, ≥25-≤30, n (%)	3 (8.6)	2 (11.8)	.801
Obesity, >30, n (%)	1 (2.9)	1 (5.9)	.801
Clinical-laboratory characteristics			
Time since diagnosis of JIA, mo, median (IQR)	140.5 (85.4-216.0)	98.2 (49.7-147.0)	.037*
Diagnostic delay, mo, median (IQR)	3.0 (2.0-3.0)	3.0 (2.8-3.7)	.943
Type of JIA			
Systemic-onset, n (%)	2 (5.7)	1 (5.9)	.963
Oligoarticular, n (%)	21 (60.0)	9 (52.9)	
Polyarticular RF+, n (%)	1 (2.9)	0 (0.0)	
Polyarticular RF−, n (%)	5 (14.3)	3 (17.6)	
Psoriatic, n (%)	2 (5.7)	1 (5.9)	
Enthesitis-related, n (%)	4 (11.3)	3 (17.6)	
Nonspecific, n (%)	0 (0.0)	0 (0.0)	
RF > 10 U/mL, n (%)	1 (2.9)	1 (5.9)	.595
Anticitrullinated peptide antibodies > 20 U/mL, n (%)	1 (4.3)	1 (6.7)	.754
HLA-B27, n (%)	5 (14.3)	4 (23.9)	.873
ANA, n (%)	17 (48.6)	4 (23.9)	.044*
Uveitis, n (%)	7 (20.0)	2 (11.8)	.462
JADAS27, median (IQR)	7.0 (2.9-15.9)	2.9 (2.4-4.2)	.006**
DAS28 at protocol date, median (IQR)	1.7 (1.0-2.4)	1.3 (0.8-1.7)	.213
Remission-low activity, n (%)	24 (85.7)	14 (87.5)	.868
Moderate-high activity, n (%)	4 (14.3)	2 (12.5)	.868
HAQ, median (IQR)	0.0 (0.0-0.4)	0.0 (0.0-0.2)	.901
Treatment			
Synthetic DMARD, n (%)	17 (48.6)	7 (41.2)	.616
Biologic DMARD, n (%)	20 (57.1)	7 (41.2)	.280
Corticosteroids, n (%)	7 (20.0)	3 (17.6)	.840

Note: Abbreviations: AHT, arterial hypertension; ANA, antinuclear antibody; BMI, body mass index; DAS28, Disease Activity Score of 28 joints – erythrocyte sedimentation rate; DM, diabetes mellitus; DMARD, disease-modifying antirheumatic drug; HAQ, Health Assessment Questionnaire; HLA, human leukocyte antigen; IQR, interquartile range; JADAS, Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis.

*$P < .05$

**$P < .01$

TABLE 4 Multivariate analysis

Predictor	Odds ratio	95% CI	*P* value
University education	0.282	0.084-0.948	.041*
Time since diagnosis	1.035	1.007-1.064	.014*
JADAS27	1.941	1.003-3.757	.049*

Note: Nagelkerke $R^2 = 0.505$.

Variables included: gender, age, educational level, time since diagnosis in months, JADAS27 score, and Beck Depression Inventory score.

Abbreviation: JADAS, Juvenile Arthritis Disease Activity Score.

*$P < .05$.

more accurate with JADAS27,[24] and this is probably why we did in fact identify an association based on this instrument but not on the DAS28.

We also observed an independent association between educational level and visuospatial function in patients with JIA. Visuospatial organization was less affected in patients with a higher educational level, as reported in other studies on RA. Shin et al[17] and Katchamart et al[7] found that patients with RA and poor cognitive function were more likely to have a lower educational level. In the study by Katchamart et al[7] in particular, the cognitive function most affected was visuospatial organization.

4.1 | Limitations

Our study is subject to a series of limitations. First, given the lack of similar studies on JIA, we had to make all comparisons based on other chronic inflammatory joint diseases such as RA. However, this is the first study to evaluate cognitive function solely in adult JIA patients and to identify an association between impairment of visuospatial function and inflammatory activity and educational level. Subtle motor impairment in the hands of patients with JIA might interfere in the results of the Block Design test, even when patients with overt current or previous hand involvement are excluded. Our study is also limited by the fact that we did not apply functional and/or structural imaging tests or biological markers of neuronal damage, although our results could prove useful in future studies that do evaluate these factors.

5 | CONCLUSION

Cognitive function in adult JIA patients was poorer than in healthy controls matched for gender, age, and educational level at the expense of visuospatial function evaluated using the Block Design test. Visuospatial function performance was inversely associated with greater disease duration and inflammatory activity measured using the JADAS27. Similarly, visuospatial involvement was less pronounced in patients with a higher educational level. These results point to the importance of achieving remission of disease activity, since this may have an effect at levels other than the joints and may serve as the basis for future studies on biological markers and tests of brain function to explain the origin of these findings.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this study.

AUTHOR CONTRIBUTIONS

NM made substantial contributions to the conception and design of the work and participated in the acquisition, analysis, and interpretation of data. NM also drafted the manuscript. PC made substantial contributions to the conception and design of the work and participated in the acquisition, analysis, and interpretation of data. PC also drafted the manuscript. FO made substantial contributions to the acquisition of data and drafted the manuscript. LM made substantial contributions to the conception and design of the work and participated in the analysis and interpretation of data. GD made substantial contributions to the acquisition of data and revised the work. SM made substantial contributions to the acquisition of data and revised the work. AF made substantial contributions to the acquisition of data and revised the work.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Research Ethics Committee of Hospital Regional Universitario de Málaga (HRUM), Malaga, Spain (Project identification code 0925-N-19). All of the subjects gave their written informed consent before participating.

ORCID

Natalia Mena-Vázquez https://orcid.org/0000-0001-6173-2051
Pablo Cabezudo-García https://orcid.org/0000-0002-6421-5348
Gisela Díaz-Cordovés Rego https://orcid.org/0000-0002-0880-5319
Sara Manrique-Arija https://orcid.org/0000-0003-2284-7846
Antonio Fernández-Nebro https://orcid.org/0000-0002-2962-9844

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Vaspin deficiency failed to promote the proliferation of BMSCs in osteoarthritis

Junfeng Wang[1,2] | **Zhongqiang Chen**[1,2] | **Zhenpeng Guan**[3]

[1]Department of Orthopedics, Peking University Third Hospital, Beijing, China

[2]Department of Orthopedics, Peking University International Hospital, Beijing, China

[3]Department of Orthopedics, Peking University Shougang Hospital, Beijing, China

Correspondence
Zhongqiang Chen, Department of Orthopedics, Peking University Third Hospital, Beijing 100191, China.
Email: pkuih2020@126.com

Zhenpeng Guan, Department of Orthopedics, Peking University Shougang Hospital, Beijing 100144, China.
Email: guanzhenpeng@qq.com

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Abstract

Objective: The aim of this study was to estimate the possible role of vaspin in the proliferation of bone mesenchymal stem cells (BMSCs) and its molecular mechanisms in the bone marrow microenvironment of osteoarthritis (OA).

Methods: This study included 15 non-obese elderly patients with severe knee OA and 15 non-obese controls with femoral neck fracture. Patients all underwent hip or knee arthroplasty surgery to restore joint shape and function. Bone marrow samples were taken during surgery to estimate vaspin and transforming growth factor (TGF)-β1 levels by enzyme-linked immunosorbent assay and to observe the effect of vaspin on BMSCs proliferation by Cell Counting Kit-8. Real-time polymerase chain reaction and western blot were performed to evaluate the effect of vaspin on the genes and proteins of Akt involved in the PI3K/AKT signaling pathway.

Results: Bone marrow vaspin levels were significantly lower in OA patients compared to controls ($P = .03$). Furthermore, we found a significant correlation between vaspin and TGF-β1 concentrations in bone marrow ($r = .60$, $P < .01$). In addition, the in vitro studies indicated the proliferation of BMSCs was significantly promoted when the vaspin treatment concentration was 150 ng/mL ($P < .01$). Meanwhile, we found that the Akt messenger RNA and pAkt protein levels in BMSCs were increased after vaspin treatment ($P < .05$).

Conclusion: The findings of this study suggest there was abnormal expression of vaspin in OA bone marrow microenvironment, and vaspin likely had a mediator role in the proliferation of BMSCs, which may work by promoting the activation of the PI3K/AKT signaling pathway.

KEYWORDS
Akt, BMSCs, osteoarthritis, vaspin

1 | INTRODUCTION

Osteoarthritis (OA) is the most common joint disorder, affecting an estimated 10% of men and 18% of women over 60 years of age.[1] OA is now known to be a very complex disease affecting the entire joint, involving articular cartilage damage, changes in the subchondral bone, and systemic and surrounding soft tissues inflammation.[2] Recent progress has significantly increased our understanding of both the risk factors involved in the initiation of OA and the mechanisms responsible for its progression. However, little attention has been paid to the role of related molecules and cells belonging to the bone marrow microenvironment in OA, in particular the interactions

between bone mesenchymal stem cells (BMSCs) and cytokines. BMSCs display a multilineage potential and can be differentiated toward the osteogenic, chondrogenic and adipogenic lineage, which makes them actively participate in cartilage and bone homeostasis.[3] BMSCs are also a potent modulator of immune responses, having anti-inflammatory capacity, which contribute to the regeneration of tissues in inflammatory and degenerative joint diseases.[4-7] Their proliferation and differentiation potential, and immune modulating function are largely dependent on bone marrow microenvironmental factors including cytokines and adipokines, in particular, specific growth factors such as transforming growth factor (TGF)-β.[8,9] So, studying bone marrow microenvironmental changes of BMSCs may be more helpful in preventing the continuous degeneration of cartilage and subchondral bone in OA.

Recent progress suggests that inflammatory mediators play a critical role in the initiation and progression of OA.[10-12] As an important component of inflammatory mediators, adipokines have also demonstrated roles in modulating pro/anti-inflammatory and anabolic/catabolic joint balance, with implications in cartilage and bone homeostasis.[13] As we all know, the proportion of adipose tissue in the bone marrow cavity gradually increases with age, and adipokines secreted from which may be involved in the regulation of the BMSC inflammatory microenvironment in OA.

Vaspin, a member of the serine protease inhibitor family, was initially identified in visceral adipose tissue, and is a beneficial adipokine counteracting insulin resistance and inflammatory processes.[14] Recent progress suggests that vaspin is also expressed in other adipose tissues and connective tissues, to exert potent anti-inflammatory effects in various endothelial, osteoblast and skin cells.[15] Meanwhile, some studies have also found that vaspin could affect the inflammation and metabolism of osteoblasts, osteoclasts and chondrocytes through a variety of signaling pathways, and participate in the internal balance of bone and cartilage.[16-18]

Considering the role of vaspin in inflammatory and metabolism regulation, whether it is expressed in the bone marrow microenvironment, as well as its effect on proliferation and differentiation of BMSCs in OA, has not yet been reported. Therefore, this study was conducted to investigate the expression of vaspin in bone marrow of OA patients, and its possible role and molecular mechanisms of vaspin on the proliferation of BMSCs.

2 | MATERIALS AND METHODS

2.1 | Subjects

Ethics approval for this study was granted by the ethics committee of Peking University International Hospital. Written informed consent was obtained from all individuals prior to their participation in this study. A total of 30 (15 severe OA patients and 15 femoral neck fracture patients as controls) non-obese individuals were enrolled to the study. Demographic data of the patients are shown in Table 1. There were no differences in term of age, gender or body mass index (BMI). According to the patient's condition, all OA and femoral neck fracture patients required hip or knee arthroplasty surgery. None of the 15 controls showed clinical or lab measurement features of OA based on the American College of Rheumatology criteria.[19]

TABLE 1 Comparison of demographic and laboratory parameters of patients and controls

Parameter	Osteoarthritis (N = 15)	Control (N = 15)	*P* value
Age, y	69.2 ± 5.7	70.2 ± 7.0	NS
Gender, f/m	13/2	13/2	NS
BMI, kg/m^2	24.7 ± 2.0	23.5 ± 3.0	NS
Vaspin, ng/mL	0.35 ± 0.29	0.68 ± 0.50	.03
TGF-β1, ng/mL	28.00 ± 10.78	36.45 ± 11.04	.04

Note: Data are given as means ± SD.

Abbreviations: BMI, body mass index; NS, not significant; TGF, transforming growth factor.

2.2 | Sample collection

We collected bone marrow fluid from patients with knee OA who needed knee replacement surgery and patients with femoral neck fractures who needed hip replacement surgery. The bone marrow fluid collection method in knee replacement surgery was performed using a 5 mL sterile pipette to aspirate through the positioning hole in the femoral condyle during intramedullary positioning. The method of collecting bone marrow fluid during hip replacement surgery was using bone piercing needles to aspirate bone marrow fluid from the inner side of the greater trochanter before reaming the proximal femur. After collection, they were centrifuged to separate supernatant from BM debris, then the supernatants were stored at −80°C until the day of measurement. BM debris were resuspended in 10 mL phosphate buffered saline before using a 40 μm strainer for filtration. After being filtered, the filtrates were centrifuged to obtain the precipitate, then which were plated and incubated in a humidified atmosphere of 5% CO_2 at 37°C, according to the method described in previous literature.[20] They were passaged using 0.05% trypsin-0.02% ethylenediaminetetraacetic acid (Gibco, USA) when adherent colonies were detached at 80%-90% confluency, and the 3rd passage cells were used in this study.

2.3 | Enzyme-linked immunosorbent assay

BM vaspin (USCN Science) and TGF-β1 (R&D Systems) levels were measured by using the commercially available enzyme-linked immunosorbent assay kits. Each assay was performed in duplicate according to the manufacturer's instructions. The assays had a minimum detectable dose of 0.056 ng/mL for vaspin and of 1.7-15.4 pg/mL for TGF-β1. None of the measured values of vaspin and TGF-β1 in any of the subjects were below the limits of detection for this assay.

The intra-assay coefficients of variation for vaspin and TGF-β1 were 5.8% and 3.5%, while the inter-assay coefficients were 6.7% and 8.3%.

2.4 | Cell proliferation by cell counting kit-8 assay

To assess the effect of vaspin on the proliferation of BMSCs, the 3rd passage BMSCs were incubated into 96-well plates at a density of 1×10^4/well, with different concentrations of vaspin in growth culture medium (0, 150 and 300 ng/mL). Twenty-four hours later, the proliferation of BMSCs was measured by Cell Counting Kit-8 assay (Dojindo). The optical density (OD) value (absorbance) of each sample was measured at 450 nm with a microplate reader.

2.5 | Real-time polymerase chain reaction (PCR)

The 3rd passage BMSCs were incubated into 6-well plates at a density of 3×10^5/well, with different concentrations of vaspin in growth culture medium (0, 150 and 300 ng/mL) for 24 hours at 37˚C. The cells were harvested for real-time PCR and western blot analyses. Total RNA was extracted from BMSCs using a total RNA extraction kit (Thermo) and all procedures complied with the manufacturer's instructions. Complementary DNA of each RNA sample was reverse transcribed (RT) with a RT reagent kit (Promega) according to the manufacturer's instruction. Real-time PCR was performed using SYBR Green Realtime PCR Master Mix (Toyobo). The reaction conditions were: 95˚C for 5 minutes, 40 cycles of 95˚C for 15 seconds and 60˚C for 60 seconds, and then 95˚C for 15 seconds. Primer sequences were as follows: Akt, forward 5′-ACGCTACTTCCTCCTCAA-3′ and reverse 5′-CTGACATTGTGCCACTGA-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward 5′-GGCAAGTTCAACGGCACAG-3′ and reverse 5′-CGCCAGTAGACTCCACGACA-3′. GAPDH was used as an internal control, and each reaction was performed in triplicate. The relative PCR data were analyzed using the $2^{-\Delta\Delta Ct}$ method.[21]

2.6 | Western blot analysis

To extract cytoplasmic and nuclear protein, cells were lysed on ice for 10 minutes in RIPA lysis buffer (Cwbio, China), and each lysate was centrifuged, then the supernatant was collected. The protein concentrations were quantified by bicinchoninic acid protein assay kit (Applygen, China). Equal amounts of proteins were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to a polyvinylidene difluoride membrane and then probed with primary antibodies against Akt (Cell Signaling), phosphorylated (p) Akt (Cell Signaling, USA) and β-actin (Affinity) overnight at 4˚C, then washed with Tris-buffered saline – Tween 20 and incubated with horseradish peroxidase-coupled goat anti-mouse and goat anti-rabbit Abs (ZSGB-BIO) for additional 2 hours at room temperature. The blots were visualized by super chemiluminescence (ECL) western blot detection system (Applygen). The signal intensity was quantified using Image J software (NIH).

2.7 | Statistical analysis

Data analysis was performed using GraphPad Prism 5.01 software (San Diego, CA, USA) and SPSS (Version 16.0). All values are expressed as mean ± SD. Non-categorical variables were compared using independent-samples *t* test or separate variance estimation *t* test (when the variance is heterogeneity). The correlation between the concentration of vaspin and TGF-β1 in BM was determined by Pearson's correlation analysis. Chi-square test was used for comparison of the frequency of variables between different groups in gender analysis. A P value < .05 was considered significant.

3 | RESULTS

3.1 | Patient characteristics

The demographic data of 2 groups are displayed in Table 1. A total of 15 OA patients (Kellgren and Lawrence grade 3 or 4, mean age 69.2 ± 5.7 years) and 15 controls (femoral neck fracture, mean age 70.2 ± 7.0 years) were enrolled in the study. No significant difference with regard to age, gender, BMI was observed in OA patients compared to controls.

3.2 | BM vaspin and TGF-β1 expression

Table 1 also shows BM levels of vaspin and TGF-β1 between OA patients and controls. The level of BM vaspin was significantly decreased in OA patients compared with controls (0.35 ± 0.29 ± 25.84 ng/mL vs 0.68 ± 0.50 ng/mL, $P = .03$). Activation of inflammatory cells and release of inflammatory cytokines play an important role in the modulation of BM microenvironment. By examining immunomodulating cytokine TGF-β1 in OA patients and controls, we found BM concentrations of TGF-β1 were also significantly decreased in OA patients compared with controls (28.00 ± 10.78 ng/mL vs 36.45 ± 11.04 ng/mL, $P = .04$). We also analyzed the correlation between the BM concentrations of vaspin and TGF-β1; the result showed a significant positive correlation between BM concentrations of vaspin and TGF-β1 in all subjects ($r = .60$, $P < .01$; Figure 1).

3.3 | Effect of vaspin on the proliferation of BMSCs

The proliferation of BMSCs showed that a significant increase in relative cell number (value of OD) was observed at 150 ng/mL vaspin compared to control (0 ng/mL), and the difference was statistically significant ($P < .01$). However, we found that at the concentration of 300 ng/mL vaspin, the BMSCs number were only increased slightly

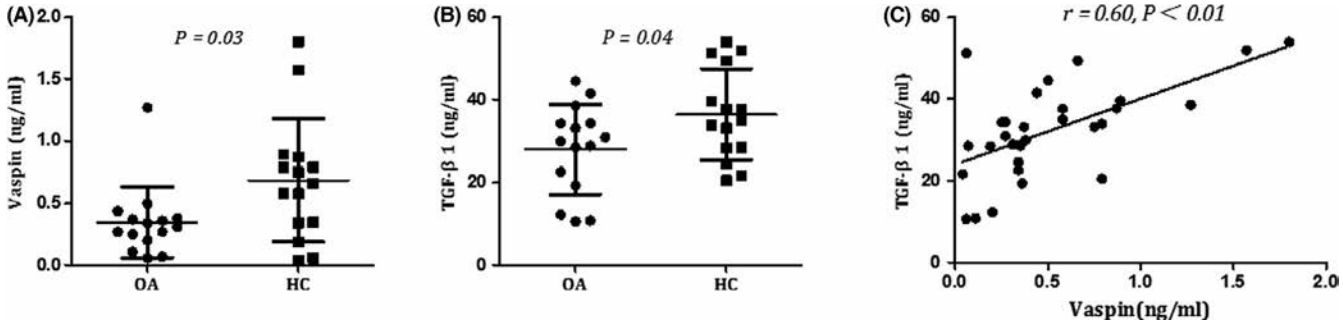

FIGURE 1 BM levels of (A) vaspin, (B) TGF-β1 in patients with OA and controls (HC), BM vaspin and TGF-β1 levels are decreased in OA patients than in controls ($P < .05$). C, Relationship between BM vaspin and TGF-β1 levels in all subjects, the result shows a significant positive correlation between BM concentrations of vaspin and TGF-β1. The error bars in this figure stands for standard deviations

compared to the control ($P = .06$). The rates of promoting BMSCs proliferation are illustrated in Figure 2. As the data indicated, only when the concentration of vaspin reaches a certain value, the proliferation of BMSCs could be promoted. However, this effect is not continuously enhanced as the concentration of vaspin increases. At least within the range we tested, the promotion effect on BMSCs proliferation was most significant when the concentration of vaspin was 150 ng/mL.

3.4 | Vaspin enhanced Akt and pAkt expression in the PI3K/AKT signaling pathway of BMSCs

As demonstrated in Figure 3A, the results of the real-time PCR analysis demonstrated that the messenger RNA (mRNA) level of Akt was markedly increased following stimulation with vaspin. This was significant at concentrations of 150 ng/mL ($P = .02$). Meanwhile, the serine phosphorylation levels of Akt protein in BMSCs plus vaspin group (150 ng/mL and 300 ng/mL) were also significantly increased when compared to the control group (Figure 3B,C). These demonstrated that vaspin could enhance the expression of Akt mRNA and pAkt protein.

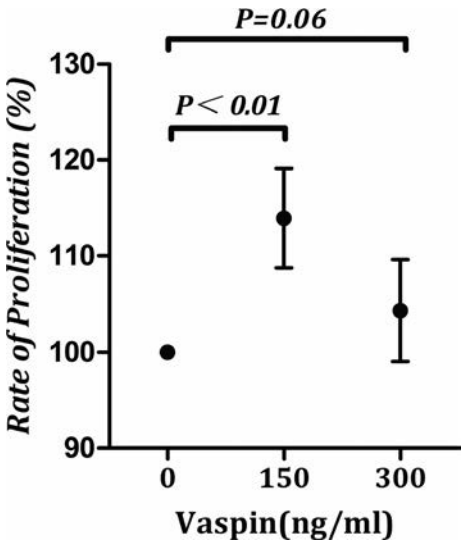

FIGURE 2 The promotion effect on BMSCs proliferation of vaspin with different concentrations. When the concentration of vaspin is 150 ng/mL, the relative cell number of BMSCs is obviously increased ($P < .01$)

4 | DISCUSSION

OA is the most common degenerative joint disease, and can affect the entire joint. The etiology of OA is very complex, including joint injury, obesity, aging and genetic factors.[22] Bone marrow adipogenesis, which is observed in aging and obesity, is associated with a set of local changes in bone marrow, which are explained by the adipocytes and adipokines.[23,24] Bone and joint tissues homeostasis are largely regulated and maintained by the bone marrow microenvironment in OA. So the changes in the bone marrow microenvironment caused by bone marrow adipogenesis and the release of adipokines, may be involved in the development and progression of OA. Adipokines have been extensively studied in the pathogenesis of OA. Among them, leptin, adiponectin, resistin and visfatin have been recognized as having the role of pro- and/or anti-inflammatory properties in OA.[25-27] Several studies have also found that vaspin could affect the inflammation and metabolism of osteoblasts, osteoclasts and chondrocytes through a variety of signaling pathways, and participate in the internal balance of bone and cartilage.[16-18] However, whether there might be abnormal expression of vaspin in the bone marrow microenvironment of OA, and plays a role in regulation of the proliferation of BMSCs, have not been reported.

In the present study, we demonstrated that BM vaspin and TGF-β1 concentration were lower in the OA patients compared with controls. Furthermore, a significant correlation between BM vaspin and TGF-β1 concentrations was observed in all subjects. This is the first study to evaluate bone marrow vaspin levels and its relationship with other cytokines in OA, and these findings indicate that vaspin could be a potential participant in bone marrow microenvironment of OA. The bone marrow microenvironment is a very active milieu, which involves multiple interactions between cells, hormones, growth factors, neural connections, and the vasculature.[28-30] In this study, as a beneficial adipokine counteracting inflammatory processes, the expression of vaspin is found to be down-regulated in OA bone marrow microenvironment, and has a positive correlation with TGF-β1,

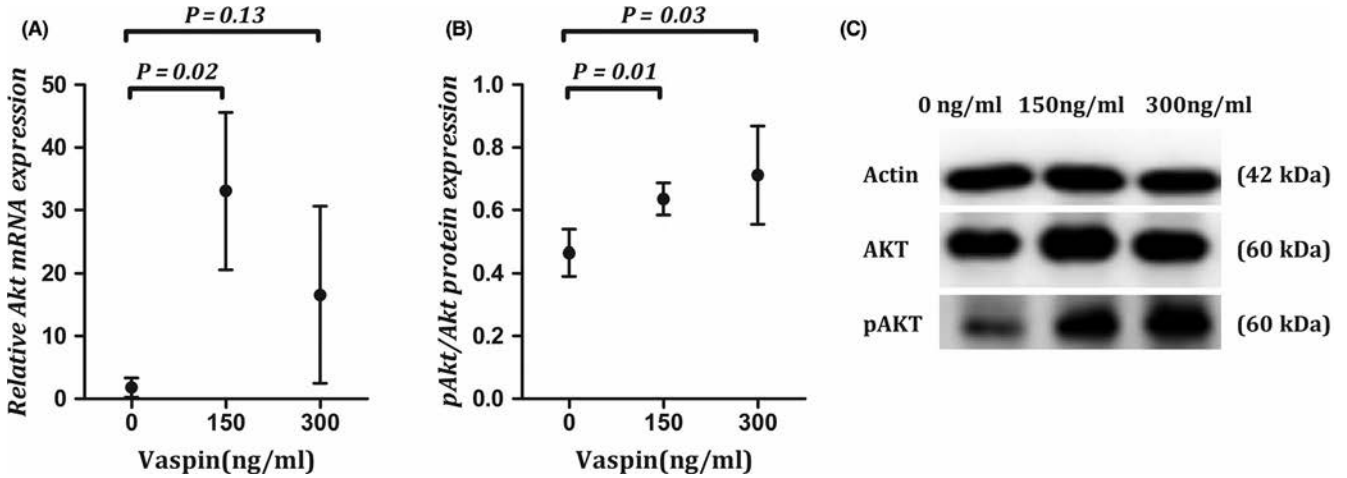

FIGURE 3 Effects of vaspin on Akt mRNA and pAkt protein levels in BMSCs. BMSCs were treated with 0 (control), 150, 300 ng/mL vaspin for 24 h. A, Effect of vaspin on Akt mRNA in BMSCs. The level of Akt mRNA was determined by Real-time PCR. B and C, Effects of vaspin on the protein levels of pAkt in BMSCs. Cell lysates were subjected to western blotting and incubated with antibodies against Akt and pAkt. The error bars in this figure stands for standard deviations

which suggest vaspin may be involved in interactions with other inflammatory cytokines and growth factors in bone marrow microenvironment, and plays a certain role in the progression of OA.

TGF-β is a multifunctioning cytokine, which not only has the function of immune regulation, but also has an anabolic effect on articular cartilage homeostasis by stimulating the production of extracellular matrix proteins and preventing terminal differentiation of chondrocytes.[31,32] TGF-β also can promote bone formation by augmenting progenitor recruitment, proliferation and differentiation into osteoblasts, helping to maintain the dynamic balance between bone resorption and bone formation.[33] So in this study, whether the correlation between vaspin and TGF-β1 in OA bone marrow implies that vaspin may has some effects on the proliferation and differentiation of BMSCs, is a problem deserving of studying. To explore this problem, we explored the effects of vaspin on the proliferation of BMSCs in OA bone marrow. Our results indicated that vaspin could promote the proliferation of BMSCs in vitro, which manifested a dose-dependent pattern. In combination with the previous results of this study, we believe that reduced expression of vaspin in OA bone marrow microenvironment may attenuate the proliferation ability of BMSCs by interacting with other cytokines such as TGF-β. However, whether vaspin affects the chondrogenic differentiation ability of BMSCs still needs to be verified in further study. The results of this experiment also suggest that enhancing vaspin activity may have therapeutic effects in OA by improving the proliferation capacity of BMSCs. However, whether this therapeutic effect requires the assistance of other relevant cytokines such as TGF-β, needs to be further verified.

Phosphoinositide-3 kinase (PI3K)/Akt signaling may regulate cell proliferation, growth, migration, death, adhesion, and tumorigenesis in various cell lineages.[34] Akt is a key protein located downstream of the PI3K/Akt signaling pathway. It can be activated by phosphorylation to be pAkt, and plays an important role in regulating cell proliferation and apoptosis through several mechanisms.[35] Vaspin can act on the PI3K/Akt signal pathway of vascular smooth muscle cells and endothelial cells, improve insulin resistance and protect free fatty acid mediated apoptosis of these cells.[36,37] This study investigated the effects of vaspin on the PI3K/Akt signaling pathway in BMSCs and whether vaspin can improve the proliferation ability of BMSCs through this pathway. The results showed that treatment with vaspin could increase levels of Akt and pAkt in BMSCs and improve the proliferation ability of BMSCs. This suggested that vaspin can improve the proliferation of BMSC cells by promoting the transduction of the PI3K/Akt signaling pathway.

5 | CONCLUSION

The findings of this study suggest there was abnormal expression of vaspin in OA bone marrow microenvironment, and vaspin likely had a mediator role in the proliferation of BMSCs, which may work by promoting the activation of the PI3K/AKT signaling pathway in BMSCs. Further studies about the function and action mechanism of vaspin on the multidirectional differentiation ability of BMSCs are necessary for further understanding the pathogenesis of OA.

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CONFLICTS OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Zhongqiang Chen and Zhenpeng Guan contributed equally to this work and should be considered co-corresponding authors. JW carried out all the experiments, acquisition of corresponding data and

their statistical analysis, prepared figures and drafted the manuscript. ZG and ZC designed the study, coordinated the research and revised the manuscript. Revising and final approval of the article: all authors.

ETHICS APPROVAL

All experimental procedures used in our study were approved by the ethics committee of Peking University International Hospital (2018-068[BMR]).

ORCID

Junfeng Wang https://orcid.org/0000-0003-2657-0936

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Is being barefoot, wearing shoes and physical activity associated with knee osteoarthritis pain flares? Data from a usually barefoot Sri Lankan cohort

Inoshi Atukorala[1] | Arunasalam Pathmeswaran[2] | Nishamani Batuwita[1] | Nimesha Rajapaksha[1] | Vishmi Ratnasiri[1] | Lalith Wijayaratne[3] | Monika De Silva[3] | Thashi Chang[1] | Yuqing Zhang[4] | David John Hunter[5,6]

[1]Department of Clinical Medicine, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka

[2]Department of Public Health, University of Kelaniya, Ragama, Sri Lanka

[3]Department of Rheumatology and Rehabilitation (General), National Hospital Sri Lanka, Colombo, Sri Lanka

[4]Division of Rheumatology, Allergy, and Immunology, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA

[5]Institute of Bone and Joint Research, Kolling Institute, University of Sydney, Sydney, Australia

[6]Rheumatology Department, Royal North Shore Hospital, Sydney, NSW, Australia

Correspondence
Inoshi Atukorala, Department of Clinical Medicine, Faculty of Medicine, University of Colombo, Sri Lanka.
Email: inoshi.atu@gmail.com

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Abstract

Aim: To identify the association between hours of being barefoot/wearing footwear, physical activity (PA) and knee osteoarthritis pain flares (KOAF).

Methods: Persons with a diagnosis of knee osteoarthritis, who reported previous KOAF, were followed up in a 3 months long telephone-based case-crossover study. Exposures to risk factors were assessed every 10 days and whenever the participants experienced a KOAF. Conditional logistic regression examined associations of KOAF with following: hours of being barefoot/using footwear and PA performed ($P < .05$).

Results: There were 260 persons recruited, of whom 183 continued longitudinal follow up. Of them, 120 persons had at least one valid KOAF and control period. Participants were female (90%) with mean (SD) age and body mass index of 59.9 (7.0) years, 28.0 (5.0) kg/m^2 respectively. Participants were barefoot for a mean duration of 12.7 hours (SD 4.6) and used footwear for 5.1 (SD 4.7) hours daily; 99% wore heel heights <2.5 cm. Duration of being barefoot, 1 and 2 days before, demonstrated reduced multivariate odds of KOAF (odds ratio [OR] = 0.85; 95% CI 0.80-0.90). Moderate PA performed 1, 2 days prior was associated with a significantly increased risk of KOAF (multivariate OR 4.29; 2.52-7.30 and OR 3.36; 2.01-5.61). Similarly, hours of using footwear 1 and 2 days before flare demonstrated increased odds of KOAF (OR 1.15; 1.07-1.23 and 1.10; 1.03-1.18).

Conclusions: Increased duration of being barefoot 1 to 2 days before is associated with reduced risk of KOAF. Performing moderate PA 1 to 2 days before was associated with an increased risk of KOAF.

KEYWORDS

knee osteoarthritis pain,

1 | INTRODUCTION

Knee osteoarthritis (KOA) is a leading cause of disability and pain in older persons with significant numbers reporting severe disabling knee pain.[1,2] This experience of pain is incapacitating with recurrent episodes of pain (flare-ups) of increasing intensity, frequency and duration occurring before the disease becomes more severe.[3-5] Pain in moderately severe KOA, including KOA pain flare-ups (KOAF), is believed to be in part mediated by aberrant/abnormal biomechanical forces and dynamic knee loading.[6-8]

As micro-trauma consequent to repeated low-level and aberrant load is pivotal in the pathogenesis of KOA, acute perturbations in joint mechanics due to physical activity (PA) or footwear could precipitate KOAF.[5,9] This fact is corroborated indirectly by evidence that patients report pain worsening with exercise and that neuromuscular exercise programs reduce acute pain due to exercise.[7,10] It is important to identify whether PA precipitates KOAF, as knee pain increasing immediately after exercise may negatively impact adherence to long-term exercise.[11,12]

Footwear use/walking barefoot is another exposure with the potential to affect knee loads and KOAF. Previous research has shown that footwear increases loads on knees, particularly if heel height is high.[13] In addition, poor flexibility of footwear increases the impact of knee load while flat flexible shoes improve knee load by promoting natural foot motion during walking.[14-16] Further, footwear significantly increases the knee adduction moment in persons with KOA while barefoot running causes reduced joint torques at the knee when compared to running with shoes.[14,17,18] Interestingly, it is established walking barefoot reduces the peak joint load at the knee and is possibly the most superior method of unloading the knee while walking.[19]

In most cultures, walking barefoot is not practical or culturally acceptable and there is a paucity of data on culturally barefoot groups and KOA to the best of our knowledge. However, in perennially warm, tropical Sri Lanka being barefoot is common and frequently the norm, particularly in poorer or more traditional communities. Therefore, observations of these persons can provide valuable insights on the effect of being barefoot on KOA.

It is not known whether KOAF is associated with the pattern of PA or footwear use (or being barefoot) in the time period preceding flare-ups. As KOAF are detrimental to patient well-being, antecedent risk factors must be identified. This is particularly true if core recommendations of exercise are to be followed. If not KOAF may discourage persons from ever engaging in PA, thereby negatively impacting their long-term disease management and outcome. In barefoot communities, including communities in Sri Lanka who are barefoot inside houses and when working-walking barefoot can be further promoted if they reduce knee pain episodes. Footwear mimicking barefoot walking could be promoted in non-barefoot cultures. Therefore, this study was designed to identify whether PA, using footwear or walking barefoot are associated with KOAF in a cohort of Sri Lankan patients.

2 | MATERIALS AND METHODS

2.1 | Study design and population

This study was conducted as a telephone-based case-crossover study using a pretested questionnaire in the participant's native language (Sinhala, English or Tamil via interpreter). A case-crossover study design, where each participant serves as his/her own control, was utilized. This design allows for self-matching preventing confounding by risk factors that are unique to a given individual but different between individuals. Additionally, this case-crossover study design has been proven useful in chronic conditions punctuated by intermittent transient exacerbations like pain flares, including KOAF.[20-22]

Using this study design, the participant's exposures to risks/risk factors during the KOAF (case periods) were compared with exposures during the period without pain (control periods). The study design, including the timing to exposures in relation to KOAF, is shown in Figure 1. As internet use/adherence is poor in middle-aged Sri Lankans and in poorer urban communities that are dominantly represented in this cohort, data collection was by mobile telephone/landline than by a web-based platform used in other studies.[23,24]

2.2 | Recruitment of participants and ascertainment of KOAF

Participants were recruited from the general rheumatology clinic at the National Hospital Sri Lanka. An interviewer-administered pretested questionnaire was used to recruit patients. Participants were considered eligible for the study if they: (a) were aged >45 years; (b) had knee pain on most days of the month which fluctuated in intensity episodically; (c) had index knee diagnosis of KOA by a rheumatologist according to American College of Rheumatology (ACR) criteria[25]; (d) with intact hearing for conversational voice; and (e) access to telephone (mobile/landline) with a good command of Sinhalese/English. Participants who spoke exclusively in Tamil were interviewed with interpreters. Participants with inflammatory joint disease, osteonecrosis with constant knee pain for all days of the month or with previous or plans for replacement of index knee were excluded. A rheumatologist (IA) examined the participant records and confirmed their diagnosis of KOA.

The participants were followed up at least at 10-day intervals for 3 months. They were contacted by phone every 10 days (control period assessment points) to ascertain exposure to risk factors using a standardized questionnaire. Case period questionnaire data for KOAF was collected as follows: patients were asked to report KOAF by telephone at the time of the flare or the data for exposures related to the KOAF was collected retrospectively within 2 days of a KOAF when the investigator called them as a reminder. At each encounter participant's pain level was assessed by a 0-10 numeric

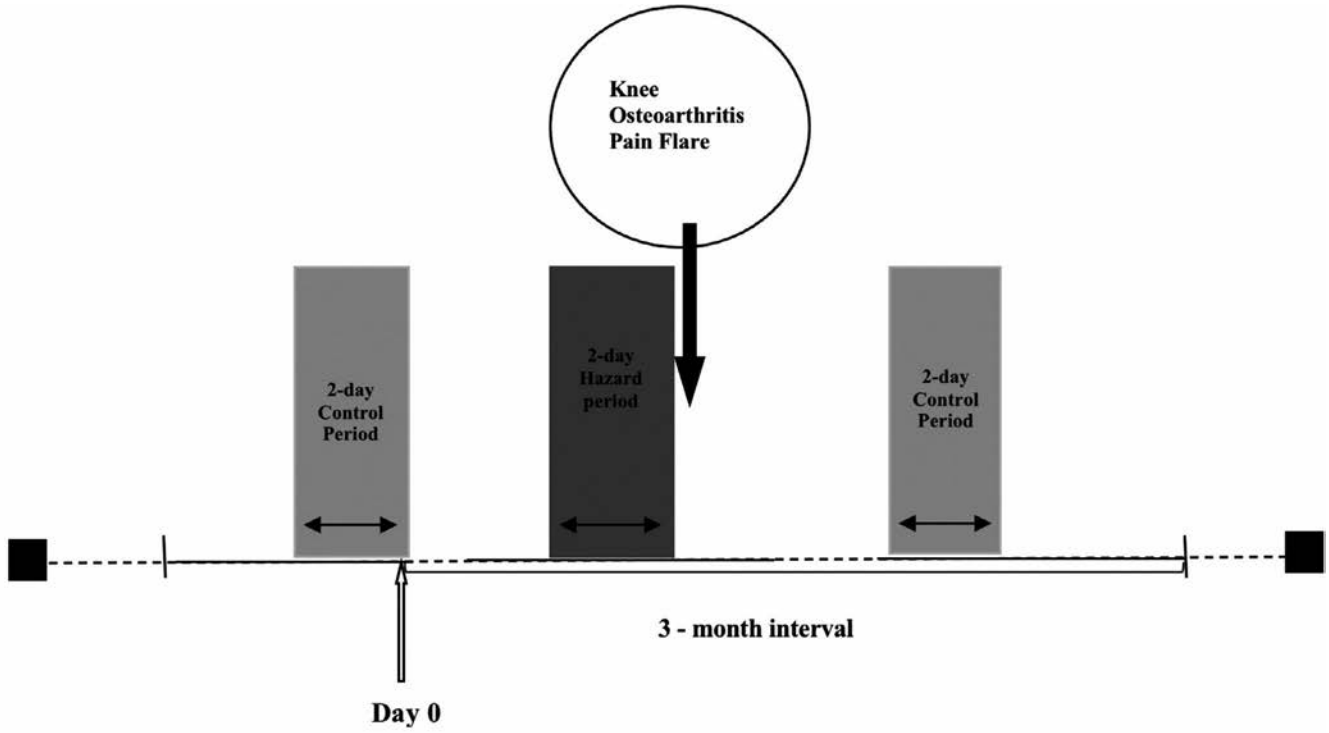

FIGURE 1 Case-Crossover Study Design Employed in this Study

rating scale (NRS) with 0 being the lowest and 10 the highest level of pain. Questionnaire details are given below

1. Demographic questionnaire

This assessed information on age, gender, height, weight, ethnicity, religious practices at baseline

2. Knee pain exacerbation questionnaire

Persons rated their pain levels by using an NRS at baseline and at control/case periods 3.

3. Control period questionnaires

The case period and control period questionnaires collected identical information though control period questionnaires were administered every 10 days

4. Case period questionnaires

A KOAF or case period was defined as an increase in NRS of ≥2 compared with the participant's rating on NRS from the usual background level of pain reported at baseline (maintained in participants' records and assessed by the investigator). This NRS-based definition of KOAF has been utilized in many previous studies of KOAF.[22] Only KOAF lasting at least 4 hours were included in the analysis, to eliminate transient pain increases in KOA.[26] Flares which did not settle within 2 control periods were omitted from the analysis.

The following risk factors (exposure measurement) were assessed in both case and control period questionnaires.

2.3 | Assessment of risk factors (predictor variables)

Exposure to the following risk factors - duration of being barefoot (or wearing footwear and shoe heel height) and PA - was assessed as below during case points (at time of KOAF) and during control points (every 10 days).

2.4 | Assessment of footwear and being barefoot

Duration of being barefoot (or wearing footwear) was assessed in hours. In addition, the hours of being barefoot (or wearing footwear) was dichotomized as <8 hours or >8 hours using the premise that people sleep at least 8 hours[27] and dividing the hours awake in half.

Self-reported shoe heel height was assessed in 3 categories <2.5 cm, 2.5-5.0 cm and >5.0 cm. Heel height of footwear was assessed at the baseline by the investigator who assessed the length between the heel cap of the footwear and the highest point of the heel. The person was informed on how to inform/assess their heel height if the footwear was changed. As the majority of participants were urban poor, most had one pair of footwear or used a similar style of footwear throughout the year. Footwear use/being barefoot was assessed 1 day and 2 days before KOAF.

2.5 | Assessment of PA

PA was assessed at 3 time points - 1 day, 2 and 3-7 days before KOAF. PA activities done were described by the patient to investigators and classified by investigators as mild, moderate or vigorous using the Centers for Disease Control (CDC) classification of PA.[28] For example, PA performed by the participant was self-reported (ie, light housework) and subsequently classified as only mild PA (reference category), any moderate or any vigorous PA according to the CDC-PA guidelines by investigators. This method of assessing PA has been previously utilized in studies.[28-30]

Ethics approval was obtained from the Faculty of Medicine, University of Colombo, Sri Lanka (EC-16-177).

2.6 | Statistical analysis description

Descriptive statistics were examined. As each person could have more than 1 case period or more than 1 control period, these periods were matched within participant using a conditional regression model. As we used the case-crossover design, persons with only case periods or only control periods during the 3 months were omitted from the case-crossover analysis. To ensure there was no overlap between case and control periods during analysis, control (or case periods) within 7 days of each assessment point for PA and control (or case periods) within 2 days of assessment point for heel height/duration of using footwear/being barefoot, were omitted from the analysis. The relationship of the predictor variables to the outcome variable was assessed by conditional logistic regression using STATA version 15 (StataCorp, College Station, YX, USA).

The outcome variable was the occurrence of KOAF (dichotomous variable Yes/No) and risk factors (exposures) were duration of wearing footwear (or being barefoot) and category of PA (reference group = mild PA). The relationship of the predictor variables to the outcome variable was assessed by conditional logistic regression using STATA version 15. Both univariate and multivariable conditional logistic regression models were used and the models were subsequently adjusted for age, and body mass index.

In the multivariable regression model, exposures 1, 2-days prior to KOAF were used Interaction between the duration of being barefoot/footwear use with PA was examined.

3 | RESULTS

Of 260 persons recruited for the study, 77 did not continue follow up. Only 120 persons had both valid control and case periods. Persons with only case periods (1 participant) or only control periods (61 participants) were not included for analysis given the case-crossover study design. Flares which did not settle within 2 control periods were excluded from the analysis. Descriptive statistics and pain scores reported at baseline in those with case-control periods, those lost to follow up and those with control only periods are given and compared in Table 1. The mean number of flares per person was 1.91 (SD 0.09).

Most (84.6%) of the participants were from urban areas near Colombo, the capital of Sri Lanka. Approximately 10.8% did not go to school, 29.6% had primary school education, 45.2% had some high school education, with 14.6% having completed high school/university education. Most (40.9%) had no permanent employment, 7.1% were retired and 25.3% were semi-skilled workers with 15.5% in service professions. The majority were Buddhists 63.5%, 11% Hindus, 13.3% practiced Islam and 13% were Christian/Catholic.

The participants were barefoot for a mean duration of 12.7 (SD 4.6, range 1-19) hours and wore footwear an average of 5.1 (SD 4.7) hours daily. The majority (99%) wore footwear with a heel height <2.5 cm.

The hours of being barefoot significantly reduced the risk of KOA pain flares (multivariate odds ratio [OR] = 0.85; 95% CI 0.80-0.90) per hour of being barefoot 1 day before or the 2 days before. Similarly, the duration of using footwear was associated with increased risk of KOAF (OR = 1.16; 95% CI 1.08-1.24) per hour for footwear worn 1 day before and 1.10 (95% CI 1.03-1.18) per hour of footwear worn 2 days before (Table 2).

Moderate PA performed 1 day, 2 days and 3-7 days prior was associated with an increased risk of KOAF (multivariate OR 4.29, 95% CI 2.52-7.30; OR 3.54, 95% CI 2.12-5.90; and OR 4.27, 95% CI 2.56-7.13) (Table 2). No association could be identified between heel heights and KOAF as only a minority wore heel heights >2.5 cm.

Similarly being barefoot for more than 8 hours (reference <8 hours) 1 and 2 days before flare was associated with a significantly reduced risk of KOAF, while wearing footwear for more than 8 hours was associated with a significantly increased risk of KOAF (Table 3). But, using footwear for >8 hours showed interaction with moderate PA performed ($P < .05$) at both time points. There was no significant interaction between being barefoot for >8 hours and moderate PA performed ($P < .05$).

4 | DISCUSSION

This study is the first to demonstrate that being barefoot is associated with a reduced risk of KOAF. It is also the first to demonstrate the independent effect of increased KOAF from using footwear for long periods of time and with moderate PA. These findings are unique, as this study is the only one, to the best of our knowledge, which contains a cohort of patients who are largely barefoot for most of the day. In addition, these findings are based on data collected from the first telephone-based longitudinal study on KOA carried out in persons from a predominantly poor socio-economic group in urban/suburban Sri Lanka, a challenging exercise and a first for this resource-poor setting.

This study demonstrates that KOAF are associated with any moderate PA in the week immediately before the flare. A similar trend toward increased risk of KOAF with vigorous exercise (during the period prior to flare) was detected although this association was

TABLE 1 Comparison of demographic characteristics between the 3 groups: Group 1 - Persons with both case and control periods, Group 2 - Persons lost to follow up, Group 3 - Persons with only control periods

Characteristics assessed at baseline	Persons with both case and control periods[a] (N = 120)	Persons lost to follow up (N = 77)	Persons with control periods only (N = 61)	
	Mean (SD)	Mean (SD)	Mean (SD)	*P**
Continuous variables				
Age, y	59.9 (7.3)	58.8 (7.4)	59.2 (6.4)	.534
Height, cm	151.2 (6.4)	148.2 (6.2)	150.4 (7.9)	**.009**
Weight, kg	64.1 (11.5)	64.3 (11.7)	65.2 (11.3)	.808
Body mass index, kg/m^2	28.0 (4.9)	29.2 (5.0)	28.7 (4.9)	.311
Age at diagnosis of knee osteoarthritis, y	54.0 (9.4)	54.3 (7.6)	52.9 (8.40)	.592
Level of background pain reported at baseline, 0-10 numeric rating scale	3.9 (1.5)	4.1 (1.5)	4.1 (1.6)	.724
Worst levels of pain reported at baseline	7.5 (1.7)	7.7 (1.5)	7.8 (1.6)	.574
Mildest levels of pain reported at baseline	2.0 (2.1)	2.1 (2.1)	1.8 (2.0)	.702
Episodes of knee osteoarthritis flares experienced in previous mo	4.0 (2.4)	4.3 (3.2)	4.0 (2.6)	**.028**
Mean duration of being barefoot per d, h	12.73 (4.60)	11.71 (5.27)	12.44 (5.40)	.444
Mean duration of wearing footwear per d, h	5.10 (4.68)	6.40 (5.28)	6.32 (5.67)	.180
Dichotomous variables (yes/no)	**n (%)**	**n (%)**	**n (%)**	
Female gender	55 (94.8)	73 (94.8)	55 (90.2)	.515
Shoes/Barefoot				
Usually barefoot, Yes	89 (74.1)	60 (77.9)	39 (63.9)	.169
Hours of being barefoot >8 h, Yes	83 (85.6)	48 (75.0)	37 (82.2)	.226
Usual heel height of footwear worn				
<2.5 cm	107 (99.1)	67 (98.5)	55 (100)	1.000
Physical activity performed in the week prior				
Only mild physical activity	98 (81.7)	66 (85.7)	47 (79.7)	.811
Any moderate physical activity	20 (16.7)	9 (11.7)	11 (18.6)	
Any vigorous physical activity	2 (1.7)	2 (2.6)	1 (1.7)	

[a]Only participants with both case and control periods are permitted for inclusion in case-crossover analysis.

*P values were estimated using analysis of variance for continuous variables, Chi-square for dichotomous (or exact test if Chi-square assumptions were not met). $P < .05$ was considered statistically significant.

not statistically significant, possibly due to the very small numbers performing vigorous PA. These findings provide a different perspective from current knowledge on KOA pain and PA.

Despite some contention as to whether PA is protective (or has no impact) or whether torsional loading and high impact increase the risk of KOA development, it has been demonstrated that following PA guidelines long-term is not associated with increased risk of incident radiographic or symptomatic KOA.[31,32] Previous studies on postulated mechanisms for reduced risk of KOA conclude that low impact activities are protective for KOA because joint loading and compression improves the cartilage matrix and chondrocyte activity. But some studies have demonstrated short-term exacerbations of KOA with PA with or without knee buckling.[10,24,33]

It is possible that increased loading, in the short term, results in pain, particularly in elderly who are overweight/obese with a maladjusted gait. Studies have shown that patients with KOA frequently report pain with daily activities such as walking, climbing steps and standing and have higher levels of self-reported pain with PA combined with poor physical functionality compared to controls.[10,34] It has been demonstrated that pain intensity in KOA is related to knee adduction moment and external knee flexion moment.[35] So, it is believed that malalignment in persons who are overweight/obese with

TABLE 2 Association between knee osteoarthritis pain flares and duration of being barefoot, wearing footwear, and physical activity category in the period prior to flare (duration in hours)

Flare	Case periods	Control periods	Univariate ratios (95% CI)	*P*	Case periods	Control periods	Multi variate odds ratio (95% CI)	*P*
Exposures 1 d before flare – Barefoot & physical activity categories								
Duration of being barefoot day before flare (hours)	224	725	0.85[a] (0.79-0.90)	<.0001	224	725	0.85[a] (0.80-0.90)	<.0001
Physical activity performed 1 d prior								
Mild physical activity only	145	608	Reference		143	585	Reference	
Any moderate	80	144	3.81 (2.35-6.19)	<.0001	77	131	4.29[a] (2.52-7.30)	<.0001
Any vigorous	4	9	2.01 (0.53-7.63)	.303	4	9	1.14[a] (0.28-4.59)	.857
Exposures 1 d before flare – Footwear & physical activity categories								
Footwear as worn 1 d prior to flare (hours)	211	645	1.16 (1.08-1.24)	<.0001	211	645	1.15[b] (1.07-1.23)	<.0001
Physical activity performed 1 d prior								
Mild physical activity only	145	608	Reference		135	522	Reference	
Any moderate	80	144	3.81 (2.35-6.19)	<.0001	72	115	3.36[b] (2.01-5.61)	<.0001
Any vigorous	4	9	2.01 (0.53-7.63)	.303	4	8	1.66[b] (0.40-6.93)	.486
Exposures 2 d before flare – Barefoot & physical activity categories								
Duration of being barefoot 2 d before flare (hours)	224	725	0.85[a] (0.80-0.90)	<.0001	224	725	0.84[b] (0.79-0.90)	<.0001
Physical activity performed 2 d before								
Mild physical activity only	145	608	Reference		143	585	Reference	
Any moderate	80	144	3.73 (2.34-5.97)	<.0001	77	131	4.27[b] (2.56-7.13)	<.0001
Any vigorous	4	9	0.49 (0.05-4.69)	.537	4	9	0.26[b] (0.03-2.52)	.246
Exposures 2 d before flare – Footwear & physical activity categories								
Footwear worn 2 d prior to flare (hours)	210	634	1.11 (1.04-1.18)	.002	210	633	1.10 (1.03-1.18)	.003
Physical activity performed 2 d before								
Mild physical activity only	145	608	Reference		134	510	Reference	
Any moderate	80	144	3.73 (2.34-5.97)	<.0001	72	115	3.54[b] (2.12-5.90)	<.0001
Any vigorous	4	9	0.49 (0.05-4.69)	.537	4	8	2.32[b] (0.57-9.34)	.238

[a]Model contained: Duration of being barefoot (hours) & physical activity category 1 d before.
[b]Model contained: Duration of wearing footwear (hours) & physical activity category 1 d before.
[c]Model contained: Duration of being barefoot (hours) & physical activity category 2 d before.
[d]Model contained: Duration of wearing footwear (hours) & physical activity category 2 d before.

KOA will increase mechanical loads on the joints, a phenomenon which may be aggravated by mechanical loading by exercise.[36]

The pathogenesis of pain with exercise is indirectly supported by imaging evidence which showed increased bone marrow lesions (associated with fluctuations of knee pain) are associated with greater medial loads on the knee. As the occurrence of bone marrow lesions is associated with increased pain and resolution of bone marrow lesions is associated with diminished pain, these findings indirectly support our results that KOAF is associated with exercise.[37,38] We believe that a similar association would be reported with vigorous exercise and that diminished statistical effect of this association in the present study was due to smaller numbers of older persons with KOA performing vigorous exercise in our cohort. If a long-term recommendation of regular exercise is to be implemented, it is imperative that this dimension of post-PA KOAF be tackled effectively by healthcare professionals. Further, patients should be encouraged to

TABLE 3 Association between knee osteoarthritis pain flares and being barefoot (>8 h/ <8 h), wearing footwear(>8 h/ <8 h) and physical activity in the period prior to flare (univariate and multivariate analysis)

Flare	Case periods	Control periods	Univariate ratios (95% CI)	*P*	Case periods	Control periods	Multivariate odds ratio (95% CI)	*P*
Exposures 1 d before flare – barefoot and physical activity categories								
Being barefoot > 8 h d before flare[e]	224	725	0.29 (0.15-0.55)	<.0001	224	725	0.31[a] (0.16-0.60)	<.0001
Physical activity performed 1 d prior[f]								
Mild physical activity only	145	608	Reference		143	585	Reference	
Any moderate	80	144	3.81 (2.35-6.19)	<.0001	77	131	4.26[a] (2.51-7.23)	<.0001
Any vigorous	4	9	2.01 (0.53-7.63)	.303	4	9	1.41[a] (0.35-5.61)	.629
Exposures 1 d before flare – footwear and physical activity categories								
Wearing footwear > 8 h 1 d prior to flare[e]	211	646	4.61 (2.52-8.85)	<.0001	211	645	3.89[b] (2.08-7.27)	<.0001
Physical activity performed 1 d prior[f]								
Mild physical activity only	145	608	Reference		135	522	Reference	
Any moderate	80	144	3.81 (2.35-6.19)	<.0001	72	115	3.10[b] (1.85-5.21)	<.0001
Any vigorous	4	9	2.01 (0.53-7.63)	.303	4	8	1.22[b] (0.28-5.28)	.788
Exposures 2 d before flare – barefoot and physical activity categories								
Being barefoot > 8 h 2 d before flare[e]	224	725	0.29 (0.15-0.55)	<.0001	224	725	0.29 (0.15-0.56)	<.0001
Physical activity performed 2 d before[f]								
Mild physical activity only	145	608	Reference		143	585	Reference	
Any moderate	80	144	3.73 (2.34-5.97)	<.0001	77	131	4.27[c] (2.56-7.10)	<.0001
Any vigorous	4	9	0.49 (0.05-4.69)	.537	4	9	0.39[c] (0.04-3.69)	.412
Exposures 2 d before flare – footwear and physical activity categories								
Wearing footwear > 8 h 2 d prior to flare[e]	210	634	3.86 (2.10-7.12)	<.0001	210	633	3.51[d] (1.86-6.61)	<.0001
Physical activity performed 2 d before[f]								
Mild physical activity only	145	608	Reference		134	510	Reference	
Any moderate	80	144	3.73 (2.34-5.97)	<.0001	72	115	3.13[d] (1.90-5.14)	<.0001
Any vigorous	4	9	0.49 (0.05-4.69)	.537	4	8	0.42 [d] (0.04-4.24)	.466

[a]Model contained: duration of being barefoot footwear (<8 h or ≥8 h) and physical activity category 1 d before.

[b]Model contained: duration of wearing footwear (<8 h or ≥8 h) and physical activity category 1 d before.

[c]Model contained: duration of being barefoot footwear (<8 h or ≥8 h) and physical activity category 2 d before.

[d]Model contained: duration of wearing footwear (h) and physical activity category 2 d before.

[e]Reference < 8 h.

[f]Reference – mild physical activity only.

pace their PA and engage with physiotherapists for muscle strengthening and biomechanical corrections.

This study also demonstrated that wearing footwear was associated with a significant risk of KOAF ($P < .0001$). Previous studies have shown that loading of the knee by footwear is significantly affected by footwear design with certain types of footwear and heel heights increasing medial knee loads more than others.[13] Further, partial lift by high heel height and stiffness of soles may increase joint torques compared to being barefoot. Also, flat flexible footwear is postulated to have reduced loading of the knee compared to other types of footwear.[39-41] Although most participants in our study habitually wore footwear with a low heel <2.5 cm (99.5%-6%), the type of shoes worn, the stability/supportive nature of the shoes worn, the flexibility of the shoes could not be ascertained due to the predominantly phone-based self-reported data collection. Therefore, our data collection was limited to heel heights and duration of footwear use.

Most importantly, this project demonstrates that being barefoot is associated with a diminished risk of KOAF. This study has the added advantage of being performed in a population of persons from a perennially warm, tropical country with a long native culture of walking barefoot compared to other laboratory-based studies. Both the duration of being barefoot 1-2 days before flares and self-reported phenomenon of being usually barefoot was associated

with diminished KOAF ($P < .0001$). The higher the duration of being barefoot, the greater the reduction in risk. The mechanical advantages of walking barefoot are manifold.

Being barefoot is different compared to using footwear because there are significant differences in movement, sensation, pressure and muscle tone. Gait analyses performed in patients with medial KOA previously have demonstrated that barefoot walking significantly reduces peak knee joint loads and knee adduction moment. Walking further changes the stride, cadence, joint range of movement and toe-out angle, although the implications of this on knee loading are uncertain.[19] Three-dimensional assessments have demonstrated adverse torque on the knee with higher hip internal rotation, knee flexion and varus torque with running shoes compared to being barefoot.[15] Literature suggests that barefoot walking is less restrictive for motion control and that it improves the sensitivity of sensory perception of feet and activates the lower leg and foot muscles.[42] All types of shoes exert adverse torque forces on lower limbs compared to walking barefoot.[41] Additionally, proprioceptive input from the skin touching the ground is possibly beneficial in barefoot walking.[42] This evidence strengthens the findings of our research by explaining some of the mechanisms by which being barefoot is associated with lower mechanical loads on the knee and can explain the negative association with KOAF.

There was also a significant correlation between the hours of footwear used and being barefoot including in models where we dichotomized the hours of footwear/being barefoot to <8 hours and >8 hours. However, the strength of association with reduced risk of flares with being barefoot persisted in the multiple regression model. Therefore, we assert that the effect of footwear and being barefoot independently affects the propensity for KOAF.

4.1 | Limitations

These findings have the following limitations. There was a nearly 30% drop-out rate given the difficulties in regularly following up this very poor socio-economic group, although it did not in any way detract from the quality of the data obtained from those who were followed up. The hours of being barefoot (or using footwear) and PA were all self-reported. It was not possible to assess the exact type of exercise, including hours of exercise, undertaken in the patients as only a brief description of the activity was obtained and the data retrospectively categorized as mild, moderate or vigorous PA. A detailed assessment of the potential weight loading or torsional forces was not possible given the frequency and burden of telephone assessments in the patient's own home environment. Because time was a limiting factor, particularly as most of the patients were daily wage earners, we could not engage in very long detailed conversations on the descriptions of the activity performed; the most valid information within these constraints was obtained. Therefore, we had no capacity to ascertain what specific activities persons were engaged in when they were barefoot. However, this study has followed up patients for a long duration of time longitudinally in real-time and this strength of the study remains.

In addition, some data were collected retrospectively and may be prone to recall bias. However, being barefoot is very common in this community and participants were unaware of the research questions. We asked each participant to recall various risk factors, including their footwear history, over the last 48 hours. Thus, we believe most of the study participants should be able to recall the footwear fairly accurately within this short period of time. Although misclassification could occur, it is likely to be non-differential and such misclassification bias, in general, would bias the effect estimates toward the null.[43] Although the NRS method to identify KOAF may be prone to recall bias, the case-crossover design will mitigate this bias and this has been demonstrated by extensive use in previous studies.[22]

Given the study design, it was only possible to assess self-reported heel heights at follow-up assessments through phone calls. The impact of the flexibility of the shoe or its stability could not be assessed with this particular study design. As the self-reported heel height worn at baseline was checked using the shoe the participant wore at baseline by the investigator, while explaining the process to the participant, it is assumed that this defect is mitigated. Moreover, the study population consisted mainly of urban poor with a maximum of 1-2 pairs of shoes of a similar style, given the constant warm climate. Although it was not possible to assess the impact of different heel heights or the different stabilities of shoes, the majority of participants used lower heel heights of a flip-flop design.

It has previously been noted that self-reporting tends to over- or underestimate the PA performed by persons due to response or recall bias. This may be noteworthy in our study group, which contained a large proportion of busy daily wage earners (ie, due to social pressures) or poor educational status (ie, inaccurate memory).[44] Another potential limitation of case-crossover studies is that confounders have potential to vary along with the exposure.

However, we assert that the impact of these limitations does not diminish the association between being barefoot (or wearing footwear) and PA on KOAF. It is recommended that further research into the exact pathogenesis of reduction in flares due to being barefoot be conducted.

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CONFLICTS OF INTEREST

DJH provides consulting advice to Merck Serono, TLC Bio, Pfizer and Lilly.

AUTHOR CONTRIBUTION

All authors fulfill the International Committee of Medical Journal Editors criteria for authorship.

ORCID

Inoshi Atukorala https://orcid.org/0000-0002-1223-6454
Arunasalam Pathmeswaran https://orcid.org/0000-0003-4065-2639
Thashi Chang https://orcid.org/0000-0002-6027-7883
Yuqing Zhang https://orcid.org/0000-0001-7638-0888
David John Hunter https://orcid.org/0000-0003-3197-752X

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ORIGINAL ARTICLE

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Joint damage in rheumatoid arthritis: Lessons learned from an extended 36-joint ultrasonography and 28-joint Disease Activity Score assessments

York Kiat Tan[1,2,3] | HuiHua Li[4] | John Carson Allen Jr[5] | Julian Thumboo[1,2,3,4]

[1]Department of Rheumatology and Immunology, Singapore General Hospital, Singapore

[2]Duke-NUS Medical School, Singapore

[3]Yong Loo Lin School of Medicine, National University of Singapore, Singapore

[4]Health Services Research Unit, Singapore General Hospital, Singapore

[5]Centre for Quantitative Medicine, Duke-NUS Medical School, Singapore

Correspondence
York Kiat Tan, Department of Rheumatology and Immunology, Singapore General Hospital, Outram Road, 169608, Singapore.
Email: tan.york.kiat@singhealth.com.sg

Abstract

Aim: To study joint damage severity in rheumatoid arthritis (RA) patients classified using ultrasound power Doppler (PD) and gray-scale (GS) joint inflammation outcomes and the 28-joint Disease Activity Score (DAS28).

Method: Ultrasound erosion scores were compared between (a) patients in group 1 (PD positive and GS ≥ median score), group 2 (PD negative and GS ≥ median score) and group 3 (PD positive and GS < median score) vs group 4 (PD negative and GS < median score) and (b) patients with high, moderate and low DAS28 scores vs those in DAS28 remission. Comparative analyses were performed using the 2-sample Student's *t* test.

Results: There were 1080 joints and 1800 joint recesses from 36 joints scanned in 30 RA adult patients (mean DAS28, 3.58; mean disease duration, 70.3 months) in this cross-sectional study. The mean and 95% CI ultrasound erosion scores were significantly higher ($P = .026$) for groups 1 (9.75, 6.69-12.81) vs 4 (3.4, 1.11-5.69) with a difference (95% CI) of 6.35 (0.78-11.83), but not significantly different (P values all $> .05$) for (a) groups 2 and 3 vs 4 and (b) patients with high, moderate and low DAS28 scores vs those in DAS28 remission.

Conclusion: Severity of ultrasound-detected bone erosions was significantly greater when both positive PD and a greater degree of GS joint inflammation were present in RA. This association was not observed when either component was absent. Single time point ultrasound joint inflammation assessment – and not DAS28 – is reflective of joint damage severity in RA patients.

KEYWORDS

bone erosion, rheumatoid arthritis, synovitis, ultrasound

1 | INTRODUCTION

Musculoskeletal ultrasound and magnetic resonance imaging (MRI) are modern imaging techniques which can be utilized for rheumatoid arthritis (RA) joint assessment. Both have superiority over clinical examination in the detection of joint inflammation[1,2] and are recommended by the European League Against Rheumatism (EULAR) as imaging techniques which should be considered for more accurate assessment of inflammation in the clinical management of RA.[3] Musculoskeletal ultrasonography is non-invasive, without risk of

radiation and has high feasibility for use in routine clinical practice. When compared to MRI, it is cheaper and relatively easy to set up for use in the rheumatology outpatient setting.[4] In musculoskeletal ultrasonography, 2 common modes of imaging are power Doppler (PD) and gray-scale (GS) imaging. Both attempt to evaluate different aspects of joint inflammation in RA. PD imaging assesses synovial vascularity while GS imaging helps identify synovial proliferation or hypertrophy.[2] Despite ultrasound imaging being available for more than a decade, there remains unanswered questions which need to be addressed in order for ultrasound to be used more effectively in the office setting. For example, in the routine clinical setting, 1 key unanswered question pertains to the clinical significance of GS joint inflammation. This can be appreciated in a small scale retrospective study (n = 37) by Tan et al,[5] evaluating the utility of ultrasonography in guiding modification of disease-modifying antirheumatic drugs (DMARDs) and steroid therapy for RA/inflammatory arthritis (IA) patients in routine clinical practice, whereby PD joint inflammation was found to be especially helpful in influencing physician decision-making in RA drug therapy, whereas the clinical significance of GS joint inflammation alone requires further investigation. Specifically, all/majority of patients who were (a) initiated and/or escalated on DMARDs had positive PD and GS joint inflammation and (b) ceased and/or tapered on their DMARDs and steroid therapy, were PD negative. In contrast, out of 6 patients who were positive for GS joint inflammation and negative for PD joint inflammation, 3 had cessation and/or dose reduction of DMARDs, 4 had dose reductions of steroids and none had DMARDs or steroid escalation. Other relevant questions that remain unanswered include the timing of ultrasound imaging assessment(s), the specific joints to be assessed and defining key threshold of ultrasound findings to guide treatment.[3,6,7] To answer all these questions is beyond the scope of our current study. Rather, this proposed study specifically addresses the important question on the clinical significance of GS joint inflammation (in relation to PD joint inflammation) by comparing the severity of ultrasound-detected bone erosions in RA patients who were categorized based on their ultrasound PD and GS joint inflammation findings and their 28-joint Disease Activity Score (DAS28). We have chosen to include DAS28 in this cross-sectional study, as this mimics what happens in routine clinical practice, whereby rheumatologists often rely on routine clinical measures (like DAS28) for disease activity assessment in patients with RA.[8,9]

2 | METHODS

Rheumatoid arthritis patients with at least 1 tender and/or swollen joint from the Singapore General Hospital (which is a tertiary referral hospital) were enrolled into this pilot study from an outpatient setting (eg, during their routine outpatient follow-ups) during the time period March 2016 through January 2017. All participants provided informed consent before being recruited into this cross-sectional study which was institutional review board approved and conforms to the relevant research ethics guidelines.

TABLE 1 Joint sites scanned by ultrasound

Joints scanned bilaterally	Joint recesses
Elbows	Humeroradial; posterior fossa
Wrists	Dorsal radiocarpal and intercarpal; distal radioulnar
MCPJs (1 to 5)	Dorsal; volar
Thumb IPJs	Dorsal; volar
PIPJs (2 to 5)	Dorsal; volar
Ankles	Anterior tibiotalar
MTPJs (1 to 5)	Dorsal

Abbreviations: IPJs, interphalangeal joints; MCPJs, metacarpophalangeal joints; MTPJs, metatarsophalangeal joints; PIPJs, proximal interphalangeal joints.

2.1 | Baseline characteristics of the patients

Patient baseline characteristics were obtained through hospital medical records. These included the following: gender, age, ethnicity, disease duration prior to study enrolment, DAS28 score, DMARDs and corticosteroids use.

2.2 | Musculoskeletal ultrasonography

All ultrasonography was performed at the same study site using a Philips Medical Systems EPIQ 5G ultrasound machine along with a 5-18 MHz multi-frequency linear probe. Standardized ultrasonography was based on the previously published EULAR guidelines.[10] Each patient had the same set of joints and recesses scanned (see Table 1) as follows: bilateral elbows at the (i) humeroradial and (ii) posterior fossa joint recesses; bilateral wrists at the (i) dorsal radiocarpal and intercarpal and (ii) distal radioulnar joint recesses; bilateral metacarpophalangeal joints (MCPJs) of the thumb through to the little finger at the (i) dorsal and (ii) volar joint recesses; bilateral thumb interphalangeal joints (IPJs) at the (i) dorsal and (ii) volar joint recesses; bilateral proximal interphalangeal joints (PIPJs) of the index finger through to the little finger at the (i) dorsal and (ii) volar joint recesses; bilateral ankles at the anterior tibiotalar joint recesses; and bilateral metatarsophalangeal joints (MTPJs) of the big toe through to the little toe at the dorsal joint recesses. Ultrasound settings (pre-set at the various peripheral joint sites) were as follows: (1) pulse repetition frequencies ranging from 700 to 850 Hz and (2) Doppler frequency ranging from 8 to 9.3 MHz. A single rheumatologist experienced in musculoskeletal ultrasonography acquired the ultrasound images and graded these images using previously validated grading methods.[11,12] At the joint recesses, ultrasound PD and GS joint inflammation gradings were performed using a semi-quantitative 0-3 severity scale (none = 0/ mild = 1/ moderate = 2/ severe = 3). The definitions by Backhaus et al[11] were adopted for ultrasound PD joint inflammation grading.

A reference ultrasonographic atlas[12] was used for ultrasound GS joint inflammation grading. As the finger joint volar recesses were not included in the ultrasonographic atlas, grading of ultrasound GS joint inflammation at these volar recesses was based on the definitions provided by Backhaus et al[11] At the joint recesses, ultrasound-detected bone erosions were graded dichotomously (No = 0/ Yes = 1) according to the Outcome Measures in Rheumatology Clinical Trials (OMERACT) consensus definition of bone erosion.[13] The OMERACT definition for bone erosion was adopted, as presently there is no general consensus on the use of more sophisticated methods for bone erosion grading (such as semi-quantitative grading). For each patient, the sub-scores for ultrasound PD joint inflammation (score range of 0-3 at each joint recess), GS joint inflammation (score range of 0-3 at each joint recess) and bone erosion at the joint recesses (score range of 0-1 at each joint recess) were summed to derive the respective ultrasound PD score (score range of 0-180 for each patient), GS score (score range of 0-180 for each patient) and erosion score (score range of 0-60 for each patient) at the patient level.

2.3 | Statistical analysis

The patients were categorized into (a) 4 ultrasound patient groups: group 1 (PD positive and GS ≥ median score of 35.5); group 2 (PD negative and GS ≥ median score); group 3 (PD positive and GS < median score); and group 4 (PD negative and GS < median score) and (b) those with high DAS28 (>5.1), moderate DAS28 (≥3.2 and ≤ 5.1), low DAS28 (≥2.6 and < 3.2) scores and those who were in DAS28 remission (<2.6).[9] We arbitrarily utilized the median (50th percentile) GS joint inflammation score as a cut-off to help categorize patients into the 4 patient groups as there is presently no general consensus on what constitutes a high or low inflammatory burden on GS ultrasonography at the patient level. Ultrasound erosion scores were compared (a) between ultrasound patient groups 1, 2 and 3 vs group 4 and (b) between patients with high, moderate and low DAS28 scores vs those in DAS28 remission. Comparative analyses were performed using the 2-sample Student's *t* test. All analyses were done using R 3.6.2 (https://www.r-project.org).

3 | RESULTS

3.1 | Baseline characteristics of patients

In total, 1080 joints and 1800 joint recesses were scanned in 30 RA patients who participated in this cross-sectional study. The baseline characteristics of the patients were as follows: 23 out of 30 (76.7%) patients were Chinese; 28 out of 30 (93.3%) patients were female; mean (SD) age of 61.7 (9.1) years; mean (SD) DAS28 of 3.58 (1.20); mean (SD) disease duration of 70.3 (61.2) months; 22 out of 30 (73.3%) patients were on prednisolone; 27 out of 30 (90%) patients were on conventional DMARDs (methotrexate, sulfasalazine, hydroxychloroquine and/or leflunomide). None of the participants were on biological DMARDs.

3.2 | Comparison of ultrasound erosion scores between the ultrasound patient groups

Table 2 summarizes the comparison of ultrasound erosion scores between the 4 ultrasound patient groups. The mean and 95% CI ultrasound erosion scores were found to be significantly higher ($P = .026$) for groups 1 (9.75, 6.69-12.81) vs 4 (3.4, 1.11-5.69) with a difference (95% CI) of 6.35 (0.78-11.83) between the 2 groups. However, ultrasound erosion scores were not significantly different (*P* values all >.05) for patients in group 2 vs 4 and group 3 vs 4.

TABLE 2 Comparison of ultrasound erosion scores between the ultrasound patient groups

Ultrasound patient groups	Ultrasound erosion score, Mean (95% CI)	Differences (95% CI) Group 4 vs	*P* value Group 4 vs
Group 1 (PD positive and GS ≥ median score)	9.75 (6.69-12.81)	Group 1:6.35 (0.78-11.83)	Group 1: .026*
Group 2 (PD negative and GS ≥ median score)	7.33 (−4.11-18.78)	Group 2:3.93 (−19.95-27.81)	Group 2: .572
Group 3 (PD positive and GS < median score)	3.3 (2.17-4.43)	Group 3: −0.10 (−2.58-2.38)	Group 3: .932
Group 4 (PD negative and GS < median score)	3.4 (1.11-5.69)		

Abbreviations: GS, gray-scale; PD, power Doppler.

*Statistically significant: $P < .05$.

TABLE 3 Comparison of ultrasound erosion scores between the DAS28 categories

DAS28 categories	Ultrasound erosion score, Mean (95% CI)	Differences (95% CI) Remission vs	*P* value Remission vs
HDA (DAS28 > 5.1)	5.25 (2.32-8.18)	HDA: −0.08 (−6.74-6.58)	HDA: .978
MDA (3.2 ≤ DAS28 ≤ 5.1)	7.93 (4.63-11.24)	MDA: 2.60 (−3.67-8.87)	MDA: .396
LDA (2.6 ≤ DAS28 < 3.2)	3.4 (1.81-4.99)	LDA: −1.93 (−7.46-3.59)	LDA: .449
Remission (DAS28 < 2.6)	5.33 (1.2-9.47)		

Abbreviations: DAS28, 28-joint Disease Activity Score; HDA, high disease activity; LDA, low disease activity; MDA, moderate disease activity.

3.3 | Comparison of ultrasound erosion scores between patients in various DAS28 categories

Table 3 summarizes the comparison of ultrasound erosion scores between patients in various DAS28 disease activity categories. The ultrasound erosion scores of patients with high, moderate and low DAS28 disease activity categories were not significantly different (*P* values all >.05) from that of patients in DAS28 remission.

4 | DISCUSSION

The main finding from our study addresses the important question on the clinical significance of GS joint inflammation in RA (in relation to PD joint inflammation) by showing that severity of ultrasound-detected bone erosions was significantly greater when both a positive PD and a greater degree of GS joint inflammation were present in RA. Greater severity of bone erosion was not observed when only 1 component was present. Single time point ultrasound joint inflammation assessment – and not DAS28 – is reflective of severity of joint damage in RA patients. To the best of our knowledge, this present study is the first to utilize an extended 36-joint ultrasonography covering 60 joint recesses per patient to study the severity of joint damage (as measured by ultrasound-detected bone erosions) in an RA cohort whereby patients were classified based on (a) their ultrasound PD and GS joint inflammation findings and (b) DAS28 disease activity categories.

Our study suggests that both ultrasound PD and GS joint inflammation play an important role in assessing RA joint damage. Ultrasound PD synovial vascularity, being an important factor in bone erosion in RA is, unsurprisingly, largely consistent with the existing literature, whereby ultrasound PD joint inflammation has been shown to predict progressive bone erosion.[14] Apart from being a predictor of structural progression in RA, PD joint inflammation has also been shown to predict disease relapse[14,15] and failure in tapering of biological DMARDs.[16] Perhaps not as widely known is that ultrasound GS joint inflammation has also been previously reported in the literature to have association with, or be predictive of RA joint damage, as shown by the following 3 studies.[17-19] In a small scale cross-sectional RA study (n = 30) by Tan et al, it was shown that GS joint inflammation scores correlated with ultrasound-detected erosions (r = .64, P = .0001) using a 36-joint ultrasonography, although there was no significant association with DAS28 scores.[17] In the next study by Bøyesen et al,[18] 84 RA patients (with disease duration of less than 1 year) were followed up for a year. Multiple imaging modalities were compared which included GS ultrasonography and MRI performed at the patients' dominant wrists. GS ultrasound inflammation and MRI bone marrow edema were found to be independent predictors of MRI erosive progression in this cohort of early RA patients on a group level. In the last study by Moller et al[19] which consisted of an RA cohort nested in the Swiss RA register, patients with sequential hand radiographs at their first ultrasound assessment were included in the study. Imaging data from the wrists and 16 finger joints per patient were analyzed. After a median of 35 months, 75 of 287 patients with available GS ultrasound data (26%) demonstrated joint damage progression. GS ultrasound scores at the 50th and 75th percentiles (as well as PD ultrasound score beyond upper limit of normal, ie, 1/54), were significantly associated with radiographic progression of joint damage (both in crude and adjusted models). Through comparing the severity of ultrasound-detected bone erosions in RA patients categorized systematically based on their ultrasound PD and GS joint inflammation findings, our study adds to the existing knowledge on ultrasound PD and GS joint inflammation by showing that RA joint damage is significantly more severe at the patient level when a greater degree of GS joint inflammation occurs together with a positive PD joint inflammation status. This was not the case when either PD or GS component was absent. Future RA studies should examine whether additional treatment benefits may be derived from targeting both PD and GS components of joint inflammation vs targeting either one alone. This will be especially important to address in light of recent randomized controlled trials (RCTs)[20,21] relying on ultrasound PD joint inflammation (and not its GS counterpart) as a target to help escalate treatment in RA yielding results largely seen as unfavorable to ultrasound.

Our study also revealed that ultrasound joint inflammation assessment has an advantage over DAS28 assessment in RA when these were performed as single time point measures. Specifically, the former and not the latter was shown to be reflective of the severity of joint damage in patients with RA. In the above mentioned RA study by Moller et al,[19] whereby GS ultrasound scores (at the 50th and 75th percentiles) and PD ultrasound score (beyond upper limit of normal, ie, 1/54) were significantly associated with radiographic

progression of joint damage, various baseline clinical disease activity measures including DAS28 did not show any significant association with radiographic progression. Taken together, the results of the study by Moller et al and our study suggest that single time point DAS28 measurements, unlike ultrasound joint inflammation assessment, are neither reflective of joint damage nor predictive of damage progression in RA.

Our study is not without its limitations. Although it is a relatively small scale study, its strength lies in the use of an extended 36-joint ultrasonography covering 60 joint recesses which, we believe, will better reflect the overall joint inflammation and erosion burden of the individual patient, thereby allowing a more robust analysis in relation to our study objectives. The use of an extended ultrasonography examination of the joints would require more time spent for the imaging assessment, which is likely to represent a limitation to its widespread use in routine clinical settings. Nonetheless, there is presently no universal consensus on the optimal reduced joint set(s) for use in ultrasound inflammatory joint assessment in the context of RA, and more research in this area will be required. Another limitation is that ultrasonography was performed as the sole imaging modality at a single time point in a study with a cross-sectional design. Therefore, future larger scale prospective studies will be necessary to elucidate the relationship between serially performed ultrasound PD and GS joint inflammation and RA joint damage. This should ideally be studied in conjunction with various other clinical disease activity measures and imaging modalities (such as conventional radiography, MRI and/or computed tomography) for comparative analysis.

In summary, we have added to the RA literature by showing that the severity of ultrasound-detected bone erosions was significantly greater when both positive PD and a greater degree of GS joint inflammation were present. This association was not observed when either component was absent. Ultrasound joint inflammation assessment – and not DAS28 – is reflective of joint damage severity in RA patients when performed as single time point measures. Future RA studies should look into whether additional treatment benefits may be derived from targeting both PD and GS components of joint inflammation vs targeting either alone.

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CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

YKT led the study and was responsible for the overall design and conduct of the study. YKT performed the ultrasonography. JCA and HHL were involved in the statistical analysis. All authors were involved in interpretation of the results as well as the drafting and preparation of the manuscript. The manuscript has been approved by all authors for publication.

ORCID

York Kiat Tan https://orcid.org/0000-0002-6944-8220

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ORIGINAL ARTICLE

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Effect of radiographic disease severity in high-resolution quantitative computed tomography assessment of metacarpophalangeal joint erosion and cysts

Anne-Birgitte Garm Blavnsfeldt[1,2] | Rasmus Klose-Jensen[1,2] | Jesper Thygesen[3] | Philip Therkildsen[1,2] | Bente Lomholdt Langdahl[2,4] | Kresten Krarup Keller[1,5] | Ellen-Margrethe Hauge[1,2]

[1]Department of Rheumatology, Aarhus University Hospital, Aarhus N, Denmark

[2]Department of Clinical Medicine, Aarhus University, Aarhus N, Denmark

[3]Department of Procurement and Clinical Engineering, Central Denmark Region, Aarhus C, Denmark

[4]Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus N, Denmark

[5]Diagnostic Center, Silkeborg Regional Hospital, Silkeborg, Denmark

Correspondence
Anne-Birgitte Garm Blavnsfeldt, Department of Rheumatology, Aarhus University Hospital, Palle Juul Jensens Boulevard 59, 8200 Aarhus N, Denmark.
Email: annebirgittegn@gmail.com

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Abstract

Aim: Bone erosions are the hallmark of rheumatoid arthritis (RA). High-resolution peripheral quantitative computed tomography (HR-pQCT) enables 3-dimensional visualization of arthritic bone erosions at a high resolution. However, the degree of erosive disease could influence the reliability of HR-pQCT evaluation. We aim to assess the intra- and inter-reader variability of identification of erosions in the metacarpophalangeal (MCP) joints using HR-pQCT in healthy controls and patients with RA, stratified according to van der Heijde-modified Sharp Score (HSS) of radiographic erosions.

Method: We analyzed HR-pQCT images from 78 patients with RA and 25 healthy controls. Patients were allocated to one of three groups of mild, moderate or severe disease according to HSS of MCP joints 2 and 3. Total HR-pQCT scans were analyzed twice in random order by three experienced readers, blinded to group distribution. The number of cortical interruptions and their classification as either erosions or cysts according to predefined criteria were recorded. Intraclass correlation coefficients (ICC) for cortical interruptions, erosions and cysts were calculated for each group using a 2-way random-effects model for inter-reader ICC and a 2-way mixed-effects model for intra-reader ICC.

Results: The intra- and inter-reader ICC were good to moderate for cortical interruptions and moderate for erosions throughout disease severity groups. The ICCs for the identification of cysts decreased with increasing degree of erosive disease.

Conclusion: The detection of cortical interruptions is only minimally affected by the degree of erosive damage, whereas the distinction between erosions and cysts is more complex in patients with extensive erosive disease.

KEYWORDS

bone erosions, cortical interruptions, cysts, Heijde-modified Sharp Score, high-resolution peripheral quantitative computed tomography, inter- and intra-reader reliability

1 | INTRODUCTION

Erosions are a consequence of inflammation in rheumatoid arthritis (RA), and their number and size are correlated to disease activity.[1] Current treatment guidelines utilize treat-to-target strategies for inflammatory control, and visualizing inhibition of disease progression on radiographs is considered pivotal in risk profiling, management of the disease, and in clinical trials.[2] The van der Heijde-modified Sharp Score (HSS) is the gold standard for the radiographic evaluation of RA.[3] It allows the assessor to semi-quantitatively score the erosive damage and joint space narrowing in selected joints of the hands and feet. However, the resolution of radiographs is limited and minor erosions or erosive progression will not be detected. Furthermore, radiographs are 2-dimensional and small changes in, for example, the rotation of the hand, may lead to superimposition and affect the detectability of erosions or mimic cartilage loss.[4] Hence, sensitivity to presence and progression of radiographic erosions in early or minimal disease, as well as in minimal progression in advanced disease, poses a challenge.

High-resolution peripheral computed tomography (HR-pQCT) is a 3-dimensional imaging modality, recently applied in rheumatology, which operates at a high resolution with an isotropic voxel size of 82 μm^3, minimal radiation and a short scan time. HR-pQCT detects more erosions than conventional radiography, ultrasound and magnetic resonance imaging.[5-9] Identifying an erosion begins with the identification of a cortical interruption, which is then classified as either an erosion, a cyst or a vessel channel.[10] However, in patients with aggressive disease, the anatomy of the joints may be deformed by large erosions and secondary osteoarthritis with formation of osteophytes, which may challenge the characterization. A recently published scoring method for cortical interruptions excludes joints with severe deformity.[11] Additionally, cysts are a common finding in both healthy joints and in patients with RA and may be mistaken for erosive bone changes.[12-14]

HR-pQCT may be a valuable tool in the assessment of erosive progression in a clinical setting. Previous studies have found good intra- and inter-reader agreement of erosion measurement after, for example, repositioning and excellent inter-reader agreement regarding erosion count.[15-17] The intra- and inter-reader reliability for the presence of cortical interruptions has been moderate in cadaveric samples.[18] However, several knowledge gaps exist, such as the lack of consensus regarding methodologies for erosion quantification.[19] To our knowledge, no study has assessed the reliability of erosion identification on HR-pQCT stratified according to the erosive damage.

The purpose of this study is to assess the variability of the identification of cortical interruptions and the distinction between erosions and cysts using HR-pQCT in healthy controls and patients with RA, stratified according to erosive damage on conventional radiographs. We hypothesize that the intra- and inter-reader variability of the identification of cortical interruptions, erosions and cysts increases with the degree of bone damage according to HSS.

2 | METHODS

We identified a total of 79 RA patients who had a HR-pQCT scan as part of two prospective studies carried out at the Department of Rheumatology, Aarhus University Hospital (NCT03429426 RACTX, NCT02944799 ALOSTRA). Patients were selected if they had a plain radiograph of the corresponding hand within 6 months of a HR-pQCT scan with acceptable image quality.[20] We also utilized images from 25 healthy controls who were recruited using a Danish webpage for research subjects (www.forsøgsperson.dk), and who had undergone HR-pQCT scans as part of a case-control study (QTB).[14] We collected the following demographic variables from all participants: age, gender and smoking status. For patients with RA we also recorded time since diagnosis, rheumatoid factor (RF) and anticyclic citrullinated peptide antibodies (ACPA) positivity, conventional disease-modifying anti-rheumatic drugs (cDMARDs)/biologic DMARDs (bDMARDs) treatment, Disease Activity Score of 28 joints – C-reactive protein (DAS28-CRP). The studies were approved by the local Ethics Committee (1-10-72-452-15 [ALOSTRA], 1-10-72-437-17 [RACTX] and 1-10-72-466-12 [QTB]) and the Danish Data Protection Agency (1-16-02-473-13 [ALOSTRA], 1-16-02-33-18 [RACTX] and 1-16-02-449-12 [QTB]). All patients and healthy controls gave informed consent before any study procedures were undertaken and the studies were performed in agreement with the Declaration of Helsinki.

2.1 | HR-pQCT

All images were acquired using the same HR-pQCT scanner (Xtreme CT 1, Scanco Medical AG) by trained operators. In brief, the patient's hand was placed in a cast and a 2.7 cm-long volume of interest was scanned, comprising a region of 80 slices (6.56 mm) distal and 250 slices (20.5 mm) proximal to the distal end of the third metacarpal head. The images were acquired using a spatial resolution of 82 μm^3, an X-ray tube voltage of 59.4 kVp, a current of 900 μA, and an integration time of 100 ms Digital Imaging and Communications in Medicine (DICOM) images were exported from the HR-pQCT scanner and analyzed using a DICOM viewer (Osirix version 9.0.1; Pixmeo). All HR-pQCT images were analyzed in random order and assessed twice by three assessors (JB, RKJ, ABB), blinded to participant characteristics. The assessors had experience from the analysis of +400 patient scans and were all trained under supervision by the same expert reader (KK). Prior to the analyses for this study, a calibration session was performed in 16 moderately to severely damaged RA joints, not included in this study, to ensure consensus between the three readers.

First we assessed all images for cortical interruptions according to the Study group for Extreme Computed Tomography in Rheumatoid Arthritis (SPECTRA) definition.[21] If the interruptions were regularly delineated, without loss of surrounding trabecular structure, we identified them as vascular channels, as per the SPECTRA definition, and they were not included in the analysis.[22] Second, the remaining

cortical interruptions were classified as either erosions or cysts, if they occurred with a loss of underlying trabecular structure. Erosions were defined according to the SPECTRA definition[21] as a cortical break, visible in two or more consecutive slices and in at least two planes, with loss of the underlying trabecular structure and a nonlinear shape. Cysts were defined according to the SPECTRA definition as a small cortical break with a disproportional area of absence of the underlying trabecular bone.[22] In addition to the SPECTRA definition, we included a rounded and clearly delineated boundary toward the surrounding trabecular structure.

All scans were presented to the readers in total (330 slices) without annotations. We assessed eight surfaces of the second and third metacarpal joints: the palmar, dorsal, radial or ulnar surface of the metacarpal head and of the proximal phalanx, yielding a total of 16 surfaces for each participant.[23] For each subject we recorded the presence, location and number of cortical interruptions and classified them as either erosions or cysts.

2.2 | Radiographs

Radiographs were obtained using standard dorso-palmar (PA) projection of the hands and wrist at a focus distance of 100-115 cm, and an exposure of 50-55 kV.

The radiographs were randomized and anonymized before analysis by a single assessor (RKJ). The 2nd and 3rd metacarpophalangeal (MCP) joints of the hands corresponding to the HR-pQCT images were scored according to the HSS method, yielding a total score of 0-18 points for each individual.[3]

2.3 | Data management and statistical analysis

Data were collected using the REDCAP database.[24] We divided the participants into four groups: healthy controls and, based on HSS scores of the 2nd and 3rd MCP joints of the patients, low, moderate and high degree of disease severity. The 33 and 66 percentiles of HSS scores were used as cutoffs between patient groups. We used STATA 2014 (StataCorp LP) for statistical analysis. Results were expressed as means (standard deviations) or as median (interquartile range) according to the distribution of the data. Normality was assessed using QQ-plots. We calculated the inter-reader intraclass correlation coefficient (ICC) for each group, using a 2-way random-effects model, and intra-reader ICCs using a 2-way mixed-effects model. ICCs were interpreted as poor if below 0.5, moderate if 0.5-0.75 and good if above 0.75.[25]

2.4 | Role of the funding sources

The funding sources played no role in the study design, in the collection, analysis or interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

3 | RESULTS

We analyzed images from 103 individuals. One RA patient was excluded due to lack of access to the radiographs.

Age, gender, use of tobacco, RF and ACPA status were similar among patient groups, whereas the mean age for healthy controls was slightly higher than for the patient groups (Table 1). Based on the defined grouping criteria, the median (interquartile range) HSS for the patient groups were 1 (0-1) for the low disease severity group, 3 (1-5) for the moderate and 9 (5-18) for the high disease severity group (Table 1; Figure 1D).

The inter-reader ICCs remained moderate to good throughout the disease severity groups for cortical interruptions (Table 2). For erosions, the inter-reader ICCs were moderate. The ICCs for cysts varied substantially, with the lowest ICCs in the moderate and high disease severity groups (Table 2; Figure 1F-J). Intra-reader ICCs showed the same patterns with good results for cortical interruptions and erosions but poor results for cysts in the high disease severity group (Table 3). An exploratory analysis of the 11 patients with the highest HSS scores (HSS > 11) yielded poor inter-reader ICC for cortical interruptions (0.43 [0.08,0.76]). The intra-reader ICC for cortical interruptions remained good for readers 1 and 2 and moderate for reader 3 (0.83 [0.49, 0.95]; 0.81 [0.45, 0.95] and 0.61 [0.06, 0.88]).

The median number of cortical interruptions, erosions and cysts per reader are illustrated in Figure 2A-C. The variation in the median number per reader was larger for cysts than for cortical interruptions and erosions. Most cortical interruptions were located at the radial surfaces of the metacarpal heads (see Figure 2D-F).

4 | DISCUSSION

The ICC remained moderate to good when assessing cortical interruptions across the groups of healthy controls and patients. Similar results were seen for erosions, with inter-reader ICCs in the moderate range. In contrast, the ICCs for cysts showed the expected pattern of great agreement for healthy controls and poorer results with increasing degree of erosive damage. For the patients with the highest HSS scores, inter-reader ICC for cortical interruptions was poor, but the intra-reader ICC remained good to moderate, supporting the assumption that for an individual reader, the detection of cortical interruptions is largely unaffected by disease severity.

Our results imply that the readers, even though they correctly identify a break in the cortex, do not agree on the distinction between erosions and cysts. Cysts are fluid-filled cavities found in the juxta-articular areas of both healthy controls and patients with RA.[12,14,26] In patients with RA, erosions and cysts are most frequently located at the radial surface, and erosions may expand into already existing cysts. Consequently, a singular lesion may present erosion qualities in some slices and cyst qualities in others (Figure 3). The variation in the median number of erosions and cysts per reader was more pronounced for the high disease severity group. This

TABLE 1 Baseline characteristics

	Healthy controls (n = 25)	Radiographic disease severity group[a]		
		Low (n = 25)	Moderate (n = 26)	High (n = 27)
Mean age, y (range)	56 (39-70)	62 (42-80)	70 (54-87)	67 (43-80)
Women, n (%)	19 (76)	21 (84)	19 (73)	17 (63)
Mean disease duration, y (SD)	NA	18.52 (10.3)	16.54 (9.1)	27.8 (14.3)
ACPA positive, n (%)	NA	18 (72)	18 (72)	21 (78)
RF positive, n (%)	NA	17 (68)	17 (65)	13 (48)
cDMARD, current treatment, n (%)	NA	21 (84)	22 (84)	23 (85)
bDMARD, current treatment, n (%)	NA	9 (36)	13 (50)	11 (41)
Percentage ever use of tobacco, n (%)	13 (52)	18 (72)	14 (54)	12 (46)
Mean DAS28-CRP (SD)	NA	2.1 (0.59)	1.99 (0.62)	2.41 (0.92)
Number of cortical interruptions, median (IQR)[b]	0 (0-1)	1 (0-3)	4 (2-6)	8 (3-14)
Number of erosions, median (IQR)[b]	0 (0-0)	1 (0-2)	3 (1-4)	7 (2-13)
Number of cysts, median (IQR)[b]	0 (0-1)	0 (0-1)	1 (0-2)	1 (0-1)
Median HSS[c] (range)	NA	1 (0-1)	3 (1-5)	9 (5-18)

Abbreviations: ACPA, anticyclic citrullinated peptide antibodies; bDMARD, biologic DMARDs; cDMARD, conventional disease-modifying anti-rheumatic drugs; DAS28-CRP, Disease Activity Score of 28 joints – C-reactive protein; HSS, van der Heijde-modified Sharp Score; IQR, interquartile range; RF, rheumatoid factor.

[a]HSS range for disease severity groups: low = 0-1, moderate = 1-5, high = 5-18.

[b]Median of all scores (three readers, two readings).

[c]HSS score for the 2nd and 3rd metacarpophalangeal joints corresponding to the High-resolution peripheral quantitative computed tomography images.

indicates that a slight discrepancy among readers in how cortical interruptions and the underlying loss of trabecular structure are perceived, becomes increasingly pronounced as the amount of erosive damage increases. This may either be due to the larger degree of joint damage making it more difficult to analyze, or merely because the number of cortical interruptions increases, and with it the possibility for disagreement. Also, in joints with a high degree of damage, the presence of osteophytes can affect the rims of bone defects and alter their appearance, resulting in a severely deformed joint. In some cases osteophytes shaped like forceps may form what could be seen as pseudo-erosions.[7] Furthermore, if erosions present healing, the rims become smooth, resembling cyst walls. This challenge was targeted by the calibration sessions prior to analysis. Patient age and symptom duration may act as a proxy for erosive damage which might explain that the variability of measurement of width and volume of erosions increased with patient age and symptom duration in a previous study, consistent with our results.[15]

Interestingly, the intra-reader agreement was less affected than the inter-reader agreement when the patients with the most damaged joints were analyzed separately. This is clinically relevant, since monitoring erosive progression is challenging in very eroded joints when using conventional radiographs due to the ceiling effect of the HSS system.

Most cysts were located at the radial surface of the head of metacarpal bones two and three, as were most erosions. This is in accordance with previous publications.[23] However, we observed cysts in all locations of metacarpal heads and phalangeal bases. Hence, based on the current definitions, location does not aid in the identification of cysts.

The definition of erosions on HR-pQCT has changed over time, reflecting the emerging body of evidence.[6,21,23,27,28] In 2016 the SPECTRA group (an international collaboration of HR-pQCT users) recommended the following definition for erosions: "a definite cortical break in two consecutive slices in at least two perpendicular planes nonlinear delineation of the cortical perforation with underlying loss of trabecular structure". A cyst is defined as a "disproportional area of absence of trabecular bone at the site of a small cortical break".[21,22] Reflecting the challenge of identification, recent

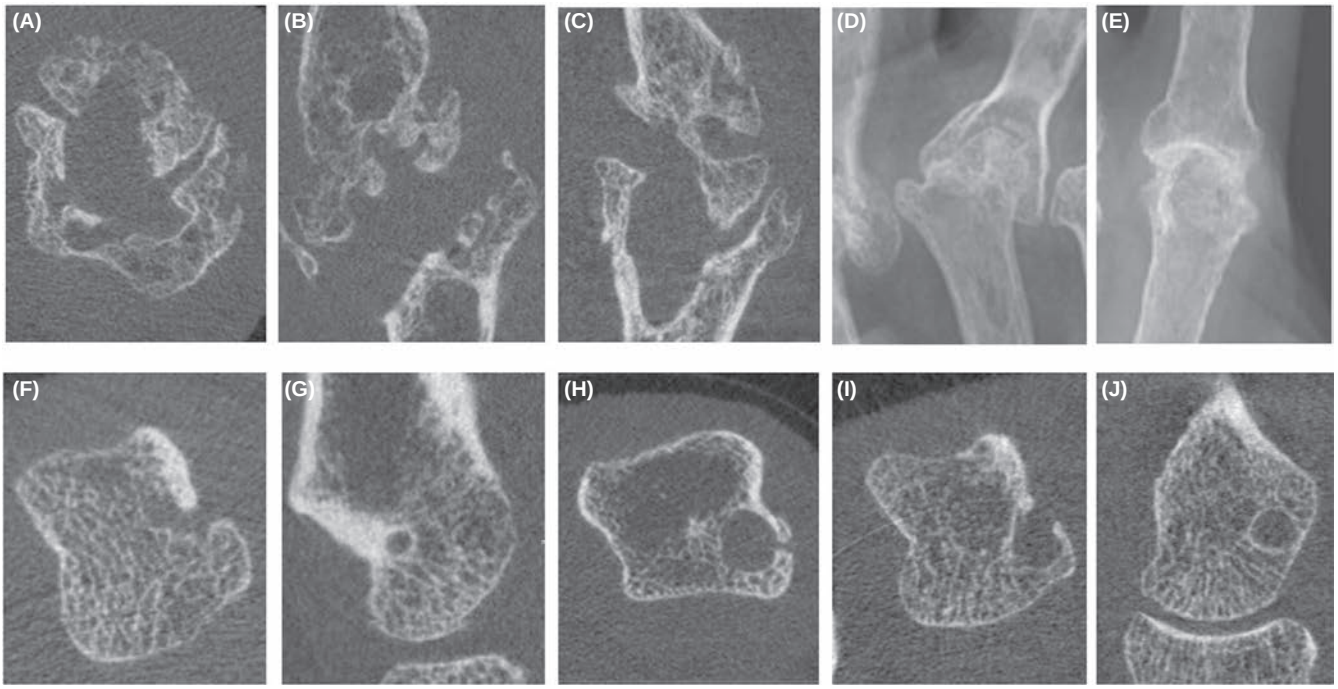

FIGURE 1 High-resolution peripheral quantitative computed tomography (HR-pQCT) images in axial (A), sagittal (B) and coronal (C) planes of a severely eroded metacarpophalangeal (MCP) joint. The proportion of damaged joint surface exceeds 50%. Cortical interruptions are few compared to the degree of bone loss. To the right, a conventional radiograph (D) of similar degree of destruction, which was graded as nine (maximum) on the van der HSS scale. Far right, a radiograph (E) of a lesser damaged joint showing superimposition of the metacarpal and phalangeal bones, leading to reduced visibility of erosions. Panels F-J show images of cysts in a healthy control subject (F and G), a patient with seronegative rheumatoid arthritis (RA) (H) and a patient with seropositive RA (I and J). The cysts demonstrate disproportional area of absence of trabecular structure at the site of a small cortical interruption (F and H) and a clear impression of a cyst wall (G and I)

publications use the term "cortical breaks" or "cortical interruptions" instead of erosions.[29]

Some limitations need to be discussed. First, it is possible that one reader registered one erosion and a second reader registered another. The current study was not performed at the level of individual lesions. This might have led to over-estimation of inter-reader agreement. However, the identification of a singular erosion is not standard and, to our knowledge, no study has been able to verify that individual lesions are identified consistently by multiple readers, as this is very time-consuming.[17,30] In the future, automatic identification algorithms may prove valuable and may enable counting, measurement and tracing over time of the individual lesions.[31] Second, the RA patients were evenly assigned to disease severity groups according to the predefined distribution of HSS scores, and overlap of HSS scores between groups was present. Therefore, a possible effect of disease severity may have been diluted by the fact that patients with the same score were represented in two groups. An alternative approach could have been to primarily define HSS ranges of each severity group and assign patients to groups after analysis of HSS. However, this was considered in an exploratory analysis which yielded very few cases in the high disease severity group. Third, the advantage of the high resolution also leads to identification of very small cortical interruptions. Some small cortical interruptions are known to be the entry points of minute arterial or venous vessels.[32] Thus, very small erosions may be mistaken for such vessel channels and vice versa.

Among the strengths of the present study is the inclusion of a group of healthy controls, and the fact that we utilized three

TABLE 2 Inter-reader intraclass correlation coefficients for numbers of cortical interruptions, erosions and cysts

		Radiographic disease severity group[a]		
	Healthy controls (n = 25)	Low (n = 25)	Moderate (n = 26)	High (n = 27)
Cortical interruptions	0.90 [0.82, 0.95]	0.82 [0.67, 0.91]	0.84 [0.69, 0.92]	0.72 [0.52, 0.85]
Erosions	0.65 [0.44, 0.81]	0.63 [0.39, 0.81]	0.60 [0,29, 0,79]	0.58 [0.27, 0.78]
Cysts	0.94 [0.90, 0.97]	0.47 [0.23, 0.62]	0.37 [0.08, 0.57]	0.10 [−0.03, 0.30]

Note: Data presented with [95% confidence intervals].

[a]van der Heijde-modified Sharp Score range for disease severity groups: low = 0-1, moderate = 1-5, high = 5-18.

TABLE 3 Intra-reader intraclass correlation coefficients for numbers of cortical interruptions

	Healthy controls (n = 25)	Radiographic disease severity group[a]		
		Low (n = 25)	Moderate (n = 26)	High (n = 27)
Cortical interruptions				
Reader 1	0.93 [0.85, 0.97]	0.96 [0.92, 0.98]	0.92 [0.83, 0.96]	0.91 [0.81, 0.96]
Reader 2	0.92 [0.82, 0.96]	0.84 [0.67, 0.93]	0.92 [0.84, 0.97]	0.90 [0.79, 0.95]
Reader 3	0.88 [0.74, 0.94]	0.96 [0.92, 0.98]	0.80 [0.60, 0.90]	0.80 [0.61, 0.91]
Erosions				
Reader 1	0.80 [0.60, 0.91]	0.93 [0.84, 0.97]	0.91 [0.80, 0.96]	0.92 [0.84, 0.96]
Reader 2	0.60 [0.28, 0.80]	0.68 [0.40, 0.84]	0.67 [0.39, 0.84]	0.78 [0.58, 0.90]
Reader 3	0.59 [0.26, 0.79]	0.97 [0.93, 0.99]	0.80 [0.60-0.90]	0.81 [0.63, 0.91]
Cysts				
Reader 1	0.92 [0.84, 0.97]	0.68 [0.40, 0.85]	0.77 [0.55, 0.89]	0.37 [0, 0.66]
Reader 2	1 [0, 0]	0.94 [0.86, 0.97]	0.83 [0.66, 0.92]	0.63 [0.34, 0.81]
Reader 3	0.93 [0.86, 0.97]	0.71 [0.45, 0.86]	0.66 [0.38, 0.83]	0.08 [−1.03, 0.41]

Note: Data presented with [95% confidence intervals].

[a]van der Heijde-modified Sharp Score range for disease severity groups: low = 0-1, moderate = 1-5, high = 5-18.

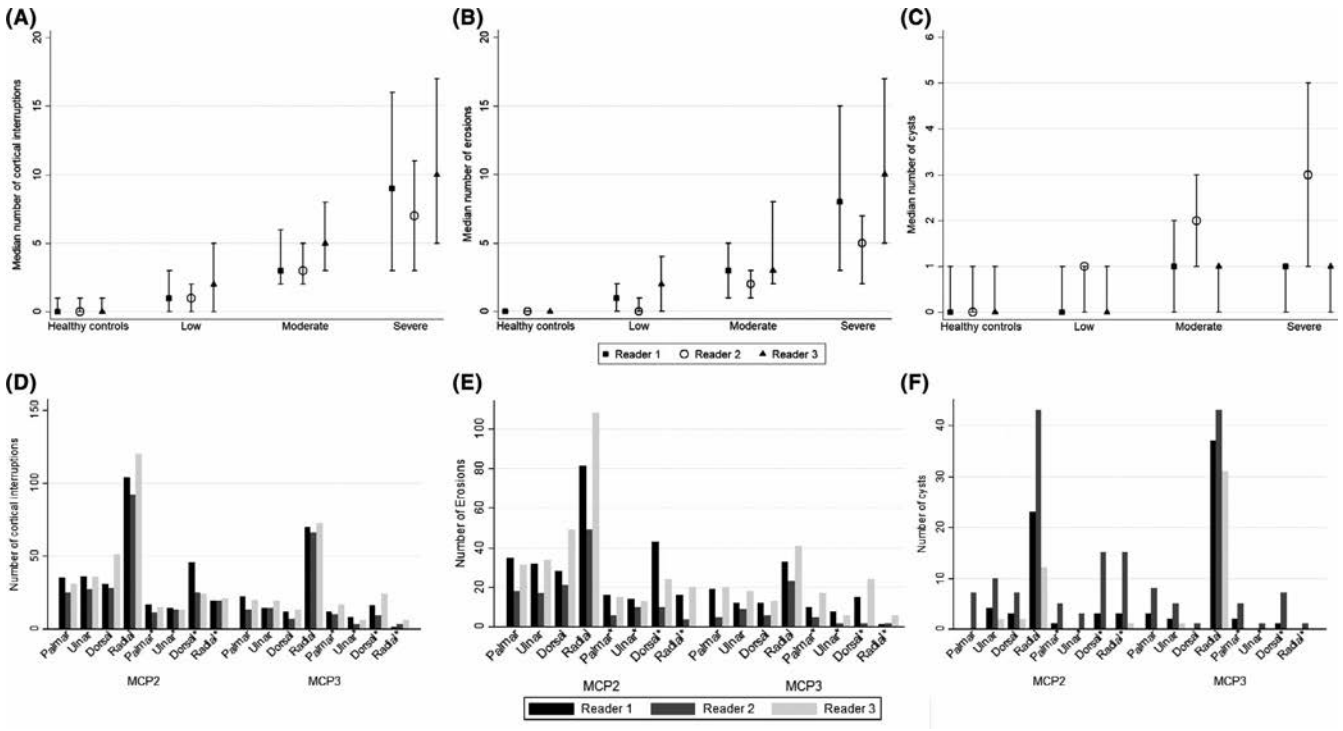

FIGURE 2 Median (interquartile range) numbers of cortical interruptions (A), erosions (B) and cysts (C) per reader. Number of cortical interruptions (D), erosions (E) and cysts (F) per surface for each reader. *Base of proximal phalangeal bone. MCP, metacarpophalangeal joint

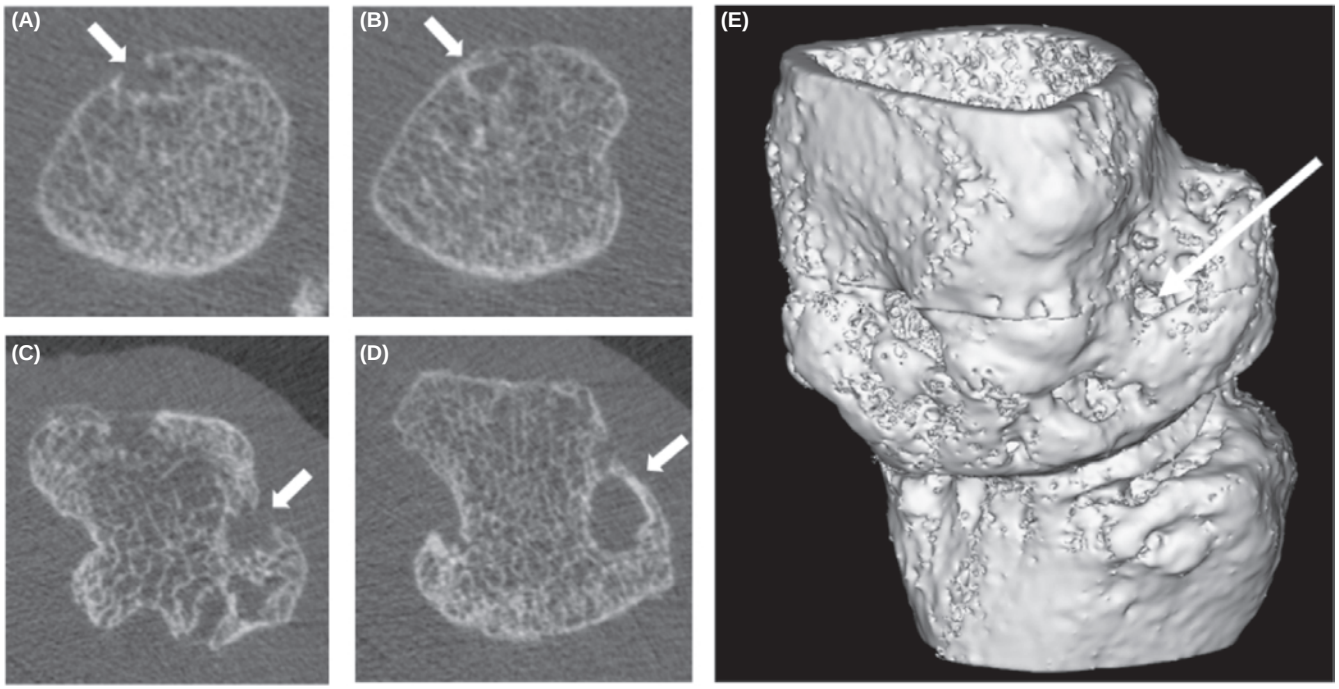

FIGURE 3 High-resolution peripheral quantitative computed tomography (HR-pQCT) images of two cortical interruptions, indicated by arrows, of the metacarpal bone. In panel A (dorsal surface) and C (radial surface), the lesions display sharp edges and open trabecular structure at the bottom of the cavity and would hence be identified as erosions. A few slices further distally (B and D) the rounded shape is clearly delineated and gives the impression of a cystic wall and the lesions would hence be identified as cysts. Panel E is the 3D segmentation of the joint from panels C and D, arrow indicating the cortical interruption in the radial surface of the metacarpal head

readers instead of two. HR-pQCT has several advantages over conventional radiographs. It operates at a higher resolution, enabling visualization of much smaller changes. It is 3-dimensional, which eliminates the problem of superimposition as seen on conventional radiographs. However, at present, no method for scoring the severity of bone deformation has been agreed upon for HR-pQCT. Thus erosions are counted and the maximum width, depth or volume are estimated.[8,9,27] This is in contrast to the HSS, which takes the percentage of the damaged joint surface into account, meaning that an erosion can contribute with 1, 2 or 3 points to the total score according to its size and whether it transcends the midline of the joint. Such distinctions have not been agreed upon for HR-pQCT. An automated algorithm to detect and measure surface abnormalities, both erosions and osteophytes has been suggested.[33]

In conclusion, our results suggest that the detection of cortical interruptions in HR-pQCT scans from patients with RA is only minimally affected, whereas the distinction between erosions and cysts is more complex in patients with extensive erosive disease. Given the current definitions, location does not aid in this process. The intra-observer agreement on cortical interruptions seems particularly robust, and thus patients with high degree of erosive disease should not necessarily be precluded from follow-up studies. Further studies should address the issue of robust definitions of erosions and cysts, and definite identification, retrieval, counting and measurement of individual erosions.

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AUTHOR CONTRIBUTIONS

Conceptualization: ABB, KKK, EMH; Provision of study materials: BL, KKK, EMH; Data collection: ABB, RKJ, KKK; Data curation: JT; Image analysis: ABB, RKJ; Statistical analysis: ABB, PT; Visualization: PT; Writing – original draft: ABB; Writing – review and editing, ABB, RKJ, PT, JT, KKK, BL, EMH; Supervision: EMH.

ORCID

Anne-Birgitte Garm Blavnsfeldt https://orcid.org/0000-0002-4590-8085

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ORIGINAL ARTICLE

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Ovarian antibodies among SLE women with premature menopause after cyclophosphamide

Martha Tsaliki[1,2,3] | Kristi A. Koelsch[1,2,3] | Ambre Chambers[1,2,3] | Mitali Talsania[4] | R. Hal Scofield[1,2,3] | Eliza F. Chakravarty[1,2]

[1]Arthritis & Clinical Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

[2]Department of Medicine and Pathology, College of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

[3]Oklahoma City US Department of Veterans Affairs Medical Center, Oklahoma City, OK, USA

[4]Section of Endocrinology, Department of Medicine, Loma Linda University School of Medicine, Loma Linda, CA, USA

Correspondence
R. Hal Scofield, Arthritis & Clinical Immunology Program, Oklahoma Medical Research Foundation, 825 NE 13th Street, Oklahoma City, OK 73104, USA.
Email: hal-scofield@omrf.org

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Abstract

Background: Women with systemic lupus erythematosus (SLE) are at risk of premature ovarian failure when treated with cyclophosphamide. This risk is increased when autoimmune thyroid disease is present. We undertook this study to determine whether the presence of ovarian autoimmunity also increased the risk of early ovarian failure among women receiving cyclophosphamide.

Methods: We examined the records of women enrolled in the Lupus Family Registry and Repository, a cross-sectional study of ~3300 SLE subjects, for treatment with cyclophosphamide as well as menopausal status. We defined premature menopause as permanent, spontaneous cessation of menstruation before age 45. We measured anti-ovarian antibodies by enzyme-linked immunosorbent assay using stored sera.

Results: There were 258 women treated with cyclophosphamide in whom presence of absence or premature menopause could by defined. A total of 169 (65.6%) had premature ovarian failure, while 89 (34.6%) did not. While anti-ovarian antibodies were present in a small percentage of patients, there was no association of premature menopause to either level of these antibodies (16.2 ± 20.3 units vs 17.4 ± 21.7 units, P = NS by Fisher's exact test), or positivity on this testing (11 of 169 [6.5%] positive vs 8 of 89 [8.9%], $\chi^2 = 0.53$, $P = .46$, 95% CI 0.95-1.1). Neither renal disease nor hypothyroidism increased the risk of premature ovarian failure in these women receiving cyclophosphamide.

Conclusion: Anti-ovarian antibodies among women with SLE are not associated with premature ovarian failure after treatment with cyclophosphamide.

KEYWORDS
clinical aspects, epidemiology

1 | INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease with a relapsing-remitting course that affects multiple organs and tissues leading to a wide range of clinical manifestations.[1] SLE affects women more commonly than men with a ratio of 9:1, most commonly occurring in the child bearing age.[2,3]

One of the most serious manifestations of SLE is renal disease (lupus nephritis), ranging from asymptomatic hematuria or proteinuria to serious nephritic and nephrotic syndrome, which can progress to acute and chronic renal failure.[4] There is a high risk of morbidity and mortality associated with renal involvement in SLE; thus, this manifestation can require immediate and intensive management.[5] Glucocorticoids are used for acute treatment, but more aggressive

induction therapy with a combination of glucocorticoid with additional immunosuppressant therapy is often required for more serious disease with a declining renal function.[4,5] Lupus nephritis can be difficult to control and often relapses; thus, patients often require long-term management with aggressive immunosuppressive therapy.

Cyclophosphamide was standard of care in managing serious lupus nephritis for many decades.[6] Due to its toxicity, safer alternatives are continuously sought. So far, mycophenolate mofetil has been shown to be non-inferior to cyclophosphamide with considerably improved tolerability, but cyclophosphamide remains among the first-line treatments for lupus nephritis in the most current guidelines.[7] Adverse effects of cyclophosphamide include infection, leukopenia, malignancy, cardiotoxicity, bladder toxicity and ovarian toxicity resulting in premature ovarian failure.[4] In SLE, the main factors responsible for ovarian dysfunction include disease activity and cytotoxic agents. Cyclophosphamide-induced ovarian toxicity is related to the age at the start of treatment, the cumulative dose along with the length of treatment, and bone marrow suppression determined by the neutrophil count with pulse intravenous (IV) cyclophosphamide.[8] In LUMINA, a multi-ethnic SLE cohort from the USA, 37 of 316 woman had premature menopause. In a multivariable regression analysis, age at receiving cyclophosphamide, cyclophosphamide induction therapy, higher disease activity and Texas-Hispanic heritage were associated with premature ovarian failure.[9] Another study compared prolonged IV cyclophosphamide to 5-7 monthly doses followed by mycophenolate mofetil maintenance therapy. In the latter group only 1/22 women (4%) had sustained amenorrhea, while in the prolonged cyclophosphamide treated patients 20/39 (51%) had sustained amenorrhea. Age at initiation of treatment remained an important risk factor.[10]

The presence of other autoimmune diseases may play a role in premature ovarian failure. One study showed hypothyroidism as an important risk factor. This study reported 71 patients with SLE who received cyclophosphamide, of whom 11 patients developed ovarian failure. All 11 patients had hypothyroidism as evidenced by raised thyroid-stimulating hormone (TSH) levels.[11] This association has not been confirmed.

Autoimmunity directed toward more than 1 organ is commonly found in the same individual. Perhaps a mechanism for the association of hypothyroidism with premature ovarian failure could be simultaneous presence of autoimmune ovarian disease.[12] Thus, we hypothesized that premature ovarian failure might associate with anti-ovarian antibodies, and undertook this study to determine whether anti-ovarian antibodies are associated with premature menopause among SLE patients taking cyclophosphamide.

2 | METHODS

2.1 | Lupus Family Registry and Repository (LFRR)

Patient data and serum were obtained from the LFRR, which we have described in detail.[13] All subjects were enrolled and consented under protocols approved by the Institutional Review Boards of Oklahoma Medical Research Foundation. SLE was confirmed by questionnaire, interview and review of available medical records, and all subjects designated as having SLE met at least 4 of the 11 American College of Rheumatology (ACR) revised classification criteria for SLE.[14] Extensive medical history data are available including medications, menstruation and menopausal history. Premature menopause was defined as spontaneous cessation of menstrual periods prior to age 45. We recorded the highest available serum creatinine in the reviewed records. Subjects were assessed as having hypothyroidism by medical record review and interview with study personnel. Subjects with a history of cyclophosphamide administration were identified and were the basis of this report.

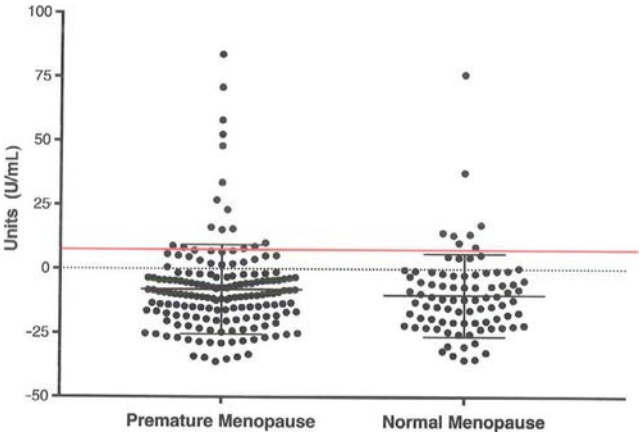

FIGURE 1 Anti-ovarian antibodies in the serum of SLE patients treated with cyclophosphamide and either with or without premature menapause. The solid line represents the cutoff for a positive test in this ELISA. P = NS by Student's t test

2.2 | Anti-ovarian antibody measurement

We measured autoantibodies binding ovarian antigens using a commercially available product (anti-ovarian antibody enzyme-linked immunosorbent assay [ELISA], product number 1B79184Immune Biological Laboratories, Minneapolis, MN, USA). Assays were performed according to the instructions provided. Positive and negative controls were provided with a cutoff for positivity established by the manufacturer, using these controls.

2.3 | Statistics

Categorical data were analyzed by Chi-square, while continuous data were analyzed by Student's t test. A P value of <.05 was considered statistically significant.

2.4 | Patient and public involvement

There was no involvement of patients or the public in this work.

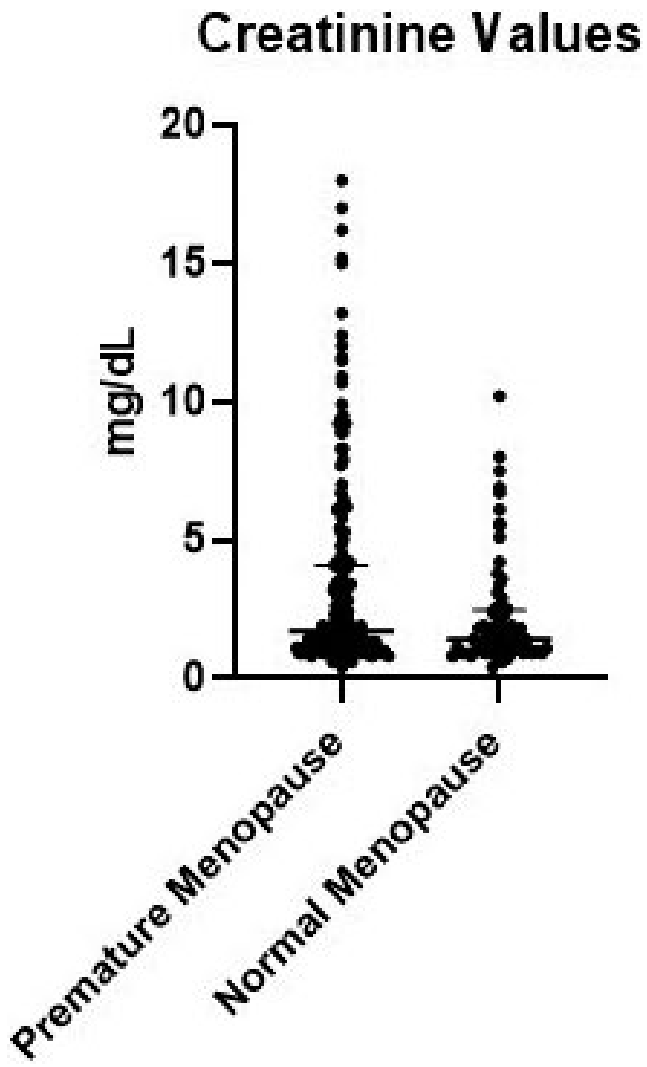

FIGURE 2 Highest serum creatinine found for the SLE women with or without premature ovarian failure. The data do not fit a Gaussian distribution and Mann-Whitney *U* testing showed no significant difference. Median plus interquartile range is shown

3 | RESULTS

Among approximately 3000 SLE women enrolled, we found 258 who had received cyclophosphamide in whom menopause status could be defined. Of these, 169 (65.5%) women had premature menopause. Seventy-three (28.3%) had menopause after age 45, while another 16 (6.2%) continued to have menses past age 45 years. Thus, there were 89 who did not have premature menopause at the time of data collection. Other women who received cyclophosphamide were under age 45 and were still menstruating at the time of evaluation were not included since the presence or absence of premature menopause could not be ascertained.

We performed the anti-ovarian antibody ELISA in these 258 subjects, 169 with premature menopause, 89 without, all of whom had received cyclophosphamide. We found the mean anti-ovarian antibody levels were 16.2 units (±20.3) among those with premature ovarian failure and 17.4 units (±21.7) among those without

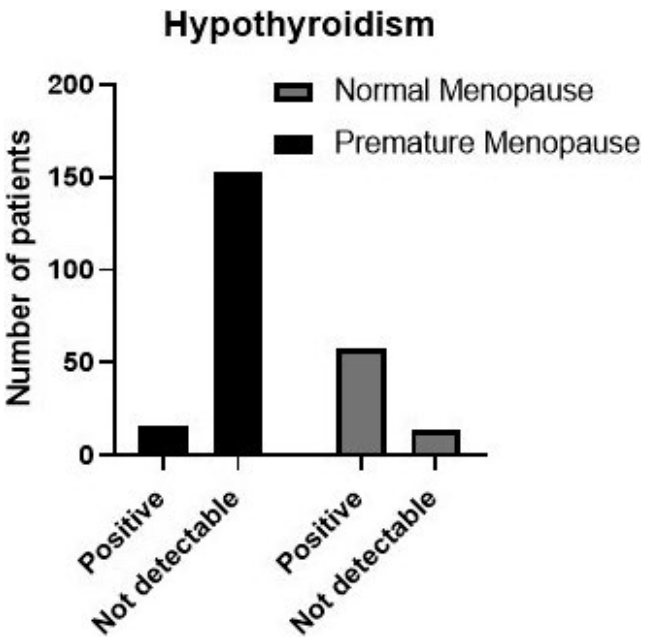

FIGURE 3 Hypothyroidism among the SLE women receiving cyclophosphamide. By Chi Square analysis, significantly more women with normal menopause had hypothyroidism than those with premature ovarian failure

premature ovarian failure (Figure 1, P = NS by Student's t test). Positive and negative controls supplied as part of the assay established the level of positivity. Using this cutoff, 11 of 169 (6.5%) with early menopause had a positive result, while 8 of 89 (8.9%) with menopause at an age > 45 had a positive result in the anti-ovarian antibody ELISA ($\chi^2 = 0.53$, $P = .46$, 95% CI 0.95-1.1).

Next we assessed 2 possibly confounding conditions, namely, hypothyroidism and renal disease, both of which might increase the possibility of premature menopause. When we compared the highest available serum creatinine from the reviewed medical records, there were high outliers among the women with premature ovarian failure. However, there was no statistical difference in serum creatinine between the women with or without premature ovarian failure after cyclophosphamide (Figure 2). Concerning hypothyroidism, among those with premature ovarian failure we found 16 of 169 (9.4%) had hypothyroidism, while among those without premature ovarian failure there were 58 of 72 (81%) with hypothyroidism (Yate's $\chi^2 = 116.6$, $P < .0001$) (Figure 3) . Thus, hypothyroidism was statistically more common among those who did not have premature ovarian failure.

4 | DISCUSSION

In this cross-sectional study of a very large cohort of women with SLE, we did not find an association between ovarian autoimmunity and premature menopause. Our analyses of these data included only those lupus-affected subjects who had received cyclophosphamide. Neither renal function nor hypothyroidism were risk factors

for premature ovarian failure in this group of SLE women. In fact, a statistically higher percentage had hypothyroidism among those without premature ovarian failure compared to those with premature ovarian failure.

SLE and its relationship to menopause has been extensively studied. Mean age of menopause in 961 women with SLE was 46.4 years and median age was 50.7 years.[15] The prevalence of premature ovarian failure in these women was 5.4%. In another study, the prevalence of premature ovarian failure was 0.6% in the absence of cyclophosphamide exposure, which is similar to the general population, compared to 16.7% in those exposed to cyclophosphamide.[16] Higher disease activity throughout the first 3 years of diagnoses was associated with premature ovarian failure.[16] However, overall factors intrinsic to SLE disease affecting ovarian function have not been described.

Cyclophosphamide use for SLE is a risk factor for premature menopause. Cyclophosphamide treatment at older age (age > 32), cumulative dose of cyclophosphamide, concurrent hypothyroidism, and neutropenia all increase risk.[16] In a cohort of 43 women with SLE, vasculitis or scleroderma, more women with cyclophosphamide use had cessation of menses in 1 year (30.4% compared to 0% in controls).[17] Cyclophosphamide is thought to primarily affect primordial follicles leading to suppression of estrogen and stimulation of gonadotropin release. This in turn leads to recruitment of new follicles, increasing the number of follicles vulnerable to toxicity of cyclophosphamide.

There are several possible associates of premature ovarian failure in SLE. Of course, patients with renal insufficiency may have early menopause. However, despite a few high outliers, there was no statistical difference in highest recorded serum creatinine between those women with or without premature ovarian failure. Thus, we did not find renal function as a contributing factor to premature ovarian failure. We also studied hypothyroidism. A previous study of 71 women with SLE found 11 with premature ovarian failure. Among these, 9 received cyclophosphamide and all had an elevated TSH. However, when reading this paper we are not sure whether the high TSH was at the time of administration of cyclophosphamide or not.[11] In any case, we did not find an association of hypothyroidism with premature ovarian failure among these SLE women who received cyclophosphamide. In fact, our data show hypothyroidism is strongly associated with protection from premature ovarian failure after cyclophosphamide. Our data and those of Medeiros et al[11] are not reconcilable as far as we can determine.

Menopause has impact on the onset and course of disease activity of SLE (reviewed in Talsania and Scofield[17]). Postmenopausal women with SLE were less likely to have malar rash, renal disease, leukopenia and positive anti-nuclear antibodies, but more likely to have arthritis, weight loss, myalgia, and myasthenia. In addition, there is a low incidence of anti-double stranded DNA and hypocomplementemia in post-menopausal onset. Thus, disease severity in post-menopausal women is milder compared to pre-menopausal women. Diagnosis of SLE is often delayed in post-menopausal women due to lack of typical symptoms and signs. Compared to onset of SLE at age of 18-50 years, onset of SLE after age of 50 was associated with lower number of ACR criteria and lower disease activity.[18] SLE disease activity may improve after menopause; however, a cause and effect relationship has not been established.[17]

Ovarian autoimmunity is thought to be responsible for 4%-30% of all premature ovarian insufficiency. In some patients with idiopathic premature ovarian insufficiency, histologic examination of ovarian tissue reveals evidence of lymphocytic oophoritis; lymphocytic infiltration is, of course, a common finding in autoimmune disorders involving other organs. The prevalence of anti-ovarian antibodies in premature ovarian failure patients varies from 3% to 66.6%.[19]

Anti-ovarian antibodies are found in the sera of women with SLE; however, while anti-corpus luteum antibodies were found in 15% of SLE patients, the presence of these antibodies was not associated with menstrual disturbances or changes in estradiol, luteinizing hormone or follicle-stimulating hormone (FSH).[20] We found anti-ovarian antibodies in less than 10% of women with SLE who had received cyclophosphamide, but there was no association with premature menopause among these patients, despite a high prevalence of premature menopause. Thus, determination of the presence of ovarian autoimmunity, at least by the method we employed, would not be useful to predict which patients are at risk of ovarian dysfunction after cyclophosphamide.

Several limitations of this study must be acknowledged. Most importantly, peripheral blood and clinical data in the LFRR were collected in a cross-sectional fashion, and there is often significant differences between the time of menopause and the time of sample collection over which detectable antibody titers may have changed. For example, neutropenia as a complication of cyclophosphamide is associated with premature menopause but we do not have neutrophil counts when cyclophosphamide was given, only at the time of study entry. Additionally, we did not have access to peripheral blood at any time before menopause in these subjects, so that a temporal correlation between antibody status and cessation of menses could not be determined, nor could we measure other important markers of menopause including FSH, estradiol, anti-Müllerian hormone, or TSH. Unfortunately we did not collect information about the age at which cyclophosphamide was given or the total dose, both of which are important correlates of premature ovarian failure among women receiving this medication.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

All authors approved the final version of the manuscript. RHS, EFC, and KAK conceived and planned the studies. MTsaliki, AC, and KAK performed experiments and along with RHS analyzed data. RHS and MTalsania wrote the first draft. KAK, EFC, and RHS edited the manuscript.

ETHICAL APPROVAL

The project was approved by the OMRF Institutional Review Board.

DATA AVAILABILITY STATEMENT

All data are available from the authors.

ORCID

R. Hal Scofield https://orcid.org/0000-0003-1015-5850

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APLAR GRAND ROUND CASE

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Cricopharyngeal bar and dermatomyositis: A cause of rapidly progressive dysphagia

Cameron Louis Adams[1,2] | Siobhan Lohan[3] | Alana Bruce[1,4] | Narainraj Kamalaraj[1,5] | Shyamini Gunaratne[1] | Ray White[6]

[1]Department of Rheumatology, Campbelltown Hospital, Sydney, NSW, Australia

[2]St George Hospital, Sydney, NSW, Australia

[3]Department of Speech Pathology, Campbelltown Hospital, Sydney, NSW, Australia

[4]Macquarie University, Sydney, NSW, Australia

[5]School of Medicine, Western Sydney University, Sydney, NSW, Australia

[6]Private Rheumatology Practice, Campbelltown, NSW, Australia

Correspondence
Cameron Louis Adams, Department of Rheumatology, Campbelltown Hospital, Sydney, NSW, Australia.
Email: cameron.adams@health.nsw.gov.au

Abstract

Background: Idiopathic inflammatory myopathies (IIM) are immune-mediated conditions that affect striated muscle, and are frequently associated with dysphagia. Dysphagia in these cases can be due to weakness of the muscles involved in swallowing or the presence of restrictive pharyngeal defects, such as cricopharyngeal bars. Treatment of dysphagia in IIM revolves around immunosuppressive therapies, and procedures to disrupt cricopharyngeus muscle when immunosuppressive therapies are unsuccessful.

Case report: A 73-year-old female presented with rapidly progressive proximal muscle weakness and dysphagia to the point she could not swallow liquids or solids. She had a rash over the extensor surfaces of the limbs, and periorbital-edema. Her creatine kinase was elevated, and skin biopsy showed an interface inflammatory reaction; however, myositis line assay revealed no autoantibodies, and a muscle biopsy was unremarkable. She was diagnosed with dermatomyositis with life-threatening dysphagia, and was admitted to our institution and treated with corticosteroids, methotrexate and intravenous immunoglobulin. A videofluoroscopic swallowing study revealed a large esophageal protrusion at the level of C5-C6, which was thought to be consistent with a cricopharyngeal bar, with large boluses unable to pass, leading to aspiration. After 10 weeks of treatment, the cricopharyngeal bar remained present, but swallowing had improved to the point that she was successfully swallowing all consistencies.

Conclusion: Dysphagia associated with IIM can be multifactorial, and can be due to the involvement of the muscles of swallowing in the inflammatory process, or due to restrictive pharyngeal defects, and determination of the cause of dysphagia can assist with management.

KEYWORDS

cricopharyngeal bar, dermatomyositis, dysphagia, idiopathic inflammatory myopathy, swallowing

1 | INTRODUCTION

Idiopathic inflammatory myopathies (IIM) are immune-mediated conditions that affect striated muscle. They can present with dysphagia in up to 25% of cases, and up to 60% of patients with an inflammatory myopathy will develop dysphagia at some point in their disease course.[1–6] Dysphagia primarily occurs when there is inflammatory infiltration of striated musculature involved in swallowing. This predominantly leads to oropharyngeal dysphagia, or in some cases early esophageal dysphagia when the upper esophageal sphincter (UES) and upper esophagus are involved.[2,7] Less commonly, dysphagia can be exacerbated by the presence of mechanical obstruction in the form of a crycopharengeal bar (CBP). A CPB is a radiological description of the prominence of the cricopharyngeus and can be found incidentally in the general population and has also been described in the presence of IIM with suggested increased incidence in this population.[1,3,8–20] In this case report we present a patient with dermatomyositis who presented with dysphagia and a CPB and discuss the significance of these findings.

2 | CASE REPORT

A 73-year-old Caucasian woman was admitted to our hospital with life-threatening dysphagia, associated with a rash and rapidly progressive muscle weakness. Her symptoms began 1 month prior to her admission, with the onset of a rash on the sun-exposed areas of her face, arms and upper back, with accompanying peri-orbital edema. Over the following week she developed dysphagia with dysphonia and proximal muscle weakness, predominantly affecting the upper limbs. Her creatine kinase was elevated at 1796 U/L, and upon review with a rheumatologist, she was commenced on prednisolone 50 mg daily, and methotrexate 20 mg weekly, for probable dermatomyositis. A myositis line immunoassay, which screened for OJ, EJ, PL-12, SRP, Jo-1, PM-Scl75, PM-Scl100, Ku, SAE1, NXP2, MDA5, TIF1 (gamma), MI-2 (beta), MI-2 (alpha), Ro-52 and HMG-CoA reductase antibodies, failed to detect any myositis-specific or associated antibodies, and she underwent a skin biopsy of the rash present on her right elbow which showed an inflammatory crust overlying a regenerating epidermis, with areas of atrophic epidermis with a loss of normal rete ridge pattern, an interface inflammatory reaction characterized by exocytosis of lymphocytes with apoptotic basal keratinocytes, and perivascular lymphocytes, without the presence of eosinophils, which in the clinical context was thought to be consistent with dermatomyositis. She also underwent a biopsy of the right vastus lateralis muscle biopsy which showed only occasional regenerative fibers, with no inflammatory cell infiltrate. A gastroscopy was performed and noted slight resistance at the cricopharyngeus, but was otherwise able to pass through the esophagus unimpeded. Despite treatment in the community with high-dose steroid and methotrexate, her dysphagia worsened over the following week and she was admitted to our hospital unable to swallow food or liquid. She was commenced on intravenous immunoglobulin at a dose of 2 mg/kg, and continued on corticosteroids and methotrexate. Speech pathology assessment showed overt aspiration with all oral trials and recommended complete avoidance of oral intake. A videofluoroscopic swallowing study (VFSS) revealed pooling of fluids in the pharynx, with multiple swallows required to clear the bolus, and a large esophageal protrusion at the level of C5-C6, with large boluses unable to pass, and subsequently held up in the pharynx leading to aspiration (Figure 1). Computed tomography of her neck and chest revealed no gross esophageal abnormality. A nasogastric tube was passed safely into her stomach, and she was commenced on enteral nutrition. Over the following 2 weeks her shoulder strength improved, and her creatine kinase normalized. A follow-up VFSS showed improvements with regard to the handling of liquids, with no aspiration on small sips of thin fluids, but persistence of delayed oral transit time with solids and the obstructive lesion at C5-C6 remained, with associated hold-up of bolus, and subsequent retrograde movement with pooling in the pharynx and aspiration. The obstruction was thought to be consistent with a cricopharyngeal bar, and a repeat gastroscopy assessed whether the lesion would be amenable to dilatation. This revealed no endoscopic evidence of a restrictive defect in the upper esophagus, and no dilatation was undertaken. Her nasogastric tube remained in situ, and she continued to receive most of her nutrition enterally. Over the course of 4 weeks her proximal upper limb power continued to improve and she was discharged home on prednisolone 25 mg daily and methotrexate 20 mg weekly, and monthly 1 g/kg intravenous immunoglobulin. At review 10 weeks later, VFSS showed she was

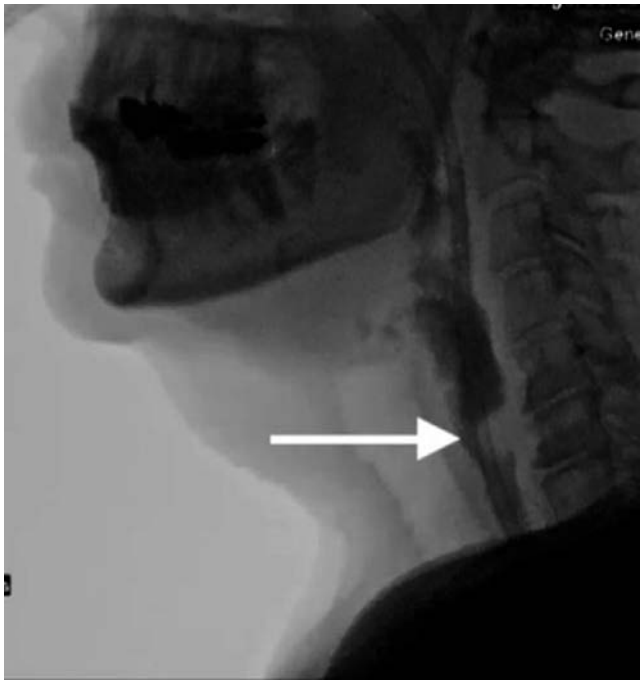

FIGURE 1 Lateral view, still image from videofluoroscopic swallowing study showing a rounded indentation arising from the posterior pharynx at C5-C6 (arrow), consistent with a cricopharyngeal bar

TABLE 1 Treatment of dysphagia in IIM

Author	Patients	Diagnosis	Manometry	Treatment	Outcome
Cherin et al[28]	4	IBM	Low UES pressure Decreased peristalsis in upper esophagus	IVIG	Improved
Marie et al[27]	73	PM/DM	Low UES pressure Decreased peristalsis in upper esophagus	IVIG	Improved • One patient found to have CPB had partial improvement and underwent myotomy with complete resolution
Dalakas et al[29]	19	IBM no CPB on VFSS	N/A	IVIG	Improved
Taira et al[20]	37 (15 with CPB, 22 without)	IBM	N/A	Various	• Similar rates of IVIG treatment between groups • 10/12 CPB + patients went on to need interventional treatment vs 0/22 CPB- patients
Murata et al[15]	1	PM with CPB	N/A	Prednisolone	• Dysphagia and impaired function of the cricopharyngeal muscle and presence of the cricopharyngeal bar persisted
Dietz et al[19]	1	PM with CPB	N/A	Prednisolone	• No improvement with prednisolone • Improved with myotomy
Wintzen et al[14]	4	IBM with CPB	N/A	Prednisolone	• No improvement with prednisolone • 3/4 improved with myotomy
Kagen et al[16]	3	PM/DM	Increased UES pressure	Prednisolone + myotomy in 2/3	• No improvement with prednisolone • Improved with myotomy
Williams et al[3]	8/9 with CPB/ stenosis	PM/DM	Normal UES pressures	Dilatation and myotomy	• Short term improvement in 4/8 • Long term improvement in 3/7
Verma et al[13]	1	IBM	N/A	Myotomy	• Limb weakness stabilized • Dysphagia failed to respond to dilatation, but improved with myotomy
Murata et al [30]	3	IBM with CPB	Low UES pressure Absent UES nadir	Intermittent balloon catheter dilatation (following IVIG)	• Short response to IVIG • Short response, but able to keep dilating

Abbreviations: CPB, cricopharyngeal bar; DM, dermatomyositis; IBM, inclusion body myositis; IVIG, intravenous immunoglobulin; N/A, not available; PM, polymyositis; UES, upper esophageal sphincter; VFSS, videofluoroscopic swallowing study.

TABLE 2 Histology of cricopharyngeal bars

Author	Patients	Diagnosis	Histology
Wintzen et al[14]	3 out of 4	IBM	• Endomysial and perivascular mononuclear infiltration • Rimmed vacuoles • Necrotic and regenerating fibers • Inclusion bodies in 1 case • Interstitial fibrosis
Danon et al[18]	1	IBM	• Perivascular and endomysial mononuclear infiltrate • Fiber necrosis • Rimmed vacuoles • Increased endomysial connective tissue
Verma et al[13]	1	IBM	• Mononuclear infiltrate • Fiber necrosis • Rimmed vacuoles • Increased endomysial connective tissue
Kagen et al[16]	1 (out of 3)	2 x PM, 1 x DM	(in 1 PM patient) • Necrosis • Inflammatory cell infiltration • Increase in connective tissue
Williams et al[3]	3 (out of 13 patients)	PM/DM	• Inflammatory cell infiltrate
Darrow et al[12]	1	IBM	• Primary myopathic process
Porubsky et al[11]	1	DM	• Round cell infiltration • Basophilia • Atrophy
Dietz et al[19]	1	PM	• Muscle fibrosis • No inflammatory cell infiltrate
Shapiro et al[31]	3 (out of 7)	Dysphagia without diagnosis of myositis	(in 3 patients) • Multiple foci of inflammatory infiltrate (lymphocytes, plasma cells, macrophages) • Necrosis • Increased connective tissue
Bachmann et al[32]	1	Dysphagia without diagnosis of myositis	• Lymphoplasmacellular florid myositis • Single-fiber atrophy • Muscle fiber necrosis with phagocytosis • Edema
Lacau et al[24]	10	Nil	• Increased connective tissue • Inflammatory cells in 9 cases
Benedict et al[33]	1	NMD	• Atrophy • Fibrosis • Chronic inflammation
Cruse et al[34]	7	Nil	• Degeneration and regeneration in the muscle fibers • Interstitial fibrosis
Leaper et al[35]	9 (out of 31 cadavers)	Nil	(in 5 CPB cases examined microscopically) • Hypertrophy of the cricopharyngeus • Proliferation of fibrous connective tissue
Watson et al[36]	1	Nil	• Increased connective tissue • Degeneration of fibers • No inflammatory cells
Siegel et al[37]	1	Granulomatous disease	• Giant cells, mononuclear cells • Granulomas
Leonard et al[38]	1	Nil	• Histiocytic infiltration
Kristmundsdottir et al[39]	3 (out of 22)	NMD	• Increased connective tissue • No inflammatory cells

Abbreviations: CPB, cricopharyngeal bar; DM, dermatomyositis; IBM, inclusion body myositis; NMD, neuromuscular degeneration; PM, polymyositis.

swallowing all consistencies successfully, and no longer required enteral nutrition, but the cricopharyngeal bar remained.

3 | DISCUSSION

Swallowing is a complex physiological action that is divided into 3 phases: the oral phase, pharyngeal phase and esophageal phase.[7] The oral phase relies on striated muscle including the tongue and muscles of mastication that facilitate formation of a food bolus and propulsion of the bolus into the pharynx. The pharyngeal phase also relies on striated muscle, including the suprahyoid muscles at the floor of the mouth, the wall of the pharynx, and the cricopharyngeus muscle which forms the UES. For swallowing to occur, the tongue and oropharynx contract to propel the bolus posteriorly. Contraction of the suprahyoid muscles elevate the hyoid bone and pull the larynx forward, closing the laryngeal inlet. The UES then relaxes allowing the bolus to pass into the upper esophagus. During the esophageal phase the bolus is transported from the UES to the lower esophageal sphincter, and into the stomach via peristalsis. The upper portion of the esophagus consists of striated muscle and the lower portion consists of smooth muscle.

Dysphagia is divided into oropharyngeal dysphagia, which occurs during the oral or pharyngeal phases of swallowing, and esophageal dysphagia, which arises during the esophageal phase of swallowing. This can develop through motor weakness of the muscles involved, impaired neurological input, or mechanical obstructions to the passage of the bolus. A CPB is one form of mechanical obstruction. It is a term used to describe the prominence of the cricopharyngeus observed in the lateral view on VFSS and appears as a smooth, rounded indentation, seen arising from the posterior pharyngeal wall around C4-C6. They represent an incomplete opening of the UES, either due to the presence of enlarged cricopharyngeus muscle or failure of other oropharyngeal muscles to exert enough force to open the UES.[21–23] The majority of cases are asymptomatic[21]; however, if symptomatic, they can be treated with either dilatation, myotomy, or botulinum toxin injection.[23–25]

Cricopharyngeal bars occur in the general population without an underlying muscle disease and are a relatively frequent finding, being found in in 5%-19% of patients who undergo a VFSS and are much more common in older people.[21–23,26] However, there have been several reports of an association between cricopharyngeal bars and IIM.[1,3,8–20] They have been found at increased rates in patients with an IIM compared to patients with a neurogenic cause of dysphagia as well as aged controls,[3] but similar to the general population, tend to occur in an older subset of patients.[3,20,26]

When CPBs are identified in IIM patients, manometry typically shows findings similar to that found in CPBs in the general population, with normal resting UES tone and increased pharyngeal and hypopharyngeal intrabolus pressures, which reflect reduced UES compliance.[3,16] This differs compared to dysphagia in IIM associated with muscle weakness from inflammatory infiltration, which tends to show a low resting UES tone.[2] For example, Marie et al[27] reported on 73 patients with IIM and dysphagia successfully treated with intravenous immunoglobulin (IVIG), where these patients tended to have low UES pressures and impaired upper esophageal peristalsis, except for 1 patient who was found to have a CPB, who only partly responded to IVIG and went on to have surgical myotomy for complete symptom resolution. This suggests that the mechanism for dysphagia in these cases reflected a weakened UES and upper esophagus from inflammation of the oropharyngeal musculature rather than a CPB obstructing the esophagus. This finding is consistent throughout the literature, which is summarized in Table 1, wherein patients with dysphagia and an IIM without evidence of CPBs tend to respond to treatment with immunomodulatory therapy,[27–29] as opposed to IIM patients with CPBs who ultimately go on to need an interventional procedure to disrupt the cricopharyngeal fibers for adequate symptom control.[3,13,14,16,18–20,26,30]

Interestingly, when biopsied, CPBs in IIM patients tend to show an inflammatory infiltrate (Table 2), which can be consistent with the underlying myositic process.[3,11–14,16,18] Several authors have observed patients with inclusion body myositis, and found histological features in the cricopharyngeal biopsies that resembled inclusion body myositis.[13,14,18] Others have commented on generally increased inflammatory infiltrate in patients with CPBs in dermatomyositis and polymyositis.[3,11,12,16] However, whether this means CPBs represent an active part of the myositic process is difficult to assess, as relatively little is known about the histology of a normal cricopharyngeus, and when reviewing the histology of patients with CPBs without an underlying IIM, there are frequently inflammatory infiltrates as well (Table 2).[24,31–33]

4 | CONCLUSION

While CPBs have been documented in IIM, they are generally found in an older subset of patients similar to CPBs identified in the general population, and although they can show inflammatory features on histology, inflammatory changes within the muscle are frequently seen in the biopsies of patients without an underlying IIM. Patients do not generally respond very well to immunomodulatory therapies and often require dilatation.

This raises the question of whether CPBs are either present prior to the diagnosis of IIM, and contribute to dysphagia in the context of new oropharyngeal muscle inflammation and loss of muscle power required to overcome the CPB or whether CPBs develop during the evolution of inflammatory myositis, either incidentally, due to chronic inflammation of the muscle leading to fibrosis and hypertrophy, or due to chronic weakness of other pharyngeal muscles to the point they can no longer open the UES. Despite their poor response to immunomodulation, CPBs clearly contribute to the development of dysphagia in some cases of IIM and represent an additional therapeutic target, through an interventional procedure to disrupt the cricopharyngeal fibers, and should be considered by clinicians involved in the management of patients with inflammatory myopathies.

AUTHOR CONTRIBUTIONS

Cameron Adams was involved in the medical care of the patient while admitted to hospital and prepared the manuscript. Siobhan Lohan was the speech pathologist involved in the care of the patient, and assisted with the parts of the manuscript relating to her assessments. Alana Bruce was involved in the medical care of the patient while admitted to hospital and revised sections of the manuscript. Narainraj Kamalaraj and Shyamini Gunaratne were staff specialists involved in the care of the patient in hospital and gave feedback on the direction of the manuscript. Ray White was involved in the care of the patient in the community. All authors have reviewed the manuscript.

ORCID

Cameron Louis Adams https://orcid.org/0000-0003-2225-2105

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COCHRANE CORNER

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Is methotrexate effective and safe in patients with psoriatic arthritis? A Cochrane Review summary with commentary

Frane Grubišić[1,2]

[1]Department of Rheumatology, Physical Medicine and Rehabilitation, School of Medicine, University of Zagreb, Zagreb, Croatia

[2]Referral Center for the Spondyloarthropathy, Ministry of Health of the Republic of Croatia, University Hospital Center "Sestre Milosrdnice", Zagreb, Croatia

Correspondence: Frane Grubišić, Department of Rheumatology, Physical Medicine and Rehabilitation, School of Medicine University of Zagreb, Zagreb, Croatia.
Email: franegrubisic@gmail.com

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The aim of this commentary is to discuss the published Cochrane Review "Methotrexate for psoriatic arthritis"[1] by Wilsdon et al[1] under the direct supervision of Cochrane Musculoskeletal Group. This Cochrane Corner is produced in agreement with the *International Journal of Rheumatic Diseases* by Cochrane Rehabilitation.

1 | BACKGROUND

Psoriatic arthritis (PsA) is an inflammatory rheumatic disease affecting approximately 30% of people with psoriasis and its estimated prevalence in the general population may vary between 0.01% and 0.19%, depending on geographical location. Men and women are equally affected across the entire age range, and PsA is more common over 40 years of age among people of both genders.[2]

Beside peripheral joint involvement, there are periarticular structures affected by inflammation leading to enthesitis, tenosynovitis, dactylitis and fingernail dystrophy.[3] Patients with PsA require input from the multidisciplinary rheumatology team (including rheumatologist, nurse, physiotherapist, occupational therapist), but also multispecialty care taking into account other key comorbidities (eg, dermatology, cardiology, psychiatry).[4] The goals of therapy for patients with PsA are to achieve minimal disease activity, improve functional status and quality of life, prevent structural damage and avoid or minimize complications. New treatment recommendations for PsA were updated in 2015 by both EULAR (European League Against Rheumatism) and GRAPPA (Group for Research and Assessment of Psoriasis and Psoriatic Arthritis) and in 2019 by American College of Rheumatology/National Psoriasis Foundation Guideline which are evidence-based and broadly suggest a similar "step up" approach to therapy..[5-7] This approach uses therapies sequentially starting with simple therapies such as nonsteroidal anti-inflammatory drugs for pain or topical therapy for psoriasis, followed by conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) in the form of monotherapy or combinations of 2 csDMARDs and finally biologic drugs if patients fail to respond to the conventional treatment.

Among csDMARDs, current guidelines favor methotrexate (dihydrofolate reductase inhibitor) as the first-choice therapy in PsA patients.[8] Despite its frequent use, the evidence for methotrexate efficacy in PsA is lacking and until recently there were only two small randomized controlled trials (RCTs) assessing its use against placebo. Furthermore, the Methotrexate in Psoriatic Arthritis (MIPA) study, which compared methotrexate versus placebo in 221 patients with PsA, found no significant difference in ACR20 (American College of Rheumatology response criteria for 20% improvement), DAS28 (Disease activity score of 28 joints) or PsARC (Psoriatic Arthritis response Criteria) at 6 months, although they did see an improvement in

[1]This summary is based on a Cochrane Review previously published in the Cochrane Database of Systematic Reviews 201, Issue 1, Art. No.: CD012722, https://doi.org/10.1002/14651858.CD012722.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.

both patient and physician assessments of PsA and psoriasis area and severity index (PASI) compared to placebo.[9,10]

2 | METHOTREXATE FOR PSORIATIC ARTHRITIS

Wilsdon TD, Whittle SL, Thynne TRJ, Mangoni AA (2019).

2.1 | What is the aim of this Cochrane Review?

The aim of this Cochrane Review is to investigate the efficacy and safety of methotrexate in adult patients with PsA.

2.2 | What was studied in the Cochrane Review?

All studies included adult patients with PsA aged 18 years or older who were recruited from rheumatology clinics. Diagnosis of PsA was confirmed by rheumatologist or by fulfillment of validated classification criteria (Classification Criteria for Psoriatic Arthritis). The average age of people included in these studies varied from 26 to 52 years and the average duration of PsA ranged from 1 to 9 years. This review included all RCTs and quasi-RCTs that compared methotrexate versus placebo or versus another csDMARD, biologic DMARD (bDMARD), nonsteroidal anti-inflammatory drugs (NSAID) or analgesics in adults with PsA. Furthermore, authors allowed co-intervention with NSAIDs or other analgesics, provided they were used in all treatment arms. The dose of methotrexate consisted of 7.5–25 mg orally, but for most studies, 15 mg was given orally per week. Five studies compared methotrexate against placebo (345 people) while four studies compared methotrexate against another csDMARD: leflunomide (61 people), cyclosporin A (35 people), gold (30 people) and sulfasalazine (24 people). The main outcomes reported were: disease response (measured by PsA response criteria-PsARC), function (measured by the Health Assessment Questionnaire for Rheumatoid Arthritis - HAQ), health-related quality of life (measured by Short Form 36, SF-36), disease activity (measured by Disease Activity Score of 28 joints with erythrocyte sedimentation rate - DAS28-ESR), radiographic progression, serious adverse events, and withdrawals due to adverse events.

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

The authors searched CENTRAL, MEDLINE, Embase, the WHO International Clinical Trials Registry Platform, and www.clinicaltrials.gov for relevant records. All databases were researched up to 29 January 2018. Moreover, authors also hand-searched included articles for additional records and contacted study authors for additional unpublished data. They applied no language restrictions.

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

The authors of this review restricted reporting of results to the comparison of methotrexate versus placebo for up to 6 months and dose of methotrexate was 15 mg weekly in most of the studies. Moreover, the level of evidence of all major outcome measures was downgraded because of bias and imprecision.

The review showed the following.

- Disease response, measured by the proportion who responded to treatment according to PsARC (response indicates improvement), may be higher in the methotrexate group (37/100 people taking methotrexate improved) compared to placebo group (21/100 people taking placebo improved); (risk ratio [RR] 1.76, 95% confidence interval [CI] 1.14-2.70).
- Function, measured by HAQ, was improved by 0.30 points (ranging from 0.09 better to 0.51 better) in patients receiving methotrexate (people taking methotrexate rated their function as 0.7 points) compared to the placebo group (people taking placebo rated their function as 1.0).
- At 6 months, disease activity measured by DAS28-ESR, improved by 0.26 points (0.65 better to 0.13 worse) on a 0 to 10 scale with patients taking methotrexate had a DAS28-ESR 3.8 points versus DAS28-ESR 4.06 points in the placebo group; mean difference was −0.26 points (95% CI −0.65 to 0.13).
- Results show 1/141 serious adverse events in the methotrexate group and 4/152 in the placebo group: RR 0.26 (95% CI 0.03-2.26) and absolute difference was 2% fewer events with methotrexate (5% fewer to 1% more).
- As for the withdrawals, 9/141 withdrawals in the methotrexate group were due to adverse events and 7/152 in the placebo group: RR 1.32 (95% CI 0.51-3.42); absolute difference was 1% more withdrawals (4% fewer to 6% more).
- No studies reported data on the radiographic progression as one of the outcome measures.
- One study measured health-related quality of life but did not report these results.

When methotrexate was compared to other DMARDs (up to 6 months), for outcomes like disease response (PsARC), function, serious adverse events, and withdrawals due to adverse events, results were informed by very low-quality evidence (downgraded due to risk of bias and imprecision). Furthermore, when compared to other DMARDs (beyond 6 months), trials did not report results. For outcomes of health-related quality of life, disease activity, and radiographic progression, trials provided no results.

2.5 | What did the authors conclude?

The authors concluded that, in spite of low-quality evidence, low-dose oral methotrexate (15 mg or less) might be slightly more

effective than placebo when taken for 6 months. Methotrexate is generally well tolerated in this population. Data comparing methotrexate versus leflunomide are of very low quality and authors do not believe they provide clinically meaningful information and these data should be interpreted and applied with extreme caution. Very low-quality evidence suggests that methotrexate may be as effective as leflunomide when taken for 6 months and is generally well tolerated, although very few adverse events have been reported and its comparative safety is uncertain. As for the comparison with other DMARDs (both conventional and biologic), head-to-head data are inadequate to inform about comparisons. Since the effects of methotrexate on other outcome measures (eg, health-related quality of life, radiographic progression, enthesitis, dactylitis, fatigue), its potential benefits beyond 6 months and/or effects of higher-dose methotrexate have not been measured or reported in randomized placebo-controlled trials, this could be a good starting point for some future long-term studies to be performed in order to see if these results could change current findings.

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

Both pharmacological and nonpharmacological interventions in patients with PsA have several important long-term goals: achieving remission or low disease activity, prevent structural progression and improving health-related quality of life and functioning (eg, increased level of independence, return to work, decreased direct and indirect medical costs). In addition to that, active participation of patients and adherence to recommended interventions help in achieving the above-mentioned goals. Low-quality evidence suggests that low-dose oral methotrexate may be more effective than placebo when taken for 6 months in terms of disease response (PsARC), function, pain, and patient and physician global assessments of disease activity. The comparative efficacy of methotrexate in terms of health-related quality of life, disease activity, radiographic progression, enthesitis, dactylitis, and fatigue, along with its efficacy beyond 6 months, has not been studied in a placebo-controlled trial. Analyzed outcome measures should also include more complex variables related to the efficacy (eg, quality of life, radiographic progression, enthesitis, dactylitis). Finally, one other variable remains unexplored - dose-dependent efficacy. Given the greater bioavailability of methotrexate at doses above 15 mg when administered parenterally rather than orally,[11] studies utilizing subcutaneous or intramuscular administration of methotrexate above 15 mg weekly might provide a suitable method for exploring dose-dependent efficacy, although this review included studies with doses ranging from 7.5 to 25 mg orally weekly.

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CONFLICT OF INTEREST

The author declares no conflicts of interest.

ORCID

Frane Grubišić https://orcid.org/0000-0002-3429-0849

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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)

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