ISSN 1756-1841 VOLUME 23 NUMBER 5 2020

International Journal of Rheumatic Diseases

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

WILEY

International Journal of Rheumatic Diseases

APL.JEB.Apr20

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International Journal of Rheumatic Diseases

Volume 23 | Number 5 | May 2020

Contents

Received: 11 February 2020 | Accepted: 17 February 2020

DOI: 10.1111/1756-185X.13818

International Journal of Rheumatic Diseases WILEY

EDITORIAL

When doing the right thing is wrong: Drug efflux pumps in steroid-resistant nephrotic syndrome

Idiopathic nephrotic syndrome (INS) is the commonest form of childhood nephrotic syndrome representing more than 90% of cases between 1-10 years of age.[1] The characteristic renal biopsy findings are diffuse foot process effacement on electron microscopy, and minimal changes (minimal change disease), focal segmental glomerulosclerosis (FSGS), or mesangial proliferation on light microscopy. The majority of these patients (>90%) respond to steroid therapy and are termed steroid-sensitive nephrotic syndrome (SSNS) but 10%-20% may be steroid-resistant (SRNS). Resistance to steroid therapy is associated with poor outcomes. In the PodoNet registry of 1354 patients, the 10-year end-stage renal disease-free survival was 92% in those with complete remission vs 43% in poor responders.[2]

Steroid resistance is commonly seen in patients with NS due to single gene mutations that affect glomerular podocyte differentiation and function. Most common mutations for the SRNS are in genes such as *NPHS1/NPHS2* that encode nephrin and podocin respectively, slit diaphragm component of the podocyte, *PLCE1* (encodes phospholipase C epsilon), also referred to as *NPHS3 WT1* encoding the transcription tumor suppressor protein, which is involved in kidney and gonad development. Other less common mutations such as *LAMB2* gene, *SMARCAL1* and so on, result in syndromic SRNS. Histological patterns of FSGS or mesangial nephropathy are most commonly associated with steroid resistance in patients in whom no genetic cause is identified.[2] Reduced glucocorticoid receptor expression in peripheral blood mononuclear cells (PBMCs) or expression of drug efflux pumps called multidrug resistance proteins (MRP) leading to decreased bioavailability of steroids, have also been postulated to be associated with steroid resistance.[3,4] Transport of molecules across the cell membrane is a normal physiological process carried out by membrane proteins called "transporters". One such group of transporters binds to and utilizes adenosine triphosphate (ATP) as a source of energy to carry out their functions and are termed ABC or ATP binding-cassette transporters. Certain members of the ABC transporters are particularly efficient in transporting xenobiotics and other molecules out of the cells to protect them from harmful chemicals.[5] However, these transporters can also efficiently pump out certain beneficial molecules (pharmaceuticals compounds) from within the cells, thereby preventing these drugs from achieving their intended effects on the cells. Since these transporters confer resistance to drugs by this efflux mechanism, this group of ABC transporters are also referred to as MDRs or multidrug resistance transporters or MDR efflux pumps. The major ABC superfamily transporters involved in MDR development in humans are P-glycoprotein (P-gp/ABCB1), multidrug resistance-associated protein 2 (MRP2/ABCC2), and breast cancer resistance protein (BCRP/ABCG2). A role for MDR in treatment failure has been postulated in several diseases like asthma, ulcerative colitis, rheumatoid arthritis and systemic lupus.[6]

Another potential mechanism of steroid resistance involves an important enzyme called histone-deacetylase 2 (HDAC2). HDAC2 belongs to a group of enzymes that remove acetyl groups from lysine residues in histones (allowing DNA to wrap tightly around them) resulting in reduced gene transcription. In addition, HDAC2 deacetylates lysine residue in glucocorticoid receptor (GR). The steroid, deacetylated GR receptor ligand complex is more efficient in suppressing the activities of nuclear factor (NF)-κB, a transcription factor that regulates the expression of several pro-inflammatory molecules. A reduction in HDAC2 expression and activity has been linked with increased inflammation in chronic obstructive pulmonary disease and bronchial asthma patients.[7] Therefore, with reference to NS, higher HDAC2 activity would translate to more efficient suppression of NF-κB activity resulting in fewer pro-inflammatory mediators and improvement in kidney functions. Conversely, lower HDAC2 would mean reduced GR deacetylation, higher NF-κB activity, heightened inflammation leading to poor or no improvement in renal functions.

In this issue of *International Journal of Rheumatic Diseases*, Singh et al carried out a prospective study to understand the relationship between P-gp, HDAC2 expression and functionality to steroid responsiveness in INS. Extensive work by this group previously has shown the important role of MDR-1/P-gp in childhood nephrotic syndrome and other autoimmune diseases.[6,8-10] In a cohort of 216 North Indian children, G2677T/A polymorphism in *MDR-1* gene was associated with steroid resistance.[9] G2677T/A and C3435T mutations in *MDR-1* gene in different combinations also increased the risk of developing steroid resistance in NS patients. They further examined the expression of HDAC2, P-gp and MRP-1 in PBMCs of INS patients.[10] A higher expression of P-gp/MRP-1 and reduced expression of HDAC2 in PBMCs were associated with steroid resistance in NS patients. In vitro experiments showed that inducers of HDAC2, namely theophylline, had a downregulating effect on P-gp/MRP-1, whereas, inhibitors of HDAC2 such as Trichostatin A, up-regulated

the expression of P-gp/MRP-1, suggesting a reciprocal relationship between the two.

In the present study, 31 children (mean age 8.42 ± 3.8 years) with NS were recruited and treated for 6 weeks with oral prednisolone. Remission was defined as urine protein/creatinine ratio < 200 mg/g (<20 mg/mmol) or <1+ of protein on urine dipstick for 3 consecutive days and steroid resistance as unresponsiveness to 60 mg/m^2 body surface area per day of prednisolone therapy for 4 weeks. Twenty-four patients achieved remission and 7 patients had resistant disease. Kidney biopsy in SRNS patients showed histological features of minimal change disease. In PBMCs from patients with SSNS, the expression of P-gp messenger RNA (mRNA) was lower and HDAC2 was higher compared to baseline and to levels seen with SRNS disease. Similarly, the functionality of P-gp was reduced and enzymatic activity of HDAC2 was higher in SSNS compared to baseline and to SRNS. Furthermore, patients with SRNS treated with tacrolimus, an immunomodulatory agent with P-gp suppressive activity, showed reduction in P-gp expression plus activity and an increase in HDAC2 expression. Of the 24 patients who initially responded to steroids, 7 relapsed and P-gp, HDAC2 mRNA expression and function, paralleled changes in the SRNS group. It can be postulated that the higher expression of P-gp and functionality make less steroid available to the cells.

While this study is informative and provides novel insights in steroid resistance, some questions require further examination. As discussed previously, patients with certain genetic mutations in podocyte proteins may not respond well to steroid and it would be helpful to know their prevalence in this group. Pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 are known to induce MDR-1/P-gp, and higher P-gp expression at the time of diagnosis could be due to ongoing inflammation. It will be useful to see the expression and function of P-gp and HDAC2 compared to their respective baseline levels in the individual groups (SSNS and SRNS) instead of the pooled data. The low P-gp and high HDAC2 expression and function may be an association seen with steroid responsiveness and further mechanistic studies are required to demonstrate causation. Nevertheless, the findings are significant and make an interesting case for modulation of HDAC2 or P-gp expression and function across a broad variety of inflammatory and autoimmune diseases.[11]

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REFERENCES

1. Wang CS, Greenbaum LA. Nephrotic syndrome. *Pediatr Clin North Am*. 2019;66(1):73-85.
2. Trautmann A, Schnaidt S, Lipska-Zietkiewicz BS, et al. Long-term outcome of steroid-resistant nephrotic syndrome in children. *J Am Soc Nephrol*. 2017;28(10):3055-3065.
3. Hammad A, Yahia S, Gouida MS, Bakr A, El-farahaty RM. Low expression of glucocorticoid receptors in children with steroid-resistant nephrotic syndrome. *Pediatr Nephrol*. 2013;28(5):759-763.
4. Gao H, Wang Q, Yu X, et al. Molecular mechanisms of glucocorticoid resistance in systemic lupus erythematosus: a review. *Life Sci*. 2018;209:383-387.
5. Vasiliou V, Vasiliou K, Nebert DW. Human ATP-binding cassette (ABC) transporter family. *Hum Genomics*. 2009;3(3):281-290.
6. Kansal A, Tripathi D, Rai MK, Agarwal V. Persistent expression and function of P-glycoprotein on peripheral blood lymphocytes identifies corticosteroid resistance in patients with systemic lupus erythematosus. *Clin Rheumatol*. 2016;35(2):341-349.
7. Ito K, Ito M, Elliott WM, et al. Decreased histone deacetylase activity in chronic obstructive pulmonary disease. *N Engl J Med*. 2005;352(19):1967-1976.
8. Agarwal V, Mittal SK, Misra R. Expression of multidrug resistance-1 protein correlates with disease activity rather than the refractoriness to methotrexate therapy in rheumatoid arthritis. *Clin Rheumatol*. 2009;28(4):427-433.
9. Jafar T, Prasad N, Agarwal V, et al. MDR-1 gene polymorphisms in steroid-responsive versus steroid-resistant nephrotic syndrome in children. *Nephrol Dial Transplant*. 2011;26(12):3968-3974.
10. Singh H, Agarwal V, Chaturvedi S, Misra DP, Jaiswal AK, Prasad N. Reciprocal relationship between HDAC2 and P-Glycoprotein/MRP-1 and their role in steroid resistance in childhood nephrotic syndrome. *Front Pharmacol*. 2019;10:558.
11. Inoue K, Gan G, Ciarleglio M, et al. Podocyte histone deacetylase activity regulates murine and human glomerular diseases. *J Clin Invest*. 2019;129(3):1295-1313.

Received: 26 March 2020 | Revised: 30 March 2020 | Accepted: 30 March 2020

DOI: 10.1111/1756-185X.13842

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases | WILEY

A systematic review of the prophylactic role of chloroquine and hydroxychloroquine in coronavirus disease-19 (COVID-19)

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Abstract

Objective: The pandemic coronavirus disease-19 (COVID-19) has pushed the global healthcare system to a crisis and amounted to a huge economic burden. Different drugs for prophylaxis against COVID-19 including chloroquine (CQ) or hydroxychloroquine (HCQ) have been tried. This study was performed to systematically review the role of CQ and HCQ in preventing the spread of COVID-19.

Methods: PubMed, EMBASE, ClinicalTrials.gov, International Clinical Trials Registry Platform and Cochrane Library databases were searched for studies that evaluated the prophylactic role of CQ or HCQ on SARS-CoV-2 (pre-clinical studies) or COVID-19 (clinical studies) until 30 March 2020. The available literature was critically appraised.

Results: A total of 45 articles were screened and 5 (3 in vitro pre-clinical studies and 2 clinical opinions) were included. The pre-clinical studies showed the prophylactic effects of CQ and HCQ against SARS-CoV-2. On the other hand, the clinical opinions advocated the prophylactic use of CQ and HCQ against COVID-19. However, no original clinical studies on the prophylactic role of CQ or HCQ on COVID-19 were available.

Conclusion: Although pre-clinical results are promising, to date there is a dearth of evidence to support the efficacy of CQ or HCQ in preventing COVID-19. Considering potential safety issues and the likelihood of imparting a false sense of security, prophylaxis with CQ or HCQ against COVID-19 needs to be thoroughly evaluated in observational studies or high-quality randomized controlled studies.

KEYWORDS

chloroquine, COVID-19, high-risk, hydroxychloroquine, prevention, SARS-CoV-2

1 | INTRODUCTION

The present world is experiencing a pandemic (coronavirus disease-19 or COVID-19) caused by a novel strain of coronavirus, called SARS-CoV-2, previously called 2019-CoV. At the time of writing this article, 3 72 757 cases spanning over 195 countries and territories and 1 international conveyance have been reported.[1] This could be an underestimate due to the lower number of diagnostic tests and case identification partly due to poor health services in most countries. The mortality rate stands at 0.5-4.4%[2]; however, this could be an overestimate as the exact denominator of actual number of cases is underreported. Diversion of all healthcare facilities toward the COVID-19 pandemic is likely to increase the morbidity and mortality due to other health problems.

In such a scenario, understanding the impact on the economy is beyond the confines of a medical expert.

Another conundrum faced is a high secondary infection rate among high-risk healthcare workers annexing the already burdened healthcare system.[3] This would not only compound the impending shortage of healthcare facilities but would also mean more pervasive spread. Prevention is thus the best strategy to not only prevent more spread and deaths but also to unburden the healthcare system. However, there are challenges involved. Although methods like mitigation, quarantine, isolation, social distancing, and so on are being employed, these are not infallible. Contact tracing for the spread of infection from asymptomatic or mild undiagnosed cases, transition to community spread, and factors such as uncertainty regarding the survival of the virus in air or fomites are cumulatively adding to the mammoth task.[4] Hence, the focus has now been shifted toward evaluating and implementing other strategies like chemoprophylaxis and vaccination besides the continued use of the barrier system. Vaccine development will take time, between 12-18 months, as human trials are under way. There is a lot of speculation on chemoprophylaxis stemming from the available data on the use of some antimalarial drugs, such as chloroquine (CQ) and hydroxychloroquine (HCQ), which have been tried for the treatment of this disease.[5]

The potential drug targets depend on the natural cycle of this virus. The virus depends on pH-dependent internalization and fusion with lysosomes. HCQ and CQ target this pathway by increasing the pH as they get concentrated into the lysosome and endosomes. This, in turn, affects viral replication and also helps in immune regulation and prevention of a cytokine storm as the antigen presentation is affected. But the challenge is the translational impact of in vitro models to in vivo ones. There are studies from China and other countries highlighting the use of antimalarial anthraquinones including mention of the same in the latest guidelines.[6,7] Recent advice issued by a national body from a South-Asian country suggested the use of prophylactic HCQ at a dose of 400 mg twice daily, followed by once weekly, for healthcare workers managing patients with COVID-19 and close contacts of proven COVID-19 cases.[8] However, these studies and guidelines differ on the prophylactic use of these drugs causing further dilemma among healthcare professionals. Hence, we aimed to systematically review the literature on the role of CQ or HCQ in preventing the spread of COVID-19.

2 | METHODS

2.1 | Study design

We aimed to include all completed and published pre-clinical as well as clinical studies, without limitations, which evaluated the prophylactic role of CQ or HCQ on SARS-CoV-2 (pre-clinical studies) or COVID-19 (clinical studies). We also looked for commentaries, reviews, viewpoints, or opinions if original clinical studies were not available. Studies which evaluated the therapeutic effects of CQ or HCQ were excluded.

2.2 | Search strategy

PubMed, EMBASE, ClinicalTrials.gov, WHO International Clinical Trials Registry Platform, and Cochrane Library (Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials [CENTRAL], and Cochrane Methodology Register) were searched from inception until 30 March 2020. The search terms used in various combinations were: "chloroquine", "hydroxychloroquine", "anthraquinone", "CQ", "HCQ", "coronavirus", "coronavirus disease", "coronavirus disease-19", "COVID-19", "severe acute respiratory syndrome", "SARS-CoV-2", "prophylaxis", and "preventive". These search terms were adapted for use with different bibliographic databases in combination with database-specific filters for studies, if available. The search strategy was used to obtain the titles and the abstracts of the relevant studies in English, and they were independently screened by 2 authors, who subsequently retrieved abstracts, and if necessary, the full text of articles to determine the suitability. Disagreement resolution was done with a third author. The systematic review protocol could not be pre-registered as the current pandemic is an ongoing public health emergency, thereby resulting in a paucity of time to permit pre-registration.

2.3 | Appraisal of the selected articles

The clinical opinions were critically appraised following the checklist of McArthur et al (2015).[9] The characteristics of the pre-clinical studies were also critically appraised. This was performed independently by 2 authors, and disagreement resolution was done with a third author. No assumptions or simplifications were made during the process.

3 | RESULTS

At total of 45 articles were screened and 3 in vitro pre-clinical studies[10-12] and 2 clinical opinions[13,14] were included in the analysis. No original clinical studies on the prophylactic role of CQ or HCQ on COVID-19 were available (Figure 1). Table 1 enumerates the findings of the in vitro pre-clinical studies and Table 2 denotes the critical appraisal of the clinical opinions. The pre-clinical studies showed the prophylactic effects of CQ and HCQ against SARS-CoV-2. While Yao et al showed that HCQ exhibited a better in vitro anti-SARS-CoV-2 activity than CQ in Vero cells derived from the African green monkey kidney, Liu et al exhibited a higher potency of CQ over HCQ in the same cell line. Xiao et al enumerated that CQ and remdesivir (which inhibits RNA polymerase), as compared to five other drugs, had a better in vitro potency in inhibiting SARS-CoV-2 in Vero cell lines. On the other hand, both Zhou et al and Colson et al provided their

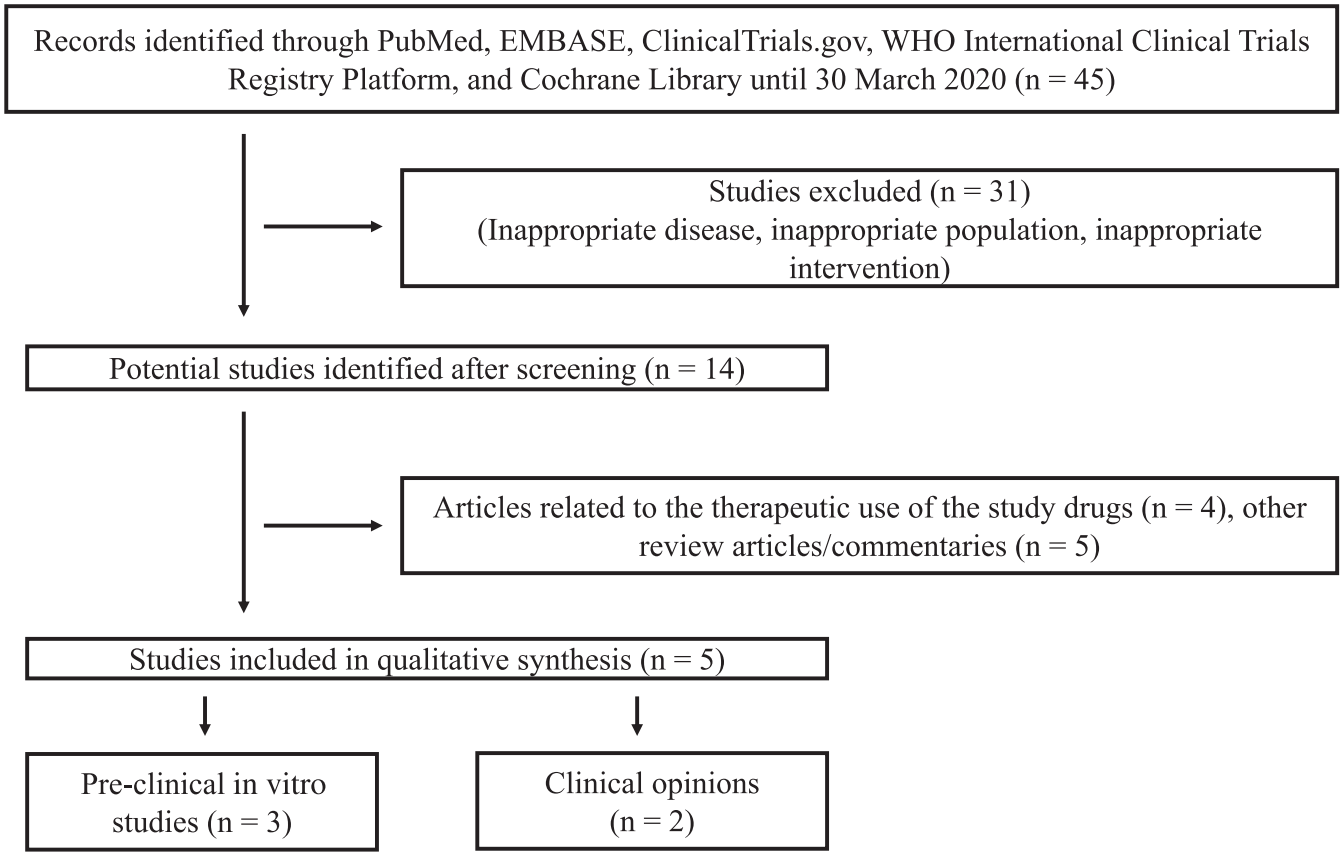

FIGURE 1 Flowchart depicting the steps of qualitative synthesis of evidence from the literature

clinical opinions advocating the possible prophylactic use of CQ and HCQ against COVID-19. On appraisal, both the articles were found to be of reasonable quality.

4 | DISCUSSION

The first in vitro study pointing toward the role of CQ and HCQ as pre-exposure prophylaxis against COVID-19 was published as a research letter by Yao et al[10] Vero cell lines derived from African green monkey kidney were treated with CQ or HCQ before exposing to a clinically isolated novel coronavirus strain (C-Tan-nCoV Wuhan strain 01) at a multiplicity of infection (MOI) of 0.05. HCQ was more potent than CQ in achieving the 50% maximal effective concentration (EC_{50}) (6.25 and 5.85 μmol/L at 24 and 48 hours, respectively). The concentration to achieve EC_{50} was >100 and 18.01 μmol/L for CQ, suggesting a higher loading dose. This study led to the enthusiasm of registration of clinical trials on the prophylactic role CQ and HCQ (Table 3). The study also highlighted the use of a high loading dose of CQ followed by a low maintenance dose to support its pharmacokinetic property of higher cellular accumulation and prolonged elimination half-life. Another in vitro study by a different group of researchers from China compared HCQ to CQ at 4 different MOI.[11] The results were contradictory to that of the previous study showing a lower EC_{50} of CQ than that of HCQ. Importantly the difference was even more striking at higher MOI, suggesting that in the presence of faster multiplication of the virus, CQ may perform better than HCQ. The possible reasons for the conflicting results are challenging to explain; however, it cautiously points toward extrapolation of in vitro evidence to clinical practice without robust clinical data. This also puts a question mark on the preventive role where the therapeutic effect of CQ might not be adequate. In another published study, Xiao et al assessed the role of multiple US Food and Drug Administration-approved antiviral drugs, including CQ (Table 2).[12] Their time-of-addition assay demonstrated that CQ functioned at both entry and post-entry stages of the SARS-CoV-2 infection in Vero E6 cells. The concentration to achieve EC_{50} and EC_{90} were 1.13 and 6.90 μmol/L, respectively.

Based on these in vitro results, some authors have adjudicated the prophylactic use of CQ and HCQ against COVID-19. Following the concept of drug repositioning, CQ and HCQ were proposed to be used against SARS-CoV-2 in an editorial published by a French group in February 2020.[14] It was also supported with the already established in vitro antiviral efficacy of CQ in other viruses, as well as against SARS-CoV-2. They emphasized the potential cost-benefit ratio of this prophylactic approach as a hope for the overburdened healthcare system during this pandemic. On 20 March 2020, researchers from China published a concise report emphasizing the role of HCQ over CQ as a prophylactic drug.[13] The report highlighted the in vitro prophylactic effects of HCQ and elaborated the molecular mechanisms of its antiviral activity. The maximum daily dose of CQ is 500 mg, while HCQ can be given at a higher daily dose

TABLE 1 Characteristics of the in vitro pre-clinical studies

Characteristics	Studies		
	Yao et al 2020	Liu et al 2020	Xiao et al 2020
Cell lines used	Vero cells derived from African green monkey kidney	Vero cells derived from African green monkey kidney	Vero E6 cells from African green monkey kidney and Huh7 human liver cancer cells[a]
Study drugs	CQ and HCQ	CQ and HCQ	CQ and others[b]
Drug concentrations and duration	0.032, 0.16, 0.80, 4, 20, and 100 μmol/L for 2 h	0.068, 0.21, 0.62, 1.85, 5.56, 16.67, and 50 μmol/L for 1 h	1.11, 3.33, and 10 μmol/L for 1 h
Comparator	None	Phosphate-buffered saline (control)	DMSO
50% maximal effective concentration (EC_{50})	Higher for CQ than that of HCQ	Lower for CQ than that of HCQ	Lower for CQ and remdesivir than others[b]
Key findings	HCQ exhibited a better in vitro anti-SARS-CoV-2 activity than CQ	The antiviral effects of HCQ seemed to be less potent than that of CQ, especially at a higher viral replication rate	CQ and remdesivir blocked virus infection at a low micromolar concentration
	Longer incubation time may provide a better antiviral effect	The entry step as well as the post-entry steps of virus infection were inhibited by HCQ	Full-time entry, as well as post-entry steps were inhibited by CQ and remdesivir

Abbreviations: CQ, chloroquine; DMSO, dimethyl sulfoxide; h, hour; HCQ, hydroxychloroquine.

[a]Remdesivir.

[b]Other drugs included ribavirin, penciclovir, nitazoxanide, nafamostat, remdesivir (GS-5734), and favipiravir (T-705).

TABLE 2 Critical appraisal of the clinical opinions[9]

Checklist	Studies	
	Zhou et al 2020	Colson et al 2020
Is the source of the opinion clearly identified?	Yes	Yes
Does the source of opinion have standing in the field of expertise?	Yes	Yes
Are the interests of the relevant population the central focus of the opinion?	Yes	Yes
Is the stated position the result of an analytical process, and is there logic in the opinion expressed?	Yes	Yes
Is there reference to the extant literature?	Unclear	Unclear
Is any incongruence with the literature/sources logically defended?	Yes	No
Is the opinion supported by peers?	Unclear	Unclear

of 1200 mg, which is equivalent to 750 mg of CQ. HCQ, at a higher dose, may have a more potent antiviral activity as compared to that of CQ. Furthermore, HCQ has a better safety profile due to lower tissue accumulation as compared to CQ. An additional advantage of HCQ is its safety in pregnancy unlike CQ.[15] Thus, if proven beneficial, HCQ may be a prophylactic drug against COVID-19.

Clinical trials are underway to assess the translational impact of the in vitro prophylactic benefits of CQ and HCQ against COVID-19. Five ongoing clinical trials are aiming to assess the prophylactic efficacy of CQ and HCQ, although there is no mention of any planned interim analysis. With the paucity of evidence on the prophylactic use of these drugs, there are additional essential concerns to address. Despite the in vitro antiviral efficacy, CQ has failed to show efficacy in an in vivo guinea pig model of Ebola,[16] and ferret model of Nipah virus[17] and influenza virus.[18] Clinical trials of CQ as prophylaxis failed in influenza[19] despite strong in vitro efficacy.[18] Even in Chikungunya, the viral replication paradoxically enhanced in animal models after CQ administration.[20] In a clinical trial, long-term musculoskeletal symptoms were more frequent in patients treated with CQ as compared to placebo.[20] Another critical concern is the toxicity of these

TABLE 3 Ongoing clinical studies evaluating the prophylactic role of CQ and HCQ against COVID-19 (search conducted on clinicaltrials.gov on 30 March 2020)

Study registration no. (country)	Recruitment status	No. of Centers and study design	Population (volunteers)	Interventional group(s)	Comparison Group(s)	Primary Outcomes
NCT04308668 (USA)	Recruiting	Multi-center randomized parallel group trial	1500 participants (contact or healthcare worker exposed to a patient with COVID-19)	HCQ	Placebo	Incidence and severity of COVID-19
NCT04304053 (Spain)	Recruiting	Multi-center cluster randomized trial	3040 participants (Contacts of patients with COVID-19)	Antiviral treatment and prophylaxis with HCQ	Standard public health measures	Incidence of secondary COVID-19 cases
NCT04303507 (Europe & Asia)	Not yet recruiting	Multi-center randomized parallel group trial	40000 participants (contact or healthcare worker exposed to a patient with COVID-19)	CQ or HCQ	Placebo	Number of symptomatic COVID-19 infections
NCT04318444 (USA)	Not yet recruiting	Community-Based Randomized Clinical Trial	1600 participants (adult household contacts of COVID-19 patients	HCQ	Placebo	Number of participants with symptomatic, lab-confirmed COVID-19
NCT04318015 (Mexico)	Not yet recruiting	Parallel group RCT	400 participants (healthcare workers attending to COVID-19 patients)	HCQ	Placebo	Symptomatic COVID-19

Abbreviations: CQ, chloroquine; HCQ, hydroxychloroquine.

drugs. CQ has a narrow safety margin and may cause several cardiovascular adverse effects, including QT prolongation, as well other unforeseen adverse reactions.[21] HCQ is relatively safer. However, unrestricted acute overdosing of these drugs can lead to serious toxicities. Moreover, these adverse events may get augmented due to potential drug inhibitors like cytochrome P-450 system inhibitors, as well as with other drugs being advocated or evaluated in COVID-19 such as azithromycin[6] and protease inhibitors.[22,23]

In the absence of robust in vivo and clinical evidence, it seems premature to recommend CQ and HCQ as a panacea for prophylaxis of COVID-19. In the current COVID-19 pandemic, quarantine, social distancing, and personal hygiene seem the only proven preventive measures.[24] It is pertinent to mention here that from the regulatory point of view, there is a mixed opinion on the prophylactic use of CQ or HCQ in different countries. Injudicious use of CQ and HCQ in the light of scarcity of evidence may indulge a false sense of protection, hampering the essential precautionary measures by the common masses. Furthermore, the pandemic hysteria leading to unrestricted off-label use of these drugs by the common masses without adhering to the guidelines may lead to deprivation of these essential drugs to other legitimate patients of lupus and rheumatoid arthritis or malaria if production does not match the demand. There are already reports of adverse effects published in newspaper including death and hospitalization.[25] Thus, further prudency is warranted in this regard.

Re-emphasizing the fact that chemoprophylaxis against COVID-19 is the need of the hour, the related socioeconomic issues need to be addressed. There are reports of the ostracization of healthcare workers and other individuals from affected places.[26,27] Hence, targeted prophylaxis of high-risk individuals can serve the purpose of social security apart from health benefits. However, the primary objective of prophylaxis is defied if a drug use, without concrete scientific evidence, leads to mass hysteria and depriving the legitimate population, such as patients with lupus and rheumatoid arthritis, for the use of these drugs.[28] If CQ and HCQ show prophylactic efficacy in ongoing clinical trials, targeted prophylaxis may be recommended over mass prophylaxis in the future.

There are limitations to our study. To date, there is a dearth of adequate data on this topic of interest. Pre-clinical and clinical studies are ongoing, and most likely new information will be added to the existing literature in the near future necessitating updating this review. Notwithstanding these limitations, we have shown that there is absence of clear evidence to support the efficacy of CQ or HCQ in preventing COVID-19.

5 | CONCLUSION

The pandemic COVID-19 has pushed the global healthcare system to a crisis and amounted to a huge economic and societal burden. Prevention of transmission of the disease in the population, particularly among high-risk individuals, is the urgent need of the hour. Different drugs for prophylaxis against COVID-19 including CQ or HCQ have been tried. Although pre-clinical results are promising, to

date there is dearth of good-quality evidence to support the clinical efficacy of CQ or HCQ in preventing COVID-19. Because of the lack of robust clinical evidence to date and duly considering the questionable efficacy, safety concerns, danger of deprivation of these essential drugs to legitimate patients due to panic stocking and instilling a false sense of protection among the common masses, the prophylactic use of CQ or HCQ against COVID-19 needs to be further reviewed as more data pour in.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest associated with this manuscript.

AUTHOR CONTRIBUTIONS

SS and VSN conceptualized the review; SS, SD, and AJ were involved in literature search and study selection; SS and DPM were involved in disagreement resolution and finalization of the included studies; SS, SD, and AJ have extracted data from the studies for qualitative synthesis of evidence; DPM, and VSN have interpreted the analyses; SS, SD, and AJ have drafted the review; DPM and VSN have provided expert inputs and updated the final review.

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REFERENCES

1. World Health Organization. Coronavirus Disease 2019 (COVID-19) Situation Report – 64 [Internet]. 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200324-sitrep-64-covid-19.pdf?sfvrsn=703b2c40_2. Accessed March 25, 2020.
2. Baud D, Qi X, Nielsen-Saines K, Musso D, Pomar L, Favre G. Real estimates of mortality following COVID-19 infection. *Lancet Infect Dis*. 2020. https://doi.org/10.1016/S1473-3099(20)30195-X
3. The Lancet null. COVID-19: protecting health-care workers. *Lancet Lond Engl*. 2020;395:922.
4. Bai Y, Yao L, Wei T, et al. Presumed asymptomatic carrier transmission of COVID-19. *JAMA*. 2020. https://doi.org/10.1001/jama.2020.2565
5. Gao J, Tian Z, Yang X. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends*. 2020;14:72-73.
6. Gautret P, Lagier J-C, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents*. 2020;20:105949.
7. China National Health Commision. Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment (7th edition) [Internet]. http://kjfy.meetingchina.org/msite/news/show/cn/3337.html#
8. Indian Council of Medical Research. Advisory on the Use of Hydroxy-Chloroquine as Prophylaxis for SARS-CoV-2 Infection [Internet]. 2020. https://www.mohfw.gov.in/pdf/AdvisoryontheuseofHydroxychloroquinasprophylaxisforSARSCoV2infection.pdf. Accessed March 24, 2020.
9. McArthur A, Klugárová J, Yan H, Florescu S. Innovations in the systematic review of text and opinion. *Int J Evid Based Healthc*. 2015;13:188-195.
10. Yao X, Ye F, Zhang M, et al. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). *Clin Infect Dis*. 2020:ciaa237. https://doi.org/10.1093/cid/ciaa237
11. Liu J, Cao R, Xu M, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. *Cell Discov*. 2020;6:1-4.
12. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res*. 2020;30:269-271.
13. Zhou D, Dai S-M, Tong Q. COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. *J Antimicrob Chemother*. 2020. [Internet]. [Cited 2020 Mar 24]; Available from https://academic.oup.com/jac/advance-article/doi/10.1093/jac/dkaa114/5810487.
14. Colson P, Rolain J-M, Raoult D. Chloroquine for the 2019 novel coronavirus SARS-CoV-2. *Int J Antimicrob Agents*. 2020;55:105923.
15. Sperber K, Hom C, Chao CP, Shapiro D, Ash J. Systematic review of hydroxychloroquine use in pregnant patients with autoimmune diseases. *Pediatr Rheumatol*. 2009;7:9.
16. Dowall SD, Bosworth A, Watson R, et al. Chloroquine inhibited Ebola virus replication in vitro but failed to protect against infection and disease in the in vivo guinea pig model. *J Gen Virol*. 2015;96:3484-3492.
17. Pallister J, Middleton D, Crameri G, et al. Chloroquine administration does not prevent Nipah virus infection and disease in ferrets. *J Virol*. 2009;83:11979-11982.
18. Vigerust DJ, McCullers JA. Chloroquine is effective against influenza A virus in vitro but not in vivo. *Influenza Other Respir Viruses*. 2007;1:189-192.
19. Paton NI, Lee L, Xu Y, et al. Chloroquine for influenza prevention: a randomised, double-blind, placebo controlled trial. *Lancet Infect Dis*. 2011;11:677-683.
20. Roques P, Thiberville S-D, Dupuis-Maguiraga L, et al. paradoxical effect of chloroquine treatment in enhancing Chikungunya virus infection. *Viruses*. 2018;10:268.
21. Vinetz J. Chemotherapy of malaria. In: Brunton L, Hilal-Dandan R, Knollmann B, eds. *Goodman & Gilman's: The Pharmacological Basis of Therapeutics*, 13th edn. New York, NY: McGraw-Hill Education; 2018:969-986.
22. Cao B, Wang Y, Wen D, et al. A Trial of lopinavir-ritonavir in adults hospitalized with severe COVID-19. *N Engl J Med*. 2020. https://doi.org/10.1056/NEJMoa2001282
23. Mason JW. Antimicrobials and QT prolongation. *J Antimicrob Chemother*. 2017;72:1272-1274.
24. Wilder-Smith A, Freedman DO. Isolation, quarantine, social distancing and community containment: pivotal role for old-style public health measures in the novel coronavirus (2019-nCoV) outbreak. *J Travel Med*. 2020;27:taaa020.
25. Coronavirus: We are now Receiving Patients Suffering from Chloroquine Poisoning, Says Lagos Govt, NCDC Cautions Nigerians [Internet]. Tribune Online. 2020. https://tribuneonlineng.com/coronavirus-we-are-now-receiving-patients-suffering-from-chloroquine-poisoning-says-lagos-govt-ncdc-cautions-nigerians/. Accessed March 24, 2020.
26. CNN HY Natasha Chen and Dushyant Naresh. What's Spreading Faster than Coronavirus in the US? Racist Assaults and Ignorant Attacks Against Asians [Internet]. CNN. https://www.cnn.

com/2020/02/20/us/coronavirus-racist-attacks-against-asian-americans/index.html. Accessed 2020 March 24, 2020.
27. While People Clapped for those in Front Line Fighting Virus, Telangana Landlords Leave Doctors Homeless [Internet]. The New Indian Express. https://www.newindianexpress.com/states/telangana/2020/mar/24/while-people-clapped-for-those-in-front-line-fighting-virus-telangana-landlords-leave-doctors-homele-2120839.html. Accessed March 24, 2020.
28. American College of Rheumatology. Hydroxychloroquine and COVID-19 [Internet]. https://www.rheumatology.org/announcements. Accessed March 25, 2020.

How to cite this article: Shah S, Das S, Jain A, Misra DP, Negi VS. A systematic review of the prophylactic role of chloroquine and hydroxychloroquine in coronavirus disease-19 (COVID-19). *Int J Rheum Dis*. 2020;23:613–619. https://doi.org/10.1111/1756-185X.13842

Received: 6 August 2019 | Revised: 13 December 2019 | Accepted: 3 February 2020

DOI: 10.1111/1756-185X.13816

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases | WILEY

Accelerated immune aging was correlated with lupus-associated brain fog in reproductive-age systemic lupus erythematosus patients

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Abstract

Aims: Cognitive impairment is common in systemic lupus erythematosus (SLE) patients with substantial adverse effects on function and quality of life. One hypothesis to understand the mechanisms of cognitive impairment in SLE is accelerated immunosenescence. The aim of this study is to observe the correlation between immunosenescence with cognitive impairment in patients with SLE.

Methods: Sixty-one female SLE patient were measured for CD4 and CD8 T cell-associated senescence markers, including percentage of end-stage differentiated T cells (CD4 and CD8 T cells expressing $CD57^+$ or loss of CD28 expression), of naïve T cells ($CD4^+CD45RA^+$ and $CD8^+CD45RA^+$), memory T cells ($CD4^+CD45RO^+$ and $CD8^+CD45RO^+$), and antigen-experienced T cells ($CD4^+KLRG1^+$ and $CD8^+KLRG1^+$) which were measured using flow cytometry. One hallmark of immunosenescence called immune risk profile (IRP) was defined by an inverted ratio of CD4 and CD8. Cognitive functions were measured by Mini-Mental State Examination (MMSE) and Montréal Cognitive Assessment (MOCA) questionnaire.

Results: Thirty-six (59.1%) SLE patients who had IRP develop significantly lower attention and recall from both MMSE ($P = .005$ and $P = .000$) and MOCA ($P = .017$ and $P = .000$) examinations. Decreased visuospatial ability was also found in patients with IRP measured by MOCA ($P = .046$). There was a negative correlation between memory $CD4^+CD45RO^+$ T cells with recall and visuospatial domain ($R = -0.204$, $P = .039$ and $R = -0.250$, $P = .033$; respectively), and negative correlation between $CD8^+CD28^-$ T cells with recall and attention domain ($R = -0.249$, $P = .027$ and $R = -0.145$, $P = .048$, respectively).

Conclusion: Systemic lupus erythematosus patients develop an accelerated immunosenescence which contributes to cognitive dysfunction, especially in attention, recall, and visuospatial domains.

KEYWORDS

immunosenescence, lupus-associated brain fog, systemic lupus erythematosus

1 | INTRODUCTION

Neuropsychiatric illness has been associated with systemic lupus erythematosus (SLE) manifestations and remains a paradox both clinically and biologically for SLE patients. Neuropsychiatric SLE (NPSLE) has been classified as several syndromes that contain a wide spectrum of neurological and psychiatric illnesses.[1] "Lupus brain fog" is a common manifestation in SLE patients which is associated with NPSLE. Lupus brain fog is a common patient complaint which refers to forgetfulness and confusion associated with cognitive impairment.[2] Cognitive impairment is one of the central NPSLE syndromes that have delirious effects on the quality of life and individual productivity in SLE patients which occurs in 20%–80% of patients with SLE.[3] However, attributions and mechanisms of cognitive impairment in SLE are extremely difficult.

Previous studies hypothesize that chronic immune activation is the key to the mechanisms of lupus brain fog. Signs for the immunological consequences in the development of NPSLE syndromes are evidence of high titers of pro-inflammatory cytokines and chemokines that have been identified in the cerebrospinal fluid (CSF) of NPSLE patients, including interleukin (IL)-1, IL-6, IL-10, interferon (IFN)α, IFNγ, tumor necrosis factor (TNF)α, B-cell activating factor, and APRIL.[4,5] Some cytokines such as IL-10, TNF-α, IL-6, IFN-γ, IL-4, and IL-13 are also used as predictors that can expect the longer disease duration and poor outcome in NPSLE.[5] Although these findings show the importance of immune activation in NPSLE, whether lupus brain fog is a central nervous system manifestation of the disease that is mediated by an immune response mechanism deserves further research.

A certain condition that contributes to the chronic inflammation condition in SLE is accelerated immune aging. Normally, the human immune system undergoes dynamic age-related changes, which continuously progress during aging, called immunosenescence.[6] However, a chronic immune activation, such as seen in autoimmune disease can accelerate the immunosenescence process.[7] The phenotype of immune aging is characterized by an increase of pro-inflammatory markers, defined as "inflammaging". Up-regulation of pro-inflammatory cytokines, such as IL-1, IL-4, IL-6, and IFN-γ is found in elderly individuals.[8] Chronic immune activation also results in a remodeling of the immune system, including decreasing numbers of naïve T cells, increasing numbers of memory T cells, and increasing numbers of terminally differentiated T cells. These remodelings also contribute to the condition of inflammaging.[9,10] However, it is still poorly understood whether this immune aging condition is correlated with lupus brain fog in SLE patients. Therefore, the aim of this study is to observe the correlation between immune aging with cognitive impairment in patients with SLE.

2 | SUBJECTS AND METHODS

2.1 | Subjects

This study was conducted from October 2017 to August 2018 and the subjects were 61 female SLE patients aged 16-45 years classified by 2012 Systemic Lupus International Collaborating Clinics classification criteria. Subjects who were pregnant and suffered from severe infections were excluded from the study. All subjects were patients who routinely reported to the Rheumatology Clinic, Department of Internal Medicine, Saiful Anwar Hospital and were treated with steroids and immunosuppressants. History and thorough physical examination were carried out on all individuals. All subjects involved in this study signed informed consent forms and all protocols were approved by Saiful Anwar General Hospital Ethics Committee.

2.2 | Peripheral blood mononuclear cells (PBMC) Isolation

We extracted 10-15 cc vein blood from all subjects, then PBMCs were isolated from peripheral blood using Lymphoprep by centrifugation (1600 *g* for 30 minutes). The formed PBMC layer was taken slowly, washed twice with 10 cc phosphate-buffered saline, the supernatant was discarded and centrifuged at room temperature (1200 *g* for 30 minutes).

2.3 | Examination of immune senescence markers

T cell senescence markers were measured using flow cytometry. Monoclonal antibodies used in this study were fluorescein isothiocyanate anti-human CD4 (Biolegend Catalog no. 300506), PerCP anti-human CD8 (Biolegend Catalog no. 344708), Phycoerythrin (PE) anti-human CD28 (Biolegend Catalog no. 302908), PE anti-human CD57 (Biolegend Catalog no. 322312), PE anti-human CD45RA (Biolegend Catalog no. 322312), PE anti-human CD45RO (Biolegend Catalog no. 302908) and Anti-Human KLRG1 Alexa Fluor® 488 (Biolegend Catalog No. 367704). The number of cells was analyzed by BD Cell Quest software. Measurements were made on 10 000 cells and results were obtained in the form of percentages (%) of cells. The patients who had immune risk profile (IRP) were defined as the inversed ratio of CD4 and CD8 percentages.

2.4 | Measurement of cognitive function

Cognitive function of SLE patients was assessed using validated questionnaires. Cognitive function was measured by the Mini-Mental State Examination (MMSE) and Montréal Cognitive Assessment (MOCA) questionnaire. Both questionnaires were the Indonesian version and already validated from a previous study.[11,12] Score from MMSE and MOCA examinations were divided according to the domain of the cognitive functions. Domains in MMSE were orientation, registration, attention, recall, language, and visuospatial, while domains in MOCA were visuospatial, naming, delayed recall, attention, language, abstraction, and orientation.

2.5 | Data analysis

The collected data were analyzed by using SPSS for Windows version 19.0. Comparison between groups was analyzed by unpaired T-test for parametric data and Mann-Whitney for non-parametric data; on the other hand, the correlations between variables were analyzed using Pearson correlation test for parametric data while non-parametric data were analyzed by Spearman. Values of $P < .05$ were considered statistically significant. Parametric data were displayed as mean ± SD while non-parametric data were displayed as median (25th-75th percentile).

3 | RESULTS

3.1 | Characteristics of subjects

Subjects were 61 female patients with SLE, age 15-45 (30.7 ± 10.9) years, with median of the duration of illness 2.8 (2.0-6.3) years. The SLE Disease Activity Index score of the subjects was 2.0-12.0 with a median score of 6.0. The most common clinical manifestations in SLE patients are presented in Table 1.

TABLE 1 Characteristics of the subjects (N = 61)

Variable	
Age, y	30.7 ± 10.9
Duration of illness, y	2.8 (2.0-6.3)
SLEDAI score	6.0 (2.0-12.0)
Clinical manifestations, n (%)	
Neuropsychiatric lupus	6 (9.8)
Nephritis	26 (42.6)
Vasculitis	14 (23.0)
Hemolytic anemia	10 (16.4)
Arthritis	18 (29.5)
Myositis	1 (1.6)
Serositis	11 (18.0)
Fever	28 (45.9)
Thrombocytopenia	14 (22.9)
Leucopenia	15 (24.6)
Mucocutaneous involvement	28 (45.9)
Fatigue	35 (57.3)

Abbreviation: SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

3.2 | Comparison of cognitive functions according to IRP status

IRP was associated with the immune aging condition in individuals. In this study, we assessed the IRP status as an inverse ratio of CD4 and CD8. According to the IRP status, there were 36 SLE patient who were IRP-positive (59.1%). Scores from MMSE and MOCA examinations were divided according to the cognitive domain according to the questionnaires. As shown in Table 2 and Figure 1, patients with IRP had significantly lower of attention (2.1 ± 1.2 vs 3.1 ± 1.4, $P = .005$) and recall (0.8 ± 0.7 vs 1.9 ± 1.1, $P = .000$) in MMSE examinations. Similarly shown in MOCA examinations in Table 3 and Figure 2, patients with IRP also had significantly lower recall (2.0 ± 1.6 vs 3.5 ± 1.4, $P = .000$) and attention (3.9 ± 1.2 vs. 4.7 ± 1.2, $P = .017$). According to the MMSE examination, SLE patients with IRP also showed a slight decrease in the visuospatial domain (4.2 ± 1.0 vs 4.7 ± 0.7, $P = .046$). However, no significant difference was found in other domains in both MMSE and MOCA examinations, including orientation, registration, language, naming, and abstractions.

TABLE 2 Comparison of MMSE domains between systemic lupus erythematosus patients according to IRP status

Domain	Patient with IRP (n = 36)	Patient without IRP (n = 25)	*P*
Orientation	9.4 ± 0.9	9.2 ± 1.1	.530
Registration	2.9 ± 0.3	3.0 ± 0.2	.396
Attention	2.1 ± 1.2	3.1 ± 1.4	**.005**
Recall	0.8 ± 0.7	1.9 ± 1.1	**.000**
Language	6.1 ± 1.2	6.4 ± 1.2	.518
Visuospatial	0.7 ± 0.4	0.9 ± 0.2	**.043**

Abbreviations: IRP, immune risk profile; MMSE, Mini-Mental State Examination. Bold values showed statistically significant comparison with $P < .05$.

3.3 | Correlation of the immunosenescence markers with the cognitive functions

There were other immunosenescence markers beside the IRP which associated with aging in the immune systems. We also examined whether these markers had correlation to the cognitive domains examined by MMSE and MOCA examinations. As shown in Table 4, some $CD4^+$ T cell senescence markers had significant correlations with the cognitive function measured by MMSE. There was a positive correlation between naïve $CD4^+$ T cells ($CD4^+$ $CD45RA^+$) percentages with recall function (R = 0.248, $P = .028$). On the other hand, negative correlations were found between memory T cell ($CD4^+$ $CD45RO^+$) percentages with recall and visuospatial domains (R = −0.204, $P = .039$ and R = −0.250, $P = .033$; respectively). As for $CD8^+$ T cell senescence markers, terminally differentiated $CD8^+$ cell ($CD8^+$ $CD28^-$) percentages had a significant negative correlation with attention and recall (R = −0.255, $P = .024$ and R = −0.326, $P = .005$; respectively). On the other hand, percentages of $CD8^+$ T cell expressed senescence markers $CD57^+$ and $KLRG1^+$ also had significant negative correlation with attention (R = −0.272, $P = .017$ and R = −0.300, $P = .009$; respectively) and recall (R = −0.358, $P = .002$ and R = −0.274, $P = .016$; respectively).

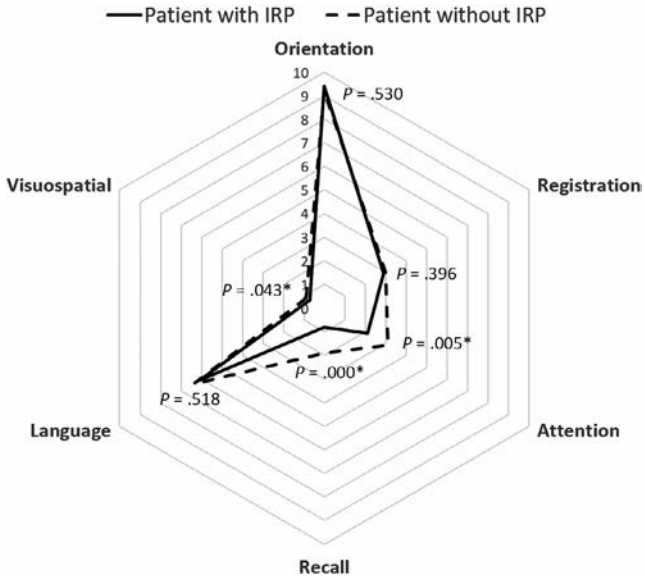

FIGURE 1 Comparison of cognitive domain examined by MMSE according to the IRP status

TABLE 3 Comparison of MOCA domain between systemic lupus erythematosus patients with and without IRP

Domain	Patient with IRP (n = 36)	Patient without IRP (n = 25)	*P*
Visuospatial	4.2 ± 1.0	4.7 ± 0.7	**.046**
Naming	2.9 ± 0.2	2.9 ± 0.2	.834
Delayed Recall	2.0 ± 1.6	3.5 ± 1.4	**.000**
Attention	3.9 ± 1.2	4.7 ± 1.2	**.017**
Language	2.5 ± 0.5	2.6 ± 0.5	.283
Abstraction	1.9 ± 0.4	1.9 ± 0.3	.758
Orientation	5.8 ± 0.4	5.9 ± 0.4	.857

Abbreviations: IRP, immune risk profile; MOCA, Montréal Cognitive Assessment. Bold values showed statistically significant comparison with $P < .05$.

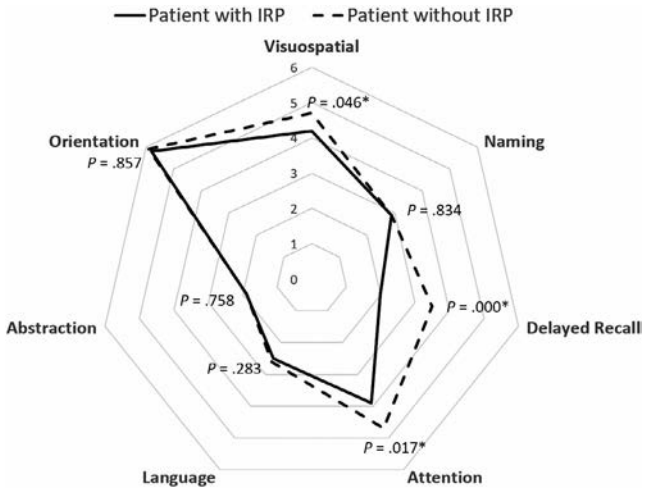

FIGURE 2 Comparison of cognitive domain examined by MOCA according to the IRP status

There were some differences found in the correlation between immunosenescence markers and cognitive domain assessed by MOCA examination (Table 5). The naïve $CD4^+$ T cells ($CD4^+$ $CD45RA^+$) percentages had significant positive correlation with language (R = 0.218, *P* = .047). Memory $CD4^+$ T cell ($CD4^+$ $CD45RO^+$) percentages had significant negative correlation with visuospatial domain (R = −0.377 and *P* = .022) and recall (R = −0.213, *P* = .049). On the other hand, percentages of $CD8^+$ T cell-associated senescence markers, including $CD28^-$ had significant negative correlation with attention (R = −0.145, *P* = .048) and recall (R = −0.249, *P* = .027). In addition, naïve $CD8^+$ T cells ($CD8^+$ $CD45RA^+$) had positive correlation with abstraction (R = 0.232, *P* = .037) while memory $CD8^+$ T cells ($CD8^+$ $CD45RO^+$) had negative correlation with language (R = −0.219, *P* = .045). However, no correlation was found between percentages of CD57 and KLRG1 in $CD4^+$ or $CD8^+$ T cells with cognitive functions examined with MOCA (Table 5).

4 | DISCUSSION

Immunosenescence is a phenomenon of the aging process in immune systems characterized by changes in the innate and adaptive immune system compartments.[10] This process mainly occurs as a natural aging process and can also be found in individuals with chronic inflammatory conditions induced by repeated antigen stimulation in T cells, such as autoimmune diseases.[13] Previous studies showed that early immunosenescence was identified in several autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, and idiopathic juvenile arthritis.[14,15]

However, early immunosenescence was also observed in the relatively younger aged women with SLE as shown in our study. One of the hallmarks for immunosenescence was the presence of IRP, characterized by a reversed ratio of CD4 and CD8.[10] IRP usually presents in individuals with age greater than 80,[16] with a prevalence of about 17%-20%.[17,18] However, our patients who were relatively young in age, the prevalence of IRP was quite high (59.1%). SLE patients presented with IRP also showed decreased cognitive functions, especially the ability to recall and attention.

Not only was there the presence of IRP, but immunosenescence also was associated with other changes, including decreased of naïve T cells, increased of memory T cells, loss of CD28 molecules, and the presence of terminally differentiated cell markers CD57 and KLRG1.[9] Our results showed that some other immunosenescence markers were correlated with the decline of cognitive function. However, there were some inconsistencies found between the examinations from the MMSE and MOCA. In spite of that, some data still showed consistencies, including the negative correlation between memory $CD4^+$ T cells with recall and visuospatial domain, and negative correlation between $CD8^+$ T cells without CD28 molecules with recall and attention domain. Our results are also consistent with a previous study. Serre-Miranda et al revealed that better cognitive performance was associated with lower numbers of effector memory

TABLE 4 Correlation of immunosenescence markers with cognitive domains in MMSE

	MMSE cognitive domain					
	Orientation	**Registration**	**Attention**	**Recall**	**Language**	**Visuospatial**
$CD4^+$ $CD28^-$	R = 0.148 *P* = .131	R = 0.065 *P* = .313	R = 0.002 *P* = .493	R = 0.126 *P* = .170	R = 0.001 *P* = .497	R = −0.076 *P* = .283
$CD4^+$ $CD45RA^+$	R = −0.027 *P* = .418	R = 0.027 *P* = .419	R = 0.131 *P* = .159	**R = 0.248** ***P* = .028**	R = −0.017 *P* = .448	R = 0.066 *P* = .309
$CD4^+$ $CD45RO^+$	R = −0.308 *P* = .011	R = −0.187 *P* = .086	R = −0.212 *P* = .060	**R = −0.240** ***P* = .039**	R = −0.206 *P* = .066	**R = −0.250** ***P* = .033**
$CD4^+$ $CD57^+$	R = 0.084 *P* = .262	R = 0.053 *P* = .342	R = −0.002 *P* = .494	R = −0.073 *P* = .291	R = 0.051 *P* = .350	R = −0.139 *P* = .144
$CD4^+$ $KLRG1^+$	R = 0.108 *P* = .203	R = 0.085 *P* = .258	R = 0.038 *P* = .387	R = −0.042 *P* = .373	R = −0.060 *P* = .322	R = −0.142 *P* = .138
$CD8^+$ $CD28^-$	R = 0.005 *P* = .486	R = −0.076 *P* = .280	**R = −0.255** ***P* = .024**	**R = −0.326** ***P* = .005**	R = −0.019 *P* = .442	R = −0.204 *P* = .058
$CD8^+$ $CD45RA^+$	R = −0.001 *P* = .497	R = −0.122 *P* = .176	R = −0.201 *P* = .061	R = −0.170 *P* = .097	R = −0.087 *P* = .254	R = −0.216 *P* = .069
$CD8^+$ $CD45RO^+$	R = 0.013 *P* = .461	R = 0.029 *P* = .411	R = −0.198 *P* = .063	R = −0.201 *P* = .061	R = −0.051 *P* = .349	R = −0.202 *P* = .059
$CD8^+$ $CD57^+$	R = −0.056 *P* = .334	R = −0.058 *P* = .329	**R = −0.272** ***P* = .017**	**R = −0.358** ***P* = .002**	R = −0.113 *P* = .194	R = −0.237 *P* = .033
$CD8^+$ $KLRG1^+$	R = −0.042 *P* = .375	R = −0.153 *P* = .119	**R = −0.300** ***P* = .009**	**R = −0.274** ***P* = .016**	R = −0.054 *P* = .339	R = −0.367 *P* = .002

Abbreviation: MMSE, Mini-Mental State Examination. Bold values showed statistically significant correlation with $P < .05$.

TABLE 5 Correlation of immunosenescence markers with cognitive domains in MOCA

	MOCA cognitive domain						
	Visuospatial	**Naming**	**Delayed Recall**	**Attention**	**Language**	**Abstraction**	**Orientation**
$CD4^+$ $CD28^-$	R = 0.011 *P* = .467	R = −0.084 *P* = .265	R = −0.027 *P* = .421	R = −0.188 *P* = .077	R = −0.013 *P* = .462	R = −0.056 *P* = .338	R = 0.007 *P* = .479
$CD4^+$ $CD45RA^+$	R = 0.131 *P* = .160	R = 0.054 *P* = .341	R = 0.125 *P* = .170	R = 0.209 *P* = .055	**R = 0.218** ***P* = .047**	R = 0.193 *P* = .070	R = −0.011 *P* = .466
$CD4^+$ $CD45RO^+$	**R = −0.377** ***P* = .002**	R = −0.215 *P* = .058	**R = −0.213** ***P* = .049**	R = −0.206 *P* = .066	R = 0.116 *P* = .199	R = 0.055 *P* = .346	R = 0.085 *P* = .268
$CD4^+$ $CD57^+$	R = −0.048 *P* = .358	R = −0.080 *P* = .271	R = −0.005 *P* = .485	R = −0.057 *P* = .333	R = 0.184 *P* = .079	R = −0.068 *P* = .303	R = −0.030 *P* = .411
$CD4^+$ $KLRG1^+$	R = −0.031 *P* = .406	R = 0.051 *P* = .349	R = 0.061 *P* = .320	R = −0.138 *P* = .144	R = 0.168 *P* = .097	R = 0.047 *P* = .361	R = −0.011 *P* = .467
$CD8^+$ $CD28^-$	R = −0.167 *P* = .099	R = −0.146 *P* = .130	**R = −0.125** ***P* = .048**	**R = −0.249** ***P* = .027**	R = 0.090 *P* = .246	R = 0.024 *P* = .426	R = −0.152 *P* = .121
$CD8^+$ $CD45RA^+$	R = −0.132 *P* = .158	R = 0.059 *P* = .327	R = −0.142 *P* = .140	R = −0.068 *P* = .302	R = 0.216 *P* = .049	**R = 0.232** ***P* = .037**	R = −0.167 *P* = .101
$CD8^+$ $CD45RO^+$	R = −0.128 *P* = .163	R = 0.055 *P* = .337	R = −0.081 *P* = .267	R = −0.174 *P* = .090	**R = −0.219** ***P* = .045**	R = 0.088 *P* = .250	R = 0.052 *P* = .346
$CD8^+$ $CD57^+$	R = −0.154 *P* = .118	R = −0.024 *P* = .426	R = −0.197 *P* = .064	R = −0.171 *P* = .094	R = 0.117 *P* = .184	R = 0.045 *P* = .368	R = −0.170 *P* = .096
$CD8^+$ $KLRG1^+$	R = −0.233 *P* = .042	R = 0.030 *P* = .410	R = −0.139 *P* = .143	R = −0.145 *P* = .133	R = 0.150 *P* = .125	R = 0.122 *P* = .175	R = −0.120 *P* = .179

Abbreviation: MOCA, Montréal Cognitive Assessment. Bold values showed statistically significant correlation with $P < .05$.

$CD4^+$ and $CD8^+$ T cells and B cells. Furthermore, effector memory $CD4^+$ T cells were found to be predictors of decline in general and executive function and memory.[18]

Although it was not clear regarding the mechanisms between immune aging and lupus-associated brain fog, some hypotheses still show that neuroinflammation is the key to the development

of brain fog in lupus.[19] IRP was likely not only shown by the phenotype of the T cells but also characterized by the overproduction of pro-inflammatory cytokines.[8] Interestingly, SLE patients who developed neuropsychiatric symptoms also had higher levels of pro-inflammatory cytokines in their CSF.[4,5] Moreover, $CD4^+$ or $CD8^+$ T cells which lose the CD28 molecule display potent effector functions with oversecretion of inflammatory cytokines IFN-γ and TNF-α.[20]

Both MMSE and MOCA examinations showed significantly lower recall and attention scores in patients with IRP. Complex interaction between psycho-neuro-immunology compartments might play parts in the process of this situation: one of them is microglia. Microglia are resident macrophage-like cells in the brain that not only have surveillance and phagocytic activity, but also are important mediators in the neuron synaptic activity.[19] Some findings revealed that microglia serve as important physiological functions in learning and memory by promoting learning-related synapse formation through brain-derived neurotrophic factor signaling.[21] Microglia also contribute in the regulation of axonal and dendritic growth, promoting relocation and synapse formation, and modulating synaptic plasticity. Therefore, disruption in the microglial function could primarily affect memory formation which we measured as attention and recall.[22] Increasing levels of inflammatory cytokines, such as IL-1, IL-6, and TNF-α which occur in the individuals with IRP could affect the microglia by switching to an inflammatory phenotype with intracerebral amplification of the peripheral inflammatory milieu and resulting in the impairment of memory function.[23]

Our results showed there were weak correlations between the immunosenescence markers and the cognitive functions. It indicated the cognitive impairment in patients with SLE was not only affected by immunosenescence but also influenced by other factors. Tomietto et al revealed that cognitive impairment in SLE was associated with disease activity, antiphospholipid antibody positivity, hypertension, and magnetic resonance imaging lesions.[24] Depression was also a cofounder that influenced cognitive dysfunction in SLE.[17] Another study showed that socio-demographic status, such as educational levels, gender, and marital status was not associated with the cognitive dysfunction in the SLE.[25]

This is the first study that examined the correlation between immune aging with cognitive impairment in SLE. In spite of that, it is still a preliminary study that deserves further research in larger populations, and in a wider range of ages to confirm these initial findings. Further study also needs to find the underlying mechanisms for the role of immunosenescence in affecting cognitive impairment in patients with SLE and also to find other factors that could influence cognitive function in SLE patients.

In conclusion, our study has confirmed the presence of accelerated immune aging in SLE patients. Immunosenescence also correlated with lupus-associated brain fog in SLE. Therefore, investigations of T cell senescence profiles in SLE patients may provide implications for better understanding in SLE clinical manifestations and development of monitoring or treatment strategies for SLE.

ACKNOWLEDGEMENT

We thank Wahyudha, S.Si at Biomedic Laboratory, Brawijaya University for guiding us on flowcytometry analysis.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest regarding this study.

AUTHOR CONTRIBUTIONS

HK: conceptualize the research, supervising, wrote the manuscript. MZP: conceptualize the research, wrote the manuscript. EM: worked the flowcytometry analysis and subject recruitment, wrote the manuscript. ESW: worked the flowcytometry analysis and subject recruitment, wrote the manuscript. PAK: worked the flowcytometry analysis and subject recruitment, wrote the manuscript. KH: conceptualized the research, supervising, wrote the manuscript.

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REFERENCES

1. Liang MH, Corzillius M, Bae SC, et al. The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum*. 1999;42(4):599-608.
2. Hanly JG, Su L, Omisade A, Farewell VT, Fisk JD. Screening for cognitive impairment in systemic lupus erythematosus. *J Rheumatol*. 2012;39(7):1371-1377.
3. Hanly JG, Harrison MJ. Management of neuropsychiatric lupus. *Best Pract Res Clin Rheumatol*. 2005;19(5):799-821.
4. Ichinose K, Arima K, Ushigusa T, et al. Distinguishing the cerebrospinal fluid cytokine profile in neuropsychiatric systemic lupus erythematosus from other autoimmune neurological diseases. *Clin Immunol*. 2015;157(2):114-120.
5. Ichinose K, Arima K, Umeda M, et al. Predictors of clinical outcomes in patients with neuropsychiatric systemic lupus erythematosus. *Cytokine*. 2016;79:31-37.
6. Weyand CM, Goronzy JJ. Aging of the immune system. Mechanisms and therapeutic targets. *Ann Am Thorac Soc*. 2016;13(Supplement 5):S422-S428.
7. Le Saux S, Weyand CM, Goronzy JJ. Mechanisms of immunosenescence-lessons from models of accelerated immune aging. *Ann N Y Acad Sci*. 2012;1247:69-82.
8. Ventura MT, Casciaro M, Gangemi S, Buquicchio R. Immunosenescence in aging: between immune cells depletion and cytokines up-regulation. *Clin Mol Allergy*. 2017;15(1):21.
9. Focosi D, Bestagno M, Burrone O, Petrini M. CD57+ T lymphocytes and functional immune deficiency. *J Leukoc Biol*. 2010;87(1):107-116.
10. Ponnappan S, Ponnappan U. Aging and immune function: molecular mechanisms to interventions. *Antioxid Redox Signal*. 2011;14(8):1551-1585.
11. Sjahrir H, Ritarwan K, Tarigan S, Rambe AS, Lubis ID, Bhakti I. The mini mental state examination in healthy individuals in Medan Indonesia by age and education level. *Neurol J Southeast Asia*. 2001;6:19-22.
12. Husein N, Lumempouw S, Ramli Y. Montreal cognitive assessment versi indonesia (moca-ina) untuk skrining gangguan fungsi kognitif. *Neurona (Majalah Kedokteran Neuro Sains Perhimpunan Dokter Spesialis Saraf. Indonesia)*. 2010;27(4):4-15.
13. Hohensinner PJ, Goronzy JJ, Weyand CM. Telomere dysfunction, autoimmunity, and aging. *Aging Dis*. 2011;2(6):524.

14. Thewissen M, Linsen L, Somers V, Geusens P, Raus J, Stinissen P. Premature immunosenescence in rheumatoid arthritis and multiple sclerosis patients. *Ann N Y Acad Sci*. 2005;1051:255-262.
15. Roy S. Immunosenescence in rheumatoid arthritis: Use of CD28 negative T cells to predict treatment response. *Indian J Rheumatol*. 2014;9(2):62-68.
16. De Martinis M, Franceschi C, Monti D, Ginaldi L. Inflammation markers predicting frailty and mortality in the elderly. *Exp Mol Pathol*. 2006;80(3):219-227.
17. Ho RC, Husain SF, Ho CS. Cognitive dysfunction in patients with systemic lupus erythematosus: the challenge in diagnosis and management. *Rheum Pract Res*. 2018;3:2059902118792434.
18. Serre-Miranda C, Roque S, Santos NC, et al. Effector memory CD4+ T cells are associated with cognitive performance in a senior population. *Neurol Neuroimmunol Neuroinflammation*. 2015;2(1):e54.
19. Mackay M. Lupus brain fog: a biologic perspective on cognitive impairment, depression, and fatigue in systemic lupus erythematosus. *Immunol Res*. 2015;63(1–3):26-37.
20. Dumitriu IE. The life (and death) of CD 4+ CD 28null T cells in inflammatory diseases. *Immunology*. 2015;146(2):185-193.
21. Chen Z, Zhong D, Li G. The role of microglia in viral encephalitis: a review. *J Neuroimmunol*. 2019;16(1):76.
22. Rodríguez-Iglesias N, Sierra A, Valero J. Rewiring of memory circuits: connecting adult newborn neurons with the help of microglia. *Front Cell Dev*. 2019;7:24.
23. Chen J, Buchanan JB, Sparkman NL, Godbout JP, Freund GG, Johnson RW. Neuroinflammation and disruption in working memory in aged mice after acute stimulation of the peripheral innate immune system. *Brain Behav Immun*. 2008;22(3):301-311.
24. Tomietto P, Annese V, D'agostini S, et al. General and specific factors associated with severity of cognitive impairment in systemic lupus erythematosus. *Arthritis Care Res*. 2007;57(8):1461-1472.
25. Butt BA, Farman S, Khan SE, Saeed MA, Ahmad NM. Cognitive dysfunction in patients with Systemic Lupus Erythematosus. *Pakistan J Med Sci*. 2017;33(1):59.

How to cite this article: Kalim H, Pratama MZ, Mahardini E, Winoto ES, Krisna PA, Handono K. Accelerated immune aging was correlated with lupus-associated brain fog in reproductive-age systemic lupus erythematosus patients. *Int J Rheum Dis*. 2020;23:620–626. https://doi.org/10.1111/1756-185X.13816

Received: 17 May 2019 | Revised: 7 December 2019 | Accepted: 3 February 2020

DOI: 10.1111/1756-185X.13808

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases

WILEY

Association between COX-2 and 15-PGDH polymorphisms and SLE susceptibility

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Funding information
This project was supported by the Research Deputy in Zahedan University of Medical Sciences [IR.ZAUMS.REC.1397.322].

Abstract

Aims: Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease. Prostaglandins E2 (PGE2), the product of the cyclo-oxygenase 2 (COX-2) enzyme, has critical roles in the etiology of autoimmune diseases. PGE2 level is controlled by a balance between its synthesis mediator (COX-2 enzyme) and its catabolic key enzyme (15-hydroxyprostaglandin dehydrogenase [15-PGDH] enzyme). In the present study, the associations of genotypic polymorphisms in COX-2 and 15-PGDH with SLE were investigated.

Methods: One hundred and sixty SLE patients and 160 healthy controls participated in the study. The polymerase chain reaction - restriction fragments length polymorphism method was used for genotyping. The COX-2 rs2745557 G/A and 15-PGDH rs8752 G/A polymorphisms were investigated.

Results: Regarding the COX-2 rs2745557 single nucleotide polymorphism, there was no significant association between COX-2 rs2745557 polymorphism and SLE. However, the dominant models showed a marginally significant relation (P = .048, odds ratio = 0.63, 95% CI = 0.4-1.0). Regarding GA genotype of 15-PGDH rd8752 polymorphism, there was a significant difference between two groups with a 4.5-fold increase in SLE development (P = .0001). The frequency of the A allele was higher in SLE patients than that in controls, showing a 1.4-fold increase in SLE development (P = .018).

Conclusion: All results showed the protective effects of the dominant model of COX-2 rs2745557 polymorphism and risk factor of 15-PGDH rs8752 polymorphism on SLE development.

KEYWORDS

15-PGDH, COX-2, polymorphism, systemic lupus erythematosus

1 | INTRODUCTION

Systemic lupus erythematosus (SLE) is a heterogeneous and chronic inflammatory autoimmune disease which has important features, such as autoantibody production against host nuclear antigens and multi-organ inflammation (like skin, kidneys, and brain).[1,2] Its occurrence is more common in women than men.[3] SLE etiology is unknown. Environmental factors, such as UV radiation,

stress, medication and infection have been put forward as possible causes of SLE development;[4] however, genetic factors are proposed as a major cause of SLE development.[5] SLE is a complex disease which has a genetic background; for example, studies have shown the increased risk of SLE in first-degree relatives of affected people.[6]

In autoimmune diseases, there is an impairment in the immune system that can regulate the balance between response to pathogens and avoidance of self-attack, leading to inflammation activation without any infection.[7] SLE leads to a chronic systemic inflammation. In fact, the cooperation between several immunopathogenic factors like cell autoantigens and both cellular and environmental immune parts results in chronic inflammation and tissue damage.[4] The relationship between SLE and some inflammatory mediators, like tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), IL-1 and type I and type II interferons (IFNs) have been described.[8]

Prostaglandins (PGs), the product of the PG endoperoxide H synthase/ cyclo-oxygenase (COX) pathway, have important roles in etiology and inflammation of autoimmune disease. COX has 2 isoforms: COX-1 with permanent expression in most tissues and COX-2 as the inducible isoform at the site of inflammation. Based on its expression mode, it seems that COX-2 has a major role in inflammation and autoimmune disease.[9] PGE2 is a major product of the COX pathway. The PGE2 level is controlled by a balance between its synthesis mediator (COX-2 enzyme) and its catabolic key enzyme (15-hydroxyprostaglandin dehydrogenase [15-PGDH] enzyme).[10] PGE2 regulates the activation of mature B lymphocytes via overproduction of immunoglobulin (Ig)E and IgG.[9] Xu et al showed the upregulation of COX-2 in human lupus T cells causing resistance to energy and apoptosis. Furthermore, they also reported the apoptosis induction in energy-resistant T cells in response to COX-2 inhibition.[11]

Single nucleotide polymorphisms (SNPs) are the most frequent variation in human disease.[12] The COX-2 (*PTGS2*) gene containing 10 exons is located in a lupus susceptibility region on chromosome 1.[13,14] The rs2745557 G/A polymorphism is located in intron 1 of the COX-2 gene.[15]

15-PGDH, the rate-limiting enzyme in prostaglandins catabolism, is a 29 kDa protein with its gene (*HPGD*) is located on chromosome 4 (16). 15-PGDH inactivates the prostaglandins through forming their 15-keto metabolites, so it play a main role in inflammation regulation.[16,17] The *HPGD* rs8752 G/A polymorphism is located in the 3′-UTR (untranslated region) of the gene and is involved in the miR-485-5p binding site.[18]

This study aimed to evaluate, for the first time, the effects of COX-2 rs2745557 G/A and 15-PGDH rs8752 G/A polymorphisms on SLE susceptibility.

2 | MATERIALS AND METHODS

2.1 | Study subjects

The protocol of the present case-control study was approved by the ethical committee of Zahedan University of medical science. Written informed consent was obtained from all participants. This study was conducted on 160 SLE patients (case group) and 160 age-matched controls. Patients with a history of other systemic diseases, malignancy and hypothyroidism were excluded from the study. In the control group, subjects who had a family history of SLE occurrence and a history of other systemic diseases were also excluded from the study.

2.2 | DNA extraction and genotyping

Two milliliters of peripheral blood were collected in ethylenediaminetetraacetic acid-containing tubes. The salting-out and polymerase chain reaction restriction fragments length

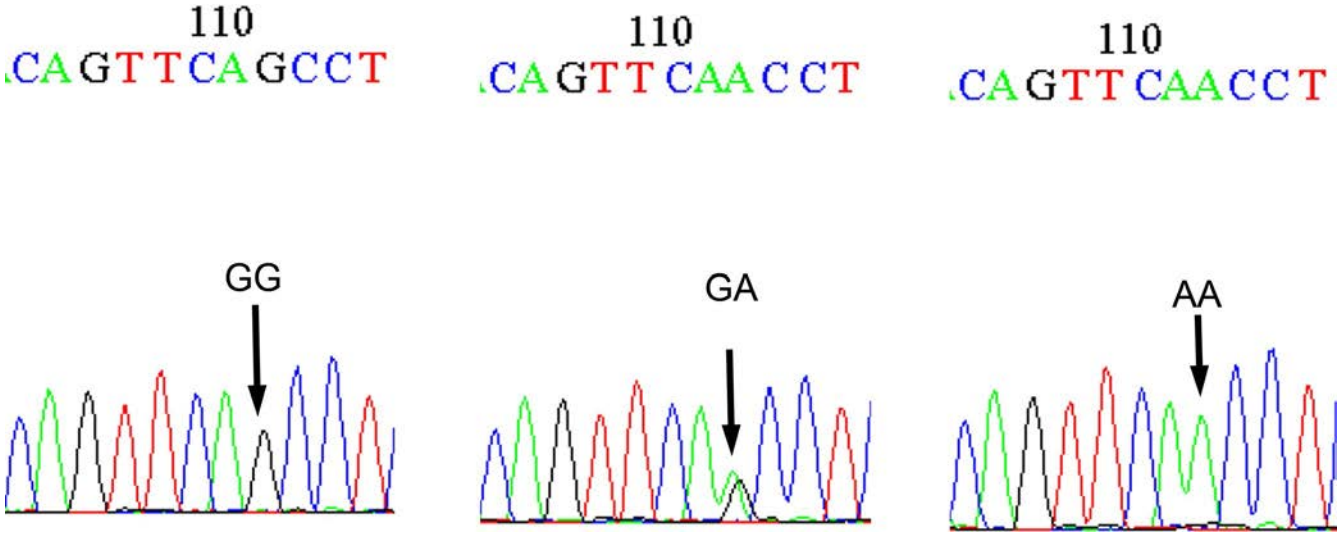

FIGURE 1 Sequencing analysis of 15-hydroxyprostaglandin dehydrogenase rs8752 polymorphism

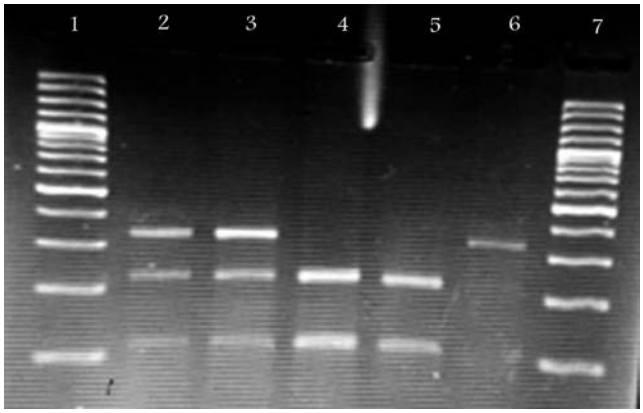

FIGURE 2 Electrophoresis images of polymerase chain reaction restriction fragments length polymorphism (PCR-RFLP) for 15-hydroxyprostaglandin dehydrogenase rs8752 polymorphism. One hundred base-pair DNA ladder (lane 1, 7); GG: 110 + 222 bp (lane 4, 5); GA: 332 + 110+222 bp (lane 2, 3); AA: 332 bp (lane 6)

polymorphism (PCR-RFLP) methods were used for DNA extraction and genotyping, respectively. PCR conditions were: 95°C for 30 seconds, followed by 30 cycles: 95°C (30 seconds), annealing temperature (30 seconds) and 72°C (30 seconds). At the final step there was a final extension at 72°C for 3 minutes. Annealing times were 59 and 54°C for COX-2 and 15-PGDH, respectively. The primer sequences for COX-2 rs2745557 polymorphism genotyping were: F: GAGGTGAGAGTGTCTCAGAT and R: TCTCGGTTAGCGACCAATT.[19] Then, PCR product (439 bp) was digested by Taq I. For the first time, we designed a PCR-RFLP method (confirmed by direct sequencing that is showed in Figure 1) for 15-PGDH rs8752 polymorphism genotyping. The used primers were: F: TTGGGGGCAGTCAAGGAATAAAC and R: AGGGTAGGCACTTTTGAAATTTGG. The PCR product (332 bp) was digested by CviKI-1. Figures 2 and 3 show the sizes of fragments of 15-PGDH and COX-2 polymorphisms, respectively. Genotyping was further confirmed by random samples.

2.3 | Statistical analysis

Data analysis was done using IBM SPSS statistics 23. The independent effect of each polymorphism on SLE risk was calculated using logistic regression. Significant estimation of allele and genotype relations was analyzed by odds ratios (OR) with 95% confidence intervals (CI). Clinical and demographic differences between groups were tested using independent Student's *t* test or Fisher's exact test whenever seasonable. *P* values of <.05 were considered as statistically significant.

3 | RESULTS

The demographic data of participants are reported in Table 1. A total of 320 individuals, including 160 SLE patients (12 male, 148 female, mean age 31.9 ± 5 years) and 160 age-matched healthy controls (13

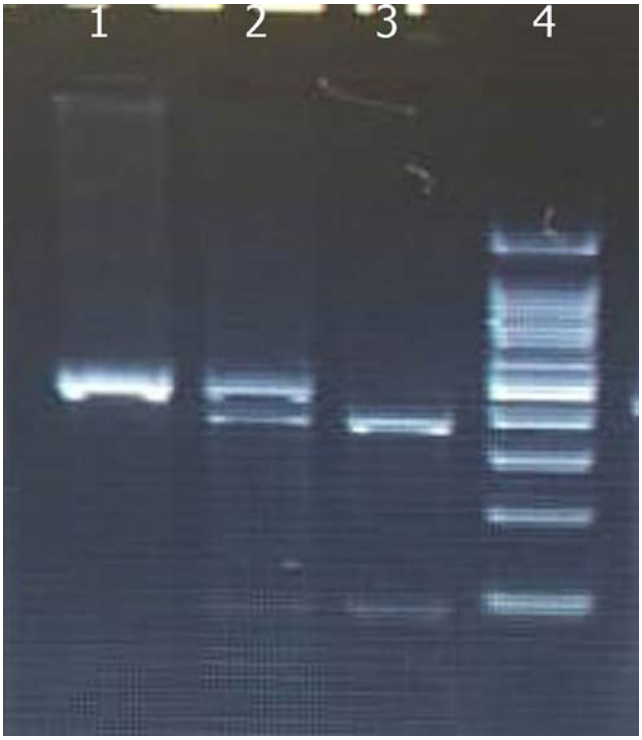

FIGURE 3 Electrophoresis images of polymerase chain reaction restriction fragments length polymorphism (PCR-RFLP) for cyclooxygenase-2 rs2745557 polymorphism. GG: 439 bp (lane 1); GA: 439 + 86+353 bp (lane 2); AA: 86 + 353 bp (lane 3); 100 bp DNA ladder (lane 4)

TABLE 1 Clinical and demographic characteristics of SLE and control groups

	SLE (N = 160)	Controls (N = 160)	*P* value
Age ± SD, y	31.9 ± 5	31.2 ± 4.5	.285
Gender (M/F)	12/148	13/147	.835
Malar rash, n (%)	77 (48.1)		
Photosensitivity, n (%)	79 (49.4)		
Discoid rash, n (%)	6 (3.8)		
Mouth ulcers, n (%)	38 (23.8)		
Hair loss, n (%)	42 (26.3)		
Arthritis, n (%)	116 (72.5)		
Leukopenia, n (%)	30 (18.8)		
Thrombocytopenia, n (%)	35 (21.9)		
Renal disease, n (%)	21 (13.1)		
Antiphospholipid syndrome, n (%)	7 (4.4)		
Anti-double-stranded DNA, n (%)	108 (67.5)		
Antinuclear antibodies, n (%)	111 (69.4)		

Abbreviation: SLE, systemic lupus erythematosus.

TABLE 2 Allelic and genotypic frequencies of COX-2 and 15-PGDH polymorphisms in SLE and control groups

Polymorphism	SLE N (%)	Control N (%)	*P* value*	OR (95% CI)
COX-2 (rs2745557)				
Codominant				
GG	66 (41)	49 (30.6)		1
GA	48 (30)	60 (37.5)	.054	0.59 (0.35-1.01)
AA	46 (29)	51 (31.9)	.148	0.67 (0.38-1.15)
Dominant				
GG	66 (41)	49 (30.6)		1
GA + AA	94 (59)	111 (69.4)	.048	0.63 (0.4-1.0)
Recessive				
GG + GA	114 (71)	109 (68.1)		1
AA	46 (29)	51 (31.9)	.543	0.93 (0.73-1.18)
Over-dominant				
CC + AA	112 (70)	100 (62.5)		1
CA	48 (30)	60 (37.5)	.157	0.71 (0.45-1.13)
Allele				
G	180 (56.2)	158 (49.4)		1
A	140 (43.8)	162 (50.6)	.096	0.75 (0.55-1.03)
15-PGDH (rs8752)				
Codominant				
GG	56 (35)	93 (58.1)		1
GA	69 (43.1)	25 (15.6)	.0001	4.5 (2.6-8.0)
AA	35 (21.9)	42 (26.3)	.254	1.3 (0.79-2.4)
Dominant				
GG	56 (35)	93 (58.1)		1
GA + AA	104 (65)	67 (41.9)	.207	1.3 (0.85-2.1)
Recessive				
GG + GA	125 (78.1)	118 (73.7)		1
AA	35 (21.9)	42 (26.3)	.36	0.78 (0.47-1.3)
Over-dominant				
GG + AA	91 (56.8)	135 (84.3)		1
GA	69 (43.1)	25 (15.7)	.001	2.2 (1.4-3.6)
Allele				
G	181 (56.6)	211 (66)		1
A	139 (43.4)	109 (34)	.018	1.4 (1.07-2.0)

Abbreviations: COX, cyclo-oxygenase; OR, odds ratio; PGDH, hydroxyprostaglandin dehydrogenase; SLE, systemic lupus erythematosus.

male, 147 female, and mean age 31.2 ± 4.5 years) were evaluated. There was no statistically significant difference between the 2 group with respect to age and gender ($P > .05$).

As shown in Table 2, the genotype frequencies of COX-2 rs2745557 polymorphism were GG (30.6%), GA (37.5%) and AA (31.9%) and GG (41%), GA (30%) and AA (29%) for control and SLE groups, respectively. There was no significant association between COX-2 rs2745557 genotypes and SLE. However, the dominant model (GG vs GA + AA) showed a marginally significant association between SLE and COX-2 rs2745557 polymorphism with a 0.63-fold decreased risk of SLE occurrence ($P = .048$, OR = 0.63, 95% CI: 0.4-1.0). Finally, there was no significant difference in the allelic distribution between the two groups ($P = .96$).

The frequencies (%) of 15-PGDH rs8752 polymorphism were GG (58.1), GA (15.6) and AA (26.3) in the control group and GG (35), GA (41.3) and AA (21.9) in the case group (Table 2). However, regarding GA genotype, there was a significant difference between the 2 groups with a 4.5-fold increase in SLE development ($P = .0001$). The over-dominant additive model also showed the same effect on SLE (GG + AA vs GA, $P = .001$, OR = 2.2, 95% CI = 1.4-3.6). Finally, we found a significant

difference between the two group with respect to the allelic distribution, so that the frequency of the A allele was higher in SLE patients (43.3%) than that in controls (34%), showing a 1.4-fold increase in SLE development. (OR = 1.4, 95% CI = 1.07-2.0, P = .018).

4 | DISCUSSION

Chronic inflammatory systemic diseases like SLE can lead to an impairment in immunologic, endocrine, neurologic and reproductive functions.[20] Chronic inflammation in SLE can have several consequences, such as anemia[21] and vasculitis.[22]

Overproduction of proinflammatory cytokines is one of the main hallmarks of SLE, triggering autoantibodies secretion from activated B-cells. Target organ inflammation in SLE is a result of autoantibody production.[23] There is important evidence of a relationship between the PG pathway and SLE inflammation. For example, PGE2, as an inflammatory modulator, and its receptor have an ability to develop autoimmune disease and regulate inflammation mediators, such as TNF-α and IL-6 expression in lupus.[24] Chae et al showed the role of PGE2 on high levels of cytokines in lupus animal models.[23] Furthermore, urinary high levels of PGE2 were also was found in lupus nephritis which was the result of increased activity of COX-2.[25] PGE2 level was regulated by both synthetic (COX-2 enzyme) and catabolic (15-PGDH enzyme) pathways.[10] Human lupus T cells have high levels of COX-2 expression that help them escape from apoptosis; therefore it is proposed that inhibition of COX-2 may be a therapeutic strategy for SLE treatment via induction of apoptosis in lupus autoreactive immune cells.[11] Some COX-2 inhibitors are able to induce apoptosis in autoreactive T cells of lupus and one of the main results of this is reduction of pathogenic anti-DNA autoantibodies.[11]

The relations among polymorphisms of several inflammatory regulatory genes, such as *STAT4*,[26] TNF-α-Induced Protein 3 Interacting Protein 1 (*TNIP1*),[27] Interferon regulatory factor 5 (*IRF5*),[28] interleukin-10 promoter,[29] TNF-α Promoter[30] and SLE were conducted. We evaluated the effect of COX-2 rs2745557 gene polymorphism on SLE. The functional effect of this intronic polymorphism on COX-2 activity is unknown.[15] Our results showed the protective effect of the dominant model of the COX-2 rs2745557 SNP. For the first time, Her et al evaluated the association between COX-2 -765G/C polymorphism and SLE. They found there was no significant correlation between COX-2 -765G/ C polymorphism and SLE risk in a Korean population.[31] Yun et al reported the protective effect of COX-2 6365T/C and -899G/C polymorphisms on rheumatoid arthritis (RA) in Koreans.[32] However, we suggest the need for further research on other populations with different ethnic backgrounds and sample sizes to confirm our results.

Regarding 15-PGDH rs8752 polymorphism, our results indicated an increased risk of developing SLE in precipitants who carried the GA genotype and A allele of 15-PGDH rs8752 polymorphism. So far, the overall effect of the rs8752 polymorphism on 15-PGDH function has not been established; however, this polymorphism is located in the miR-485-5p binding site. He et al reported that the A allele could disrupt the interaction between the miR-485-5p and *HPGD*, leading to overexpression of 15-PGDH. They proposed the *HPGD* variant as a risk factor for breast cancer development in Chinese women.[33] There is little information on the impact of 15-PGDH on autoimmune diseases. Kim et al showed that the expression of 15-PGDH in normal synovium was higher in comparison with RA synovium. The findings of the study also indicated that hydroxychloroquine, an anti-arthritis and anti-lupus drug, induces the expression of 15-PGDH in RA fibroblast-like synoviocytes (RA-FLS).[34]

Several Studies have shown the inverse effects of 15-PGDH (as a tumor suppressor) and COX-2 (as an oncogene) on several cancer cells.[10,35-38] However, we did not find such an inverse relationship in our results, although we showed the risk factor effect of 15-PGDH SNP on SLE development.

5 | CONCLUSION

All results showed the protective effect of the dominant model of COX-2 rs2745557 polymorphism and risk factor effect of 15-PGDH rs8752 polymorphism on SLE development.

ACKNOWLEDGEMENT

The authors acknowledge the Research Deputy of Zahedan University of Medical Sciences for financial support.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

ETHICS APPROVAL

All proceeding performed in the present study containing human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

INFORMED CONSENT

Informed consents were gained from the study subjects. Also, the study protocol was approved by the ethics committee of ZAUMS.

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REFERENCES

1. Bernknopf A, Rak K, Bailey T. A review of systemic lupus erythematosus and current treatment options. 2011:178-194.
2. Kazemipour N, Qazizadeh H, Sepehrimanesh M, Salimi S. Biomarkers identified from serum proteomic analysis for the differential diagnosis of systemic lupus erythematosus. *Lupus*. 2015;24(6):582-587.
3. Podolska MJ, Biermann MH, Maueröder C, Hahn J, Herrmann M. Inflammatory etiopathogenesis of systemic lupus erythematosus: an update. *J Inflamm Res*. 2015;8:161.

4. Gottschalk TA, Tsantikos E, Hibbs ML. Pathogenic inflammation and its therapeutic targeting in systemic lupus erythematosus. *Front Immunol*. 2015;6:550.
5. Mok CC, Lau CS. Pathogenesis of systemic lupus erythematosus. *J Clin Pathol*. 2003;56(7):481-490.
6. Stojan G, Petri M. Epidemiology of systemic lupus erythematosus: an update. *Curr Opin Rheumatol*. 2018;30(2):144-150.
7. Wahren-Herlenius M, Dorner T. Immunopathogenic mechanisms of systemic autoimmune disease. *Lancet (London, England)*. 2013;382(9894):819-831.
8. Ohl K, Tenbrock K. Inflammatory cytokines in systemic lupus erythematosus. *Biomed Res Int Res Int*. 2011;2011:1-14.
9. Myers LK, Kang AH, Postlethwaite AE, et al. The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. *Arthritis Rheum*. 2000;43(12):2687-2693.
10. Mohammadi A, Yaghoobi MM, Gholamhoseinian Najar A, Kalantari-Khandani B, Sharifi H, Saravani M. HSP90 Inhibition Suppresses PGE2 Production via Modulating COX-2 and 15-PGDH Expression in HT-29 Colorectal Cancer Cells. *Inflammation*. 2016;39(3):1116-1123.
11. Xu L, Zhang L, Yi Y, Kang HK, Datta SK. Human lupus T cells resist inactivation and escape death by upregulating COX-2. *Nat Med*. 2004;10(4):411-415.
12. Hashemi M, Moradi N, Ziaee SAM, et al. Association between single nucleotide polymorphism in miR-499, miR-196a2, miR-146a and miR-149 and prostate cancer risk in a sample of Iranian population. *J Adv Res*. 2016;7(3):491-498.
13. Tsao BP. Update on human systemic lupus erythematosus genetics. *Curr Opin Rheumatol*. 2004;16(5):513-521.
14. Dai ZJ, Shao YP, Ma XB, et al. Association of the three common SNPs of cyclooxygenase-2 gene (rs20417, rs689466, and rs5275) with the susceptibility of breast cancer: an updated meta-analysis involving 34,590 subjects. *Dis Markers*. 2014;2014:484729.
15. Zhang H, Xu Y, Zhang Z, Liu R, Ma B. Association between COX-2 rs2745557 polymorphism and prostate cancer risk: a systematic review and meta-analysis. *BMC Immunol*. 2012;13(1):14.
16. Eruslanov E, Kaliberov S, Daurkin I, et al. Altered Expression of 15-Hydroxyprostaglandin Dehydrogenase in Tumor-Infiltrated CD11b Myeloid Cells: A Mechanism for Immune Evasion in Cancer. *J Immunol*. 2009;182(12):7548.
17. Kishore AH, Owens D, Word RA. Prostaglandin E2 regulates its own inactivating enzyme, 15-PGDH, by EP2 receptor-mediated cervical cell-specific mechanisms. *J Clin Endocrinol Metabol*. 2014;99(3):1006-1018.
18. Qi X, Wang Y, Hou J, Huang Y. A Single Nucleotide Polymorphism in HPGD Gene Is Associated with Prostate Cancer Risk. *J Cancer*. 2017;8(19):4083-4086.
19. Wang CH, Wu KH, Yang YL, et al. Association study of cyclooxygenase 2 single nucleotide polymorphisms and childhood acute lymphoblastic leukemia in Taiwan. *Anticancer Res*. 2010;30(9):3649-3653.
20. Straub RH, Schradin C. Chronic inflammatory systemic diseases: An evolutionary trade-off between acutely beneficial but chronically harmful programs. *Evol Med Public Health*. 2016;2016(1):37-51.
21. Kunireddy N, Jacob R, Khan SA, et al. Hepcidin and Ferritin: Important Mediators in Inflammation Associated Anemia in Systemic Lupus Erythematosus Patients. *Indian J Clin Biochem*. 2018;33(4):406-413.
22. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD. Manifestations of systemic lupus erythematosus. *Maedica*. 2011;6(4):330-336.
23. Chae BS, Shin TY, Kim DK, Eun JS, Leem JY, Yang JH. Prostaglandin E2-mediated dysregulation of proinflammatory cytokine production in pristane-induced lupus mice. *Arch Pharm Respharmacal Res*. 2008;31(4):503-510.
24. Akaogi J, Nozaki T, Satoh M, Yamada H. Role of PGE2 and EP receptors in the pathogenesis of rheumatoid arthritis and as a novel therapeutic strategy. *Endocr Metab Immune Disord Drug Targets*. 2006;6(4):383-394.
25. Daza L, Kornhauser C, Zamora L, Flores J. Captopril effect on prostaglandin E2, thromboxane B2 and proteinuria in lupus nephritis patients. *Prostaglandins Other Lipid Mediat*. 2005;78(1–4):194-201.
26. Nageeb RS, Omran AA, Nageeb GS, Yousef MA, Mohammad YAA, Fawzy A. STAT4 gene polymorphism in two major autoimmune diseases (multiple sclerosis and juvenile onset systemic lupus erythematosus) and its relation to disease severity. *Egyptian J Neurol Psychiatr Neurosurg*. 2018;54(1):16.
27. Rizk MM, Elsayed ET. Association of Tumor Necrosis Factor Alpha-Induced Protein 3 Interacting Protein 1 (TNIP1) Gene Polymorphism (rs7708392) with Lupus Nephritis in Egyptian. *Patients*. 2018;56(5):478-488.
28. Hammad A, Mossad YM, Nasef N, Eid R. Interferon regulatory factor 5 gene polymorphism in Egyptian children with systemic lupus erythematosus. *Lupus*. 2017;26(8):871-880.
29. Abdallah E, Waked E, Abdelwahab MA. Evaluating the association of interleukin-10 gene promoter -592 A/C polymorphism with lupus nephritis susceptibility. *Kidney Res Clin Pract*. 2016;35(1):29-34.
30. Yang ZC, Xu F, Tang M, Xiong X. Association Between TNF-alpha Promoter -308 A/G Polymorphism and Systemic Lupus Erythematosus Susceptibility: A Case-Control Study and Meta-Analysis. *Scand J Immunol*. 2017;85(3):197-210.
31. Her MY, El-Sohemy A, Cornelis MC, Kim TH, Bae SC. Cyclooxygenase-2 polymorphisms and risk of systemic lupus erythematosus in Koreans. *Rheumatol Int*. 2006;27(1):1-5.
32. Yun HR, Lee SO, Choi EJ, Shin HD, Jun JB, Bae SC. Cyclooxygenase-2 polymorphisms and risk of rheumatoid arthritis in Koreans. *J Rheumatol*. 2008;35(5):763-769.
33. He N, Zheng H, Li P, et al. miR-485-5p binding site SNP rs8752 in HPGD gene is associated with breast cancer risk. *PLoS ONE*. 2014;9(7):e102093.
34. Kim HJ, Lee S, Lee HY, Won H, Chang SH, Nah SS. 15-hydroxyprostaglandin dehydrogenase is upregulated by hydroxychloroquine in rheumatoid arthritis fibroblast-like synoviocytes. *Mol Med Rep*. 2015;12(3):4141-4148.
35. Tong M, Ding Y, Tai HH. Reciprocal regulation of cyclooxygenase-2 and 15-hydroxyprostaglandin dehydrogenase expression in A549 human lung adenocarcinoma cells. *Carcinogenesis*. 2006;27(11):2170-2179.
36. Tai H-H, Tong M, Ding Y. 15-hydroxyprostaglandin dehydrogenase (15-PGDH) and lung cancer. *Prostaglandins Other Lipid Mediat*. 2007;83(3):203-208.
37. Liu Z, Wang X, Lu Y, et al. Expression of 15-PGDH is downregulated by COX-2 in gastric cancer. *Carcinogenesis*. 2008;29(6):1219-1227.
38. Backlund MG, Mann JR, Holla VR, et al. 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. *J Biol Chem*. 2005;280(5):3217-3223.

How to cite this article: Sandoughi M, Saravani M, Rokni M, Nora M, Mehrabani M, Dehghan A. Association between COX-2 and 15-PGDH polymorphisms and SLE susceptibility. *Int J Rheum Dis*. 2020;23:627–632. https://doi.org/10.1111/1756-185X.13808

Received: 24 May 2019 | Revised: 6 March 2020 | Accepted: 7 March 2020

DOI: 10.1111/1756-185X.13830

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases

WILEY

Use of antimalarial drugs is associated with a lower risk of preeclampsia in lupus pregnancy: A prospective cohort study

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Abstract

Introduction: Several factors have been associated with the development of preeclampsia in women with systemic lupus erythematosus (SLE).

Objective: To identify risk factors associated with preeclampsia in patients with SLE and its impact on fetal outcomes.

Patients and methods: We studied a prospective cohort of pregnancies in women with SLE from January 2009 to December 2018. Demographic, clinical, serological and drug use characteristics were compared between patients who developed preeclampsia and those who did not, as well as the main neonatal outcomes. An adjusted logistic regression analysis was performed to identify factors potentially associated with preeclampsia.

Results: We studied 316 pregnancies of 20 or more weeks of gestation. A total of 46 pregnancies (14.5%) were complicated by preeclampsia. A higher frequency of active disease before pregnancy (24.4% vs 11.3%, $P = .01$) and history of lupus nephritis (56.5% vs 30.1%, $P < .001$) were found in those patients who developed preeclampsia compared to those who did not. Preeclampsia was associated with a higher rate of prematurity, births of very low birth weight, stillbirth, and neonatal death. The multivariate analysis showed that the activity of the disease before (relative risk [RR] 2.7, 95% CI 1.04-7.4, $P = .04$) and during pregnancy (RR 3.0, 95% CI 1.0-9.1, $P = .04$) was associated with the development of preeclampsia. The use of antimalarial drugs during pregnancy was associated with a lower risk of preeclampsia (RR 0.21, 95% CI 0.08-0.53, $P < .001$).

Conclusions: Our study suggests that the use of antimalarial drugs during pregnancy reduces the risk of preeclampsia in lupus pregnancies.

KEYWORDS

antimalarials, preeclampsia, pregnancy, systemic lupus erythematosus

1 | INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease which most often affects women of reproductive age. Although the prognosis of pregnancy in women with SLE has improved, it is still associated with a higher rate of maternal and fetal complications compared with pregnant women in the general population.[1,2] Preeclampsia is a complication more frequently observed in women with SLE compared with healthy women, especially in those with lupus nephritis (with a 2 to 4 times higher risk).[2-5] In retrospective, prospective or population-based studies, preeclampsia has been reported between 10% and 30% of pregnancies in SLE patients.[6-13] The importance of preeclampsia consists of the fact that it is a complication associated with high maternal and fetal morbidity and mortality, mainly in developing countries.[14] In addition, an increased risk of future cardiovascular complications in both newborns and women complicated with preeclampsia has been reported.[15,16]

In women with SLE, several risk factors for developing preeclampsia have been described. These factors include a decreased glomerular filtration rate before conception, proteinuria at the beginning of pregnancy, elevation of blood pressure in the first trimester,[6] the presence of antiphospholipid antibodies (lupus anticoagulant and anti-β-2 glycoprotein 1),[7,8] history of lupus nephritis,[9,13] chronic hypertension,[9] active SLE before pregnancy,[9] thrombocytopenia[9] and elevated creatinine.[10] The different risk factors identified seem to vary according to the different methodological designs and populations studied. Therefore, the aim of the present study was to identify potential factors associated with preeclampsia in women with SLE and its impact on fetal outcomes.

2 | PATIENTS AND METHODS

Since January 2009, we established a prospective cohort of pregnant patients with SLE. All patients included in the cohort met the 1997 American College of Rheumatology revised criteria for the classification of SLE.[17] We excluded those patients who started their illness during the pregnancy being analyzed. A woman was considered to have antiphospholipid syndrome (APS) if she met the 2006 international consensus statement on an update of the classification criteria for definitive APS.[18] Patients were included from the first trimester of pregnancy and they were evaluated every 4-6 weeks and during the first 3 months postpartum. At each medical visit, a detailed clinical history was obtained to search for clinical manifestations of disease activity or potential complications related to pregnancy. Registered laboratory studies included blood count, blood chemistry, urinalysis, and 24-hour urine proteinuria. The immunological studies obtained included serum complements C3 and C4 (performed by nephelometry), anti-double-stranded DNA (anti-dsDNA) antibodies, anti-Sjögren's syndrome antigen A (anti-SSA)/Ro antibodies, SSB/La antibodies, anticardiolipin antibodies (performed by enzyme-linked immunosorbent assay) and lupus anticoagulant (detected by diluted Russell viper venom time [dRVVT] and the activated partial thromboplastin time [aPTT]). Immunological studies were performed during the first trimester of pregnancy; anti-dsDNA antibodies and serum complement were also determined in the second and third trimesters. Detailed information was also obtained on the drugs used by patients before and during pregnancy.

2.1 | Maternal and fetal outcomes

The main outcome studied was the development of preeclampsia. Other maternal outcomes studied included gestational hypertension, SLE flares, premature rupture of membranes (PROM), cesarean section, and maternal death; the fetal outcomes studied included live births rate, preterm birth, miscarriage, stillbirth, birth weight, low birth weight and weeks of gestation according to previously established definitions in previous reports. In short, the definitions of each complication are the following.

1. Gestational hypertension was considered when elevation of blood pressure >140/90 mm Hg was found after week 20 of gestation in the absence of proteinuria.[19]
2. Preeclampsia was defined according to WHO criteria as hypertension (either a systolic blood pressure >140 mm Hg or a diastolic blood pressure >90 mm Hg) plus proteinuria (>300 mg protein in a 24-hour urine collection) after 20 weeks gestation; for the diagnosis of eclampsia, seizures had to be present.[19] Early preeclampsia was considered if presenting at 34 weeks of gestation or earlier.
3. PROM was defined as the loss of the integrity of chorioamniotic membranes before the onset of labor.[20]
4. It was considered that one patient developed a relapse of SLE according to a definition previously used in another article.[21,22] This definition considers a new onset or the worsening of specific and associated cutaneous manifestations of SLE (acute rash, mucosal ulcers, skin vasculitis), arthritis (>1 affected joint), hematological involvement (1 or more hemocytopenias not attributed to immunosuppressive drugs including leukopenia <3000/mm^3, autoimmune thrombocytopenia <100 × 109/L, and hemolytic anemia), central nervous system involvement (such as seizures in absence of preeclampsia, transverse myelitis, psychosis, organic brain syndrome), cardiopulmonary affection (such as pleuritis, pneumonitis, diffuse alveolar hemorrhage, pericarditis) and renal manifestations (active urinary sediment, worsening or new development of proteinuria [>500 mg/24 h], elevated serum creatinine in association with low serum complement and/or elevated titers of anti-dsDNA antibodies). We also determined the SLE Disease Activity Index score adapted for pregnancy (SLEPDAI) and we considered that a woman had disease activity if she had a score higher than 3.[23]
5. Maternal death was considered according to the WHO definition as the death of a woman during pregnancy, at delivery or within 42 days after termination, due to any cause related to or

aggravated by pregnancy, delivery or puerperium or its management, but not to accidental causes.[24]
6. Preterm birth was considered such in the case of a baby born alive before the 37th week of gestation.[25]
7. Stillbirth was considered when intrauterine fetal death occurred after 20 weeks of gestation.[26]
8. Low birth weight baby was defined as one born with a weight <2500 g.[27]
9. Very low birth weight baby was defined as one born with a weight <1500 g.[27]
10. Congenital anomalies were defined according to WHO Classification of Disease (ICD) codes.[28]

2.2 | Statistical analysis

For the analysis, patients were allocated into 2 groups, with and without preeclampsia. For the analysis, each pregnancy was considered as a separate event. Continuous variables are presented as mean and standard deviation or median with range according to the distribution after performing the Kolmogorov-Smirnov test and were compared with Student's *t* or Mann-Whitney *U* tests. The categorical and ordinal variables are presented in number and percentage and were compared using Chi-square or Fisher's exact test. The sample size calculation was performed based on the results of a recently published study that found that 7.5% of patients exposed to hydroxychloroquine developed preeclampsia compared to 19.7% in those unexposed patients.[29] With these data, using an alpha error of 0.05 and a beta error of 0.2, a minimum of 196 pregnancies were obtained to have a statistical power of 80%. The multivariate analysis with binary logistic regression was performed to identify potential independent risk factors for preeclampsia. The variables with $P < .05$ in the multivariate analysis and those with clinical relevance for each outcome studied were included in the stepwise regression models and $P < .05$ was considered statistically significant. Potential predictors of preeclampsia were analyzed using the Cox regression model. We also perform a mediation analysis to adjust the use of antimalarial drugs by SLE activity.[30] All analyses used the program package SPSS version 23.0 (IBM).

3 | RESULTS

3.1 | Patient characteristics at the time of inclusion into the cohort

Until December 2018, a total of 351 pregnancies in 317 patients were included in our cohort. Of the 351 pregnancies included, 316 of them exceeded 20 weeks of gestation, which are the ones analyzed in this study. Of these 316 pregnancies, 46 (14.5%) were complicated by preeclampsia and 270 were not (85.5%). Of the pregnancies that had preeclampsia, 27 (58.6%) had early-onset preeclampsia. A higher frequency of active disease before pregnancy (24.4% vs 11.3%, $P = .01$) and history of lupus nephritis (56.5% vs 30.1%, $P < .001$) was found in those patients who developed preeclampsia compared to those who did not (Table 1). Of the 131 patients with a history of lupus nephritis, 71 of them (57.7%) had renal biopsy. WHO class III (n = 15, 21.1%) and IV (n = 43, 60.5%) lupus nephritis were the most frequent in the patients, with a similar distribution in both groups. Serum complement levels were lower in women complicated with preeclampsia compared to those who were not. Positive lupus anticoagulant was detected in 7/17 women with preeclampsia and in 34/59 patients without preeclampsia ($P = .27$). The median of pregnancies in the patients was 2 in both groups ($P = .89$). Of the patients with at least

TABLE 1 Characteristics of the patients at the diagnosis and during the pregnancy studied

	No preeclampsia (N = 270)	Preeclampsia (N = 46)	*P* value
Age (y)[a]	28 ± 6	29 ± 6	.30
Age at SLE diagnosis (y)[a]	21 ± 7	22 ± 6	.28
SLE duration (y)[a]	6.8 ± 5.5	6.5 ± 4.8	.75
Childhood-onset SLE[b]	73 (27)	10 (21.7)	.57
Primigravida[b]	121 (44.5)	22 (47.8)	.67
Previous active SLE[b]	30 (11.3)	11 (24.4)	.01
History of lupus nephritis[b]	82 (30.1)	56 (56.5)	<.001
Glomerular filtration rate[a]	106.2 ± 34.7	92.6 ± 39.9	.16
Proteinuria[a]	0.36 ± 0.84	0.85 ± 1.0	.055
History of antiphospholipid syndrome[b]	40 (14.9)	10 (22.2)	.21
Anticardiolipin positive[b]	68 (26.6)	13 (28.3)	.81
Anti-SSA/Ro positive[b]	68 (25.7)	8 (18.6)	.32
Anti-dsDNA positive[b]	94 (35.9)	22 (50)	.07
Serum C3[a]	105.8 ± 29.6	89.7 ± 30.3	.004
Serum C4[a]	16.8 ± 9.6	12.2 ± 8.4	.009
Medications during pregnancy			
Prednisone[a]	191 (71)	40 (88.9)	.01
Prednisone, mg daily[b]	10.7 ± 12.7	16.5 ± 15.6	.02
Low dose of aspirine[a]	93 (36.8)	22 (52.4)	.055
Antimalarial drug[a]	229 (84.8)	30 (68.2)	.007
Azathioprine[a]	112 (41.8)	29 (63)	.07
Low molecular weight heparin[a]	38 (15.5)	7 (18.4)	.64

Abbreviations: Anti-dsDNA, anti-double-stranded DNA; Anti-SSA, anti-Sjögren's syndrome A; SLE, systemic lupus erythematosus.

[a]Results expressed in mean (standard deviation).

[b]Results expressed in numbers and percentages.

1 previous pregnancy, 12.7% had a history of preeclampsia. Only 2.8% of the patients had a history of smoking, but none reported active smoking during the pregnancy studied. Thirty per cent of women had a body mass index higher than 25 in the first trimester of pregnancy with a similar distribution in both groups.

3.2 | Maternal evolution

During pregnancy, a higher percentage of patients who developed preeclampsia took prednisone (88.9% vs 71%, *P* = .01), and at a higher dose compared to those who did not develop it (Table 2). Interestingly, patients who did not develop preeclampsia received antimalarial drugs more frequently compared to those who did (84.8% vs 68.2%, *P* = .007). All patients took chloroquine (doses of 150-225 mg daily) during pregnancy, except for 3 who took hydroxychloroquine (200-400 mg daily). Other drugs, such as azathioprine, low doses of aspirin and low molecular weight heparin had a similar distribution in both groups (Table 1). In patients who developed preeclampsia, a higher rate of SLE flare (65.1% vs 50%, *P* = .03), especially lupus nephritis flare (53.5% vs 26.6%, *P* = .007), was observed compared to those who did not. Arthritis flare was observed more frequently in the group of patients without preeclampsia compared to those with it (16.2% vs 0%, *P* = .01). Mean SLEPDAI in each trimester of pregnancy was higher in women with preeclampsia compared to those without it (2 vs 1, 2 vs 1, 3 vs 1, *P* < .05, in the first, second, and third trimesters respectively). The majority of pregnancies complicated by preeclampsia were resolved by cesarean section (93.2% vs 68.9%, *P* = .001). Two maternal deaths were observed in the group of patients without preeclampsia, attributed to severe activity of SLE and disseminated infection.

TABLE 2 Pregnancy outcome in systemic lupus erythematosus patients

	No preeclampsia (N = 270)	Preeclampsia (N = 46)	*P* value
Maternal complications[a]	75 (27.7)	26 (56.5)	<.001
Any flare clinical[a]	135 (50)	28 (65.1)	.03
Arthritis flare[a]	22 (16.2)	0 (0)	.01
Hematological flare[a]	12 (8.8)	4 (8.6)	.48
Renal flare[a]	36 (26.6)	15 (53.5)	.007
Premature rupture of membranes[a]	34 (12.5)	1 (2.1)	.04
Cesarean section[a]	177 (58.8)	41 (93.2)	.001
Maternal death[a]	2 (0.7)	0 (0)	.26
Fetal complications[a]	93 (34.4)	31 (67.3)	<.001
Prematurity[a]	74 (27.4)	34 (73.9)	<.001
Stillbirth[a]	7 (2.5)	4 (8.6)	.05
Neonatal death[a]	4 (1.4)	3 (6.5)	.03
Weeks' gestation[b]	38 (23-42)	33 (21-40)	.01
Birth weight (g)[c]	2597 ± 592	1747 ± 820	.004
Low birth weight[a]	70 (25.9)	13 (28.2)	.72
Very low birth weight[a]	13 (4.8)	18 (39.1)	<.001
Intrauterine growth restriction[a]	19 (7.0)	3 (6.5)	.80
Apgar score at minute 1[b]	8 (0-9)	7 (1-9)	.003
Apgar score at minute 5[b]	9 (4-10)	9 (3-9)	.01

[a]Results expressed in numbers and percentages.

[b]Results expressed in median (ranges).

[c]Results expressed in mean (standard deviation).

3.3 | Effect of preeclampsia on fetal outcomes

As expected, newborns from mothers complicated with preeclampsia had a higher number of complications compared to those not affected (67.3% vs 34.4%, *P* = .001). As shown in Table 2, babies born to patients complicated with preeclampsia had a higher frequency of prematurity (73.9% vs 27.4%, *P* = .001), stillbirth, neonatal death (6.5% vs 1.4%, *P* = .03), low birth weight, and very low birth weight (39.1% vs 4.8%, *P* < .001) compared to those born from patients without preeclampsia. In addition, the babies of patients affected by preeclampsia were born with fewer weeks of gestation and with lower Apgar scores at minutes 1 and 5 compared to those not affected (Table 2). No major congenital anomalies were detected. There were also no cases of neonatal lupus.

3.4 | Multivariate analysis

The results of the multivariate analysis showed that active disease before conception (RR 2.7, 95% CI 1.04-7.4, *P* = .04) as well as active disease during pregnancy (RR 3.0, 95% CI 1.0-9.1, *P* = .04) were risk factors associated with the development of preeclampsia (Table 3). On the other hand, the use of antimalarial drugs during pregnancy was the only one associated with a lower risk of developing

TABLE 3 Results of the multivariate analysis considering preeclampsia as a variable outcome

Variable	Relative risk	95% CI	*P*
Previous active SLE	2.7	1.04-7.4	.04
History of lupus nephritis	2.0	0.83-4.8	.12
SLE flare	3.0	1.0-9.1	.04
Prednisone during pregnancy	2.0	0.6-6.5	.21
Antimalarial drug during pregnancy	0.21	0.08-0.53	.001
Azathioprine during pregnancy	0.76	0.29-2.0	.58

Abbreviation: SLE, systemic lupus erythematosus.

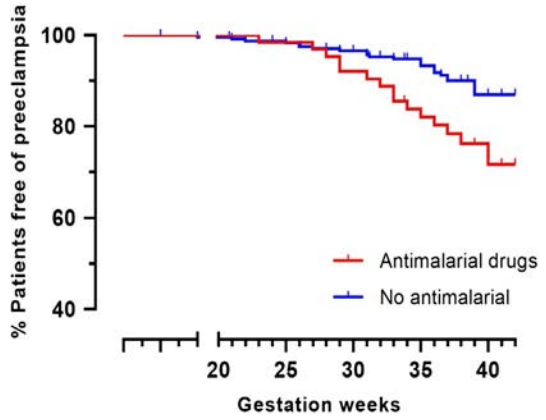

FIGURE 1 Kaplan-Meier curve showing the probability of patients remaining free of preeclampsia in women exposed or not to antimalarial drugs (P = .023, Cox regression analysis)

preeclampsia (RR 0.21, 95% CI 0.08-0.53, P < .001). After performing the analysis adjusted for SLE activity, the protective effect of antimalarial drugs on preeclampsia was maintained (RR 0.13, 95% CI 0.07-0.27, P < .001). Additionally, delivery free of preeclampsia in patients who took and did not take antimalarial drugs was evaluated through the Kaplan-Meier method and analyzed by log-rank test. The Kaplan-Meier curve showed that pregnancies exposed to an antimalarial drug were more likely to end up free of preeclampsia compared to those not exposed (Figure 1).

4 | DISCUSSION

Our results support recent information about antimalarial drugs reducing the risk of developing preeclampsia in women with SLE.[29] On the other hand, active disease before and during pregnancy is associated with an increased risk of developing preeclampsia, as it has been previously reported.[9,31] Additionally, the deleterious effects that preeclampsia have on the main fetal outcomes are confirmed. The National Institute for Health and Clinical Excellence (NICE) and the American College of Obstetricians and Gynecologists (ACOG) consider patients with SLE or APS as "high risk" to develop preeclampsia.[32] Other known risk factors for preeclampsia include kidney disease, diabetes mellitus, family history of preeclampsia, obesity, hypertension, nulliparity, multiple pregnancies, and age over 40, among others.[32]

Preeclampsia is a complex disorder.[32] Multiple risk factors, including genetic ones, have been proposed as the origin of preeclampsia. There are important advances in understanding the mechanisms involved in the development of preeclampsia and it is now accepted that early and late preeclampsia have different pathophysiologies. It has been reported that early preeclampsia is more frequent in women with SLE.[33] Alterations of the spiral arteries, aberrant placentation, imbalance of angiogenic factors (such as placental growth factor [PIGF], soluble fms-like tyrosine kinase 1 [sFlt-1], vascular endothelial growth factor [VEGF], and endoglin), endothelial dysfunction, and a dysregulation of the immune response are some of the mechanisms involved in the pathogenesis of preeclampsia.[32,34-39] In fact some of these angiogenic factors are potential biomarkers for predicting preeclampsia in patients with SLE and APS, such as endoglin, sFlt-1 and PIGF, because these factors are progressively elevated in patients who are complicated with preeclampsia.[38,39] In addition, it has been found that type I interferon can generate an antiangiogenic environment, so that high levels of this cytokine may contribute to the pathogenesis of preeclampsia in some patients with SLE.[40]

Among the different factors described associated with preeclampsia in SLE, we only identified the activity of the disease before or during pregnancy as a risk factor, as we have observed with a smaller number of patients.[41] Our results are consistent with prospective and retrospective studies published in other populations where SLE activity is a risk factor associated with preeclampsia.[9,31] The patients who developed preeclampsia had higher frequency of lupus nephritis; however, the multivariate analysis did not confirm that it was a significant risk factor as has been previously reported.[9,13] A probable explanation is that most of the patients did not present active lupus nephritis at the time of conception. In fact, it has been described that the state of lupus nephritis before pregnancy and not only the history of it, is a predictor of adverse maternal-fetal outcomes,[42] including early fetal loss, which does not allow a pregnancy beyond the second trimester. Other factors related to renal involvement, such as proteinuria, elevated creatinine or decreased glomerular filtration rate at the beginning of pregnancy have been associated with preeclampsia in patients with SLE.[6,43] These variables were not identified in our patients, perhaps due to the small number of patients with these alterations at the beginning of pregnancy. It has also been reported that abnormalities of Doppler waves of the uterine artery are associated with preeclampsia in patients with SLE or APS.[44] We did not analyze these alterations due to a lack of studies on all our patients.

With the exception of the termination of pregnancy, therapeutic options in preeclampsia are limited.[32,36] That is why the use of therapeutic interventions aimed at avoiding this complication is desirable. Several studies have shown the benefit of the use of low doses of aspirin in the prevention of early-onset preeclampsia in women at risk.[45] In pregnant women with SLE, the use of low doses of aspirin before 16 weeks gestation is also recommended as a preventive treatment for preeclampsia, although the available information is limited.[46-49] In fact, in our study we did not find that low doses of aspirin decreased the risk of developing preeclampsia. A recently published meta-analysis found that low molecular weight heparin can reduce the risk of developing preeclampsia in high-risk women.[50] The benefit of heparin on obstetric outcomes can be explained by various mechanisms of action. In our cohort, nevertheless, the use of low molecular weight heparin was not associated with a lower risk of preeclampsia. Some studies suggest that metformin or pravastatin may be useful in the prevention of preeclampsia although more information is necessary.[36] In our study, none of the patients were exposed to any of these medications during their pregnancies.

Due to its beneficial effects and good safety profile, the use of antimalarial drugs during gestation in women with SLE has increased in the present century.[51,52] Several studies have shown the beneficial effects of hydroxychloroquine on maternal-fetal outcomes, including decrease of lupus flares, prematurity rates, intrauterine growth restriction and even improvement in the rate of fetal loss.[53-58] However, information on the role of antimalarial drugs in preeclampsia is more limited. A randomized clinical trial with a small number of pregnant patients with SLE showed that those who did not receive hydroxychloroquine during pregnancy had a higher frequency of toxemia compared to those who did receive the drug, although the article does not provide further details.[59] More recently, a retrospective study of 151 pregnancies in 121 Korean women with SLE showed that the use of hydroxychloroquine was associated with a lower risk of developing preeclampsia (OR 0.106, CI 0.017-0.671).[29] Our results are consistent with this last report but with a greater number of pregnancies studied prospectively. It is worth mentioning that, despite the benefits described above, the reported use of antimalarial drugs during pregnancy ranges from 10% to 75% in contemporary studies.[8-9,11-12,29,54-55,60-63] In addition, a recently published Canadian study showed that the use of antimalarial drugs was stopped in up to 30% of patients during pregnancy. That is why we believe that our results reinforce the importance of maintaining antimalarial drugs during pregnancy.[64]

From the theoretical point of view, different effects of antimalarial drugs may explain their benefit in reducing the risk of developing preeclampsia.[65] Anti-inflammatory, antioxidants and vascular protective effects are well-known mechanisms of action of the antimalarial drugs that can influence the pathophysiology of preeclampsia.[65] Cytokines such as tumor necrosis factor alpha (TNFα) and interleukin-17 have been implicated in the pathogenesis of preeclampsia.[66] It has been found that hydroxychloroquine reduces the production of TNFα and endothelin 1 (ET-1) in an in vitro model of preeclampsia, which influences endothelial dysfunction.[67] An alteration in the balance of Th17/Treg cells has also been described in women with preeclampsia.[66] In this regard, it has been found that chloroquine can help restore the Th17/Treg balance through a mechanism of inhibition of autophagy in both an in vitro model of non-pregnant patients with SLE and in vivo studies of MRL/lpr mice.[68] As previously mentioned, type I interferon could play a role in the development of preeclampsia in women with SLE[42]; it is also known that hydroxychloroquine can inhibit the production of this cytokine in plasmacytoid dendritic cells of patients with SLE.[69]

The impact of preeclampsia on fetal outcomes has been previously described. Newborns from mothers who have suffered preeclampsia have an increased risk of presenting neonatal complications, such as growth restriction, prematurity, respiratory distress, sepsis and among others, intrauterine death.[32] In a retrospective study of 111 pregnancies in Chinese patients with SLE, it was found that preeclampsia was associated with fetal loss and preterm delivery.[70] In our study we found that newborns from pregnancies complicated with preeclampsia have a higher rate of complications, including prematurity and very low birth weight. It is known that newborns with these complications have a higher risk of hospitalization and neonatal death, as our results show.

Our study has some limitations. It is a cohort of a single center, therefore our results cannot be generalized. Nonetheless, the prospective cohort design of a significant number of patients allows for good study power. In addition, when performing the analysis adjusted for disease activity (the main co-variable associated with the development of preeclampsia), the beneficial effect of antimalarial drugs persisted. Many of our patients do not have the non-autoimmune risk factors traditionally described in the development of preeclampsia,[32] so we cannot establish the role of antimalarial drugs in preventing this complication in this group of women. But as previously commented, SLE is by itself a disease that confers a high risk of preeclampsia.[32] The vast majority of our patients took chloroquine during pregnancy, instead of hydroxychloroquine, which is the antimalarial drug most frequently used worldwide because of the lower risk of "non-serious" side effects. In our health system, the only antimalarial drug dispensed is chloroquine. However, pharmacologically both drugs show similar efficacy, so our results could be extrapolated to the use of hydroxychloroquine.[71,72] It has been suggested that serum levels of antimalarial drugs may be important on their pharmacological effect, which may reflect in part the therapeutic adherence. We did not measure the levels of antimalarial drugs in our patients, hence we do not know if the levels were adequate, although the benefit of measuring them has not been conclusively demonstrated.[73]

In conclusion, our findings suggest that antimalarial drugs may be useful in reducing the risk of developing preeclampsia in patients with SLE. It is then warranted to investigate the use of antimalarial therapy in women at high risk of developing preeclampsia.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Miguel Ángel Saavedra, study design, preparation, review and approval of the final manuscript. Dafhne Miranda-Hernández, preparation, review and approval of the final manuscript. Alejandra Lara-Mejía, preparation, review and approval of the final manuscript. Antonio Sánchez, preparation, review and approval of the final manuscript. Sara Morales, review and approval of the final manuscript. Claudia Cruz-Reyes, review and approval of the final manuscript. Pilar Cruz-Domínguez, statistical analysis and approval of the final manuscript. Luis Javier Jara, preparation, review and approval of the final manuscript.

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REFERENCES

1. Clowse ME, Jamison M, Myers E, James AH. A national study of the complications of lupus in pregnancy. *Am J Obstet Gynecol*. 2008;199:127.e1-127.e6.

2. Bundhun PK, Soogund MZ, Huang F. Impact of systemic lupus erythematosus on maternal and fetal outcomes following pregnancy: a meta-analysis of studies published between years 2001–2016. *J Autoimmun*. 2017;79:17-27.
3. Saavedra MA, Cruz-Reyes C, Vera-Lastra O, et al. Impact of previous lupus nephritis on maternal and fetal outcomes during pregnancy. *Clin Rheumatol*. 2012;31:813-819.
4. Spinillo A, Beneventi F, Locatelli E, et al. The impact of unrecognized autoimmune rheumatic diseases on the incidence of preeclampsia and fetal growth restriction: a longitudinal cohort study. *BMC Pregnancy Childbirth*. 2016;16:313.
5. Wu J, Ma J, Zhang WH, Di W. Management and outcomes of pregnancy with or without lupus nephritis: a systematic review and meta-analysis. *Ther Clin Risk Manag*. 2018;14:885-901.
6. Bramham K, Hunt BJ, Bewley S, et al. Pregnancy outcomes in systemic lupus erythematosus with and without previous nephritis. *J Rheumatol*. 2011;38:1906-1913.
7. Borella E, Lojacono A, Gatto M, et al. Predictors of maternal and fetal complications in SLE patients: a prospective study. *Immunol Res*. 2014;60:170-176.
8. Moroni G, Doria A, Giglio E, et al. Maternal outcome in pregnant women with lupus nephritis. A prospective multicenter study. *J Autoimmun*. 2016;74:194-200.
9. Phansenee S, Sekararithi R, Jatavan P, Tongsong T. Pregnancy outcomes among women with systemic lupus erythematosus: a retrospective cohort study from Thailand. *Lupus*. 2018;27:158-164.
10. Mecacci F, Simeone S, Cirami CL, et al. Preeclampsia in pregnancies complicated by systemic lupus erythematosus (SLE) nephritis: prophylactic treatment with multidisciplinary approach are important keys to prevent adverse obstetric outcomes. *J Matern Fetal Neonatal Med*. 2017;27:1-7.
11. Wu J, Ma J, Bao C, Di W, Zhang WH. Pregnancy outcomes among Chinese women with and without systemic lupus erythematosus: a retrospective cohort study. *BMJ Open*. 2018;8:e020909.
12. Chen D, Lao M, Zhang J, et al. Fetal and maternal outcomes of planned pregnancy in patients with systemic lupus erythematosus: a retrospective multicenter study. *J Immunol Res*. 2018;2018:2413637.
13. Rodrigues BC, Lacerda MI, Ramires de Jesús GR, et al. The impact of different classes of lupus nephritis on maternal and fetal outcomes: a cohort study of 147 pregnancies. *Lupus*. 2019;28:492-500.
14. Mayrink J, Costa ML, Cecatti JG. Preeclampsia in 2018: revisiting concepts, physiopathology, and prediction. *Sci World J*. 2018;2018:6268276.
15. Grandi SM, Filion KB, Yoon S, et al. Cardiovascular disease-related morbidity and mortality in women with a history of pregnancy complications. *Circulation*. 2019;139:1069-1079.
16. Thilaganathan B, Kalafat E. Cardiovascular system in preeclampsia and beyond. *Hypertension*. 2019;73:522-531.
17. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. *Arthritis Rheum*. 1997;40:1725.
18. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definitive antiphospholipid syndrome (APS). *J Thromb Haemost*. 2006;4:295-306.
19. Malik R, Kumar V. Hypertension in pregnancy. *Adv Exp Med Biol*. 2017;956:375-393.
20. Duff P. Premature rupture of the membranes in term patients: induction of labor versus expectant management. *Clin Obstet Gynecol*. 1998;41:883-891.
21. Saavedra MÁ, Miranda-Hernández D, Sánchez A, et al. Pregnancy outcomes in women with childhood-onset and adult-onset systemic lupus erythematosus: a comparative study. *Rheumatol Int*. 2016;36:1431-1437.
22. Ruperto N, Hanrahan LM, Alarcón GS, et al. International consensus for a definition of disease flare in lupus. *Lupus*. 2011;20:453-462.
23. Buyon JP, Kalunian KC, Ramsey-Goldman R, et al. Assessing disease activity in SLE patients during pregnancy. *Lupus*. 1999;8:677-684.
24. St Pierre A, Zaharatos J, Goodman D, Callaghan WM. Challenges and opportunities in identifying, reviewing, and preventing maternal deaths. *Obstet Gynecol*. 2018;131:138-142.
25. Frey HA, Klebanoff MA. The epidemiology, etiology, and costs of preterm birth. *Semin Fetal Neonatal Med*. 2016;21:68-73.
26. Blencowe H, Cousens S, Bianchi Jassir F, et al. National, regional, and worldwide estimates of stillbirth rates in 2015, with trends from 2000: a systematic analysis. *Lancet Glob Health*. 2016;4:e98-108.
27. Cutland CL, Lackritz EM, Mallett-Moore T, et al. Low birth weight: case definition & guidelines for data collection, analysis, and presentation of maternal immunization safety data. *Vaccine*. 2017;35:6492-6500.
28. World Health Organization. ICD-11 International Classification of Diseases 11th Revision [Internet]. April 8, 2018. https://icd.who.int/en. Accessed October 8, 2019.
29. Seo MR, Chae J, Kim YM, et al. Hydroxychloroquine treatment during pregnancy in lupus patients is associated with lower risk of preeclampsia. *Lupus*. 2019;28:722-730.
30. Lange T, Hansen KW, Sørensen R, Galatius S. Applied mediation analyses: a review and tutorial. *Epidemiol Health*. 2017;39:e2017035.
31. Skorpen CG, Lydersen S, Gilboe IM, et al. Influence of disease activity and medications on offspring birth weight, pre-eclampsia and preterm birth in systemic lupus erythematosus: a population-based study. *Ann Rheum Dis*. 2018;77:264-269.
32. Armaly Z, Jadaon JE, Jabbour A, Abassi ZA. Preeclampsia: novel mechanisms and potential therapeutic approaches. *Front Physiol*. 2018;9:973.
33. Lokki AI, Heikkinen-Eloranta JK, Laivuori H. The immunogenetic conundrum of preeclampsia. *Front Immunol*. 2018;9:2630.
34. Simard JF, Arkema EV, Nguyen C, et al. Early-onset preclampsia in lupus pregnancy. *Paediatr Perinat Epidemiol*. 2017;31:29-36.
35. Geldenhuys J, Rossouw TM, Lombaard HA, Ehlers MM, Kock MM. Disruption in the regulation of immune responses in the placental subtype of preeclampsia. *Front Immunol*. 2018;9:1659.
36. Phipps EA, Thadhani R, Benzing T, Karumanchi SA. Pre-eclampsia: pathogenesis, novel diagnostics and therapies. *Nat Rev Nephrol*. 2019;15:275-289.
37. Valencia-Ortega J, Zárate A, Saucedo R, Hernández-Valencia M, Cruz JG, Puello E. Placental proinflammatory state and maternal endothelial dysfunction in preeclampsia. *Gynecol Obstet Invest*. 2019;84:12-19.
38. Mayer-Pickel K, Stern C, Eberhard K, Lang U, Obermayer-Pietsch B, Cervar-Zivkovic M. Angiogenic factors in pregnancies of women with antiphospholipid syndrome and systemic lupus erythematosus. *J Reprod Immunol*. 2018;127:19-23.
39. Leaños-Miranda A, Campos-Galicia I, Berumen-Lechuga MG, et al. Circulating angiogenic factors and the risk of preeclampsia in systemic lupus erythematosus pregnancies. *J Rheumatol*. 2015;42:1141-1149.
40. Andrade D, Kim M, Blanco LP, et al. Interferon-α and angiogenic dysregulation in pregnant lupus patients who develop preeclampsia. *Arthritis Rheumatol*. 2015;67:977-987.
41. Saavedra MA, Sánchez A, Morales S, Navarro-Zarza JE, Ángeles U, Jara LJ. Primigravida is associated with flare in women with systemic lupus erythematosus. *Lupus*. 2015;24:180-185.
42. Larosa M, Del Ross T, Calligaro A, et al. Clinical outcomes and predictors of maternal and fetal complications in pregnancies of

patients with systemic lupus erythematosus. *Exp Rev Clin Immunol*. 2019;15(6):617-627.
43. Imbasciati E, Tincani A, Gregorini G, et al. Pregnancy in women with pre-existing lupus nephritis: predictors of fetal and maternal outcome. *Nephrol Dial Transplant*. 2009;24:519-525.
44. Rodríguez-Almaraz ME, Herraiz I, Gómez-Arriaga PI, et al. The role of angiogenic biomarkers and uterine artery Doppler in pregnant women with systemic lupus erythematosus or antiphospholipid syndrome. *Pregnancy Hypertens*. 2018;11:99-104.
45. Shanmugalingam R, Hennessy A, Makris A. Aspirin in the prevention of preeclampsia: the conundrum of how, who and when. *J Hum Hypertens*. 2019;33:1-9.
46. Schramm AM, Clowse ME. Aspirin for prevention of preeclampsia in lupus pregnancy. *Autoimmune Dis*. 2014;2014:920467.
47. Mendel A, Bernatsky SB, Hanly JG, et al. Low aspirin use and high prevalence of pre-eclampsia risk factors among pregnant women in a multinational SLE inception cohort. *Ann Rheum Dis Dec*. 2018;20:pii: annrheumdis-2018-214434.
48. Saavedra Salinas MÁ, Barrera Cruz A, Cabral Castañeda AR, et al. Mexican College of Rheumatology practical guidelines for the care of pregnant patients with autoinmune rheumatic diseases. Part 2. *Reumatol Clin*. 2015;11:295-304.
49. Rambaldi MP, Weiner E, Mecacci F, Bar J, Petraglia F. Immunomodulation and preeclampsia. *Best Pract Res Clin Obstet Gynaecol*. 2019;6934(19):87-96.
50. Wang X, Gao H. Prevention of preeclampsia in high-risk patients with low-molecular-weight heparin: a meta-analysis. *J Mater Fetal Neonat Med*. 2018;20:1-7.
51. Kaplan YC, Ozsarfati J, Nickel C, Koren G. Reproductive outcomes following hydroxychloroquine use for autoimmune diseases: a systematic review and meta-analysis. *Br J Clin Pharmacol*. 2016;81:835-848.
52. Bermas BL, Kim SC, Huybrechts K, et al. Trends in use of hydroxychloroquine during pregnancy in systemic lupus erythematosus patients from 2001 to 2015. *Lupus*. 2018;27(6):1012-1017.
53. Clowse ME, Magder L, Witter F, Petri M. Hydroxychloroquine in lupus pregnancy. *Arthritis Rheum*. 2006;54:3640-3647.
54. Koh JH, Ko HS, Kwok SK, Ju JH, Park SH. Hydroxychloroquine and pregnancy on lupus flares in Korean patients with systemic lupus erythematosus. *Lupus*. 2015;24:210-217.
55. Eudy AM, Siega-Riz AM, Engel SM, et al. Effect of pregnancy on disease flares in patients with systemic lupus erythematosus. *Ann Rheum Dis*. 2018;77:855-860.
56. Leroux M, Desveaux C, Parcevaux M, et al. Impact of hydroxychloroquine on preterm delivery and intrauterine growth restriction in pregnant women with systemic lupus erythematosus: a descriptive cohort study. *Lupus*. 2015;24:1384-1391.
57. Mekinian A, Lazzaroni MG, Kuzenko A, et al. The efficacy of hydroxychloroquine for obstetrical outcome in anti-phospholipid syndrome: data from a European multicenter retrospective study. *Autoimmun Rev*. 2015;14:498-502.
58. Sciascia S, Hunt BJ, Talavera-Garcia E, Lliso G, Khamashta MA, Cuadrado MJ. The impact of hydroxychloroquine treatment on pregnancy outcome in women with antiphospholipid antibodies. *Am J Obstet Gynecol*. 2016;214:273.e1-273.e8.
59. Levy RA, Vilela VS, Cataldo MJ, et al. Hydroxychloroquine (HCQ) in lupus pregnancy: double-blind and placebo-controlled study. *Lupus*. 2001;10:401-404.
60. Tedeschi SK, Massarotti E, Guan H, Fine A, Bermas BL, Costenbader KH. Specific systemic lupus erythematosus disease manifestations in the six months prior to conception are associated with similar disease manifestations during pregnancy. *Lupus*. 2015;12:1283-1292.
61. Kroese SJ, de Hair MJH, Limper M, et al. Hydroxychloroquine use in lupus patients during pregnancy is associated with longer pregnancy duration in preterm births. *J Immunol Res*. 2017;2017:2810202.
62. Zhan Z, Yang Y, Zhan Y, Chen D, Liang L, Yang X. Fetal outcomes and associated factors of adverse outcomes of pregnancy in southern Chinese women with systemic lupus erythematosus. *PLoS ONE*. 2017;12:e0176457.
63. Khan A, Thomas M, P K SD. Pregnancy complicated by systemic lupus erythematosus and its outcome over 10 years. *J Obstet Gynaecol*. 2018;38:476-481.
64. Zusman EZ, Sayre EC, Aviña-Zubieta JA, De Vera MA. Patterns of medication use before, during and after pregnancy in women with systemic lupus erythematosus: a population-based cohort study. *Lupus*. 2019;28(10):1205-1213.
65. Abd Rahman R, DeKoninck P, Murthi P, Wallace EM. Treatment of preclampsia with hydroxychloroquine: a review. *J Matern Fetal Neonatal Med*. 2018;31:525-529.
66. Saavedra MA, Romo-Rodríguez R, Gutiérrez-Ureña SR, Miranda-Hernández D, Hernández-Cruz LI, Jara LJ. Targeted drugs in spondyloarthritis during pregnancy and lactation. *Pharmacol Res*. 2018;136:21-28.
67. Rahman R, Murthi P, Singh H, et al. The effects of hydroxychloroquine on endothelial dysfunction. *Pregnancy Hypertens*. 2016;6:259-262.
68. An N, Chen Y, Wang C, et al. Chloroquine autophagic inhibition rebalances Th17/Treg-mediated immunity and ameliorates systemic lupus erythematosus. *Cell Physiol Biochem*. 2017;44:412-422.
69. Sacre K, Criswell LA, McCune JM. Hydroxychloroquine is associated with impaired interferon-alpha and tumor necrosis factor-alpha production by plasmacytoid dendritic cells in systemic lupus erythematosus. *Arthritis Res Ther*. 2012;14:R155.
70. Liu J, Zhao Y, Song Y, et al. Pregnancy in women with systemic lupus erythematosus: a retrospective study of 111 pregnancies in Chinese women. *J Matern Fetal Neonatal Med*. 2012;25:261-266.
71. Rainsford KD, Parke AL, Clifford-Rashotte M, Kean WF. Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. *Inflammopharmacology*. 2015;23:231-269.
72. Plantone D, Koudriavtseva T. Current and future use of chloroquine and hydroxychloroquine in infectious, immune, neoplastic, and neurological diseases: a mini-review. *Clin Drug Investig*. 2018;38:653-671.
73. Balevic SJ, Cohen-Wolkowiez M, Eudy AM, Green TP, Schanberg LE, Clowse MEB. Hydroxychloroquine levels throughout pregnancies complicated by rheumatic disease: implications for maternal and neonatal outcomes. *J Rheumatol*. 2019;46:57-63.

How to cite this article: Saavedra MÁ, Miranda-Hernández D, Lara-Mejía A, et al. Use of antimalarial drugs is associated with a lower risk of preeclampsia in lupus pregnancy: A prospective cohort study. *Int J Rheum Dis*. 2020;23:633–640. https://doi.org/10.1111/1756-185X.13830

Received: 21 March 2019 | Revised: 4 December 2019 | Accepted: 3 February 2020

DOI: 10.1111/1756-185X.13809

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases | WILEY

Genetic basis of relapsing polychondritis revealed by family-based whole-exome sequencing

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Funding information
Guangdong Natural Science Funds for Distinguished Young Scholar, Grant/Award Number: 2014A030306039; High-level personnel of special support program for Technology Innovative Talents and the Top Young of Guangdong Province, Grant/Award Number: 2015TQ01R516; Distinguished Young Scholar Candidates Programme for The Third Affiliated Hospital of Sun Yat-Sen University; Pearl River Nova Program of Guangzhou, Grant/Award Number: 201610010005

Abstract

Aim: Genetic factors are believed to be implicated in the pathogenesis of relapsing polychondritis (RP). However, the molecular genetic determinants remain to be elucidated. This study aimed to detect the susceptibility genes of RP with whole-exome sequencing (WES) in a Chinese family and deepen our understanding of the pathogenesis of RP thereafter.

Method: A 32-year-old Chinese female proband with RP and her family including her mother with RP were enrolled in the study. The genomic DNA of 6 human subjects was extracted from peripheral blood and then gene allele mutations were identified using WES. Candidate variants with low frequency (<0.1%) in the general population and predicted deleterious effects on gene function were identified. Sanger sequencing was applied subsequently to confirm the analyzed gene variants in 12 human blood samples.

Results: Nine single nucleotide polymorphism variants from different genes were identified to associate with RP by WES and further confirmed by Sanger sequencing, including Ring finger protein 207 (*RNF207*), collagen type XXII alpha 1 chain (*COL22A1*) rs200464636, glycosylphosphatidylinositol anchor attachment 1 (*GPAA1*) rs201424010, recQ like helicase 4 (*RECQL4*) rs757703895, folliculin (*FLCN*) NM_144606: c.G838A: p.E280K, DNA ligase 3 (*LIG3*) rs761808558, NM_207396: c.T425C:p.I142T, myosin heavy chain 15 (*MYH15*) NM_014981: c.G4462A: p.A1488T, purkinje cell protein 2 (*PCP2*) rs144974437 and coiled-coil domain containing 61 (*CCDC61*) rs777816675.

Conclusions: This study suggests that coinheritance of multigene mutation may contribute to RP predisposition. The candidate genes mutated which we discovered are potential targets for in-depth functional studies.

KEYWORDS

genetics, relapsing polychondritis, whole-exome sequencing

1 | INTRODUCTION

Relapsing polychondritis (RP) is a rare systemic disease, which is characterized by recurrent episodes of inflammation, manifested in ears, nose, larynx, tracheobronchial tree and the cardiovascular system.[1,2] It was first reported by Jaksch-Wartenhorst in 1923 as polychondropathia, and the definition "relapsing polychondritis" was first used by Pearson et al in the 1960s.[3] Its annual incidence is estimated at 3.5 per million, and RP is most frequently observed between the ages of 40 and 60 years.[1,4] It occurs equally in both genders.[2] Previous studies demonstrated that genetic and immunological factors are involved in the pathogenesis of RP. Lang et al showed that juman leukocyte antigen (HLA)-DR4 associates with RP by analyzing DR4 antigen frequency in 41 RP patients as compared with 204 unrelated controls (56.1% vs 25.5%, $P < .001$).[5] Terao et al stated that HLA-DRB1*16:02, HLA-DQB1*05:02 and HLA-B*67:01 ally with the susceptibility of RP, but they have no association with clinical phenotypes.[6] Meanwhile, they found that HLA-DR4 shows a trend of susceptibility association without significance. Immune responses against cartilage components, including collagens, matrilin-1, and cartilage oligomeric matrix protein, play a key role in the development of RP.[7] However, family occurrence has yet to be reported and it is also needed to be investigated if specific molecular genetic factors may associate with RP. Therefore, we focused on a Chinese family with a female RP proband by the recently developed whole-exome sequencing (WES) approach, which has been used extensively to reveal the genetic basis of rare diseases.

2 | PATIENTS AND METHODS

2.1 | Study subjects

A 32-year-old Chinese woman with RP and her family, including her mother with RP, were enrolled into the study. RP patients met the diagnostic criteria of McAdam et al.[2] Data about demographic and clinical features were collected by questionnaire and reviewed by well-experienced physicians. This study was approved by the Medical Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University, and permission and signed informed consent by all participants were obtained.

2.2 | Sample collection and genomic DNA extraction

Ethylenediaminetetraacetic acid-K2 anti-coagulated venous blood samples (5 mL per person) were collected from the proband and her family. Extraction of genomic DNA was performed strictly according to the manufacturer's protocol using the QIAamp DNA Blood Mini Kit (Qiagen).

2.3 | Library preparation and sequencing

According to the manufacturer's protocols, DNA from those donors whose individual IDs were L0001-L0006 was used to perform the whole-exome capture using Agilent SureSelect^{XT} Human All exon V5 kit (Agilent). Paired-end DNA library was prepared for the demands of sequencing. The adapter-modified genomic DNA fragments were enriched by polymerase chain reaction (PCR). DNA sequences of exons from genes were then captured and further applied for quality evaluation. In the final step, pooled samples were sequenced as PE150 on Illumina Hiseq 1500 platform (Illumina) according to the manufacture's protocols. The coverage of target region and the average sequencing depth are shown in Tables S1 and S2.

2.4 | Sequence alignment, variant calling and annotation

Sequencing adapters and low-quality reads were removed by using Cutadapt (v1.7.1). FastQC package was used to access the quality of sequencing. High-quality reads were aligned to the human reference genome (hg19) using Burrows-Wheeler Aligner (BWA, v0.7.12). Duplicated reads (PCR-derived duplicates) were removed using Picard tools (v1.1.127). Genome Analysis Toolkit (GATK, v3.4) was used to perform indel realignment and base recalibration in order to improve alignment accuracy. Variants were called by using HaplotypeCaller module in Genome Analysis Toolkit (GATK, v3.4) following the GATK best practice of variant detection workflow and then annotated by ANNOVAR (2015, Dec 14). Variants with read depth DP < 8 or MQ < 20 were viewed as low-quality variants and filtered. Variants were selected as candidates for subsequent assays based on the following criteria: (1) variants were found with allele frequency < 0.5% in the general population in all of the databases including 1000 genome project, Gnomad, kaviar, and in-house data (composing of 841 individuals); (2) variants were annotated to affect amino acid sequences (non-synonymous or gain/loss of stops) or located in splicing regions; (3) variants were predicted as "damaging" effect by at least 2 databases, including SIFT, PolyPhen2 HDIV, PolyPhen2 HVAR, LRT, MutationTaste, MutationAssessor, FATHMM, GERP++, PhyloP and SiPhy. The detailed process of quality control is shown in Figure S1.

2.5 | Sanger sequencing

Specific primers targeted at mutated alleles of 34 genes were designed with primer 3 software and primer sequences are listed in Table S3. The PCRs were performed in a 25 μL reaction system which contained 50 ng genomic DNA, 0.5 μL of each primer (20 μmol/L), 12.5 μL premix (R040A, Takara Bio Inc), and sterilized distilled water under the following conditions: denaturation at 98°C for 1 minute, followed by 35 thermal cycles which were composed of 98°C for 10 seconds, 65°C for 15 seconds, and 72°C for 30 seconds.

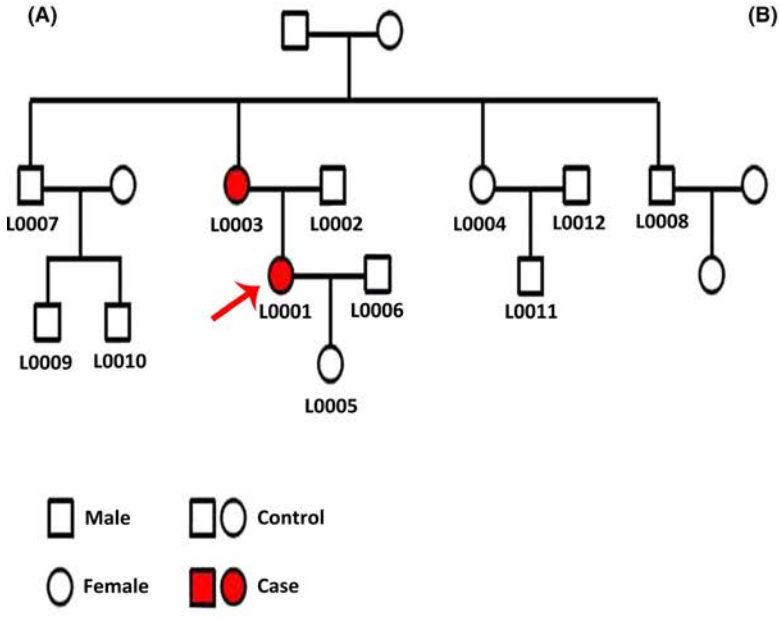
The bands of PCR products were collected if they were unique. Otherwise, the bands were collected following 2% agarose purification. Thereby they were used for big-dye terminator (BDT) reaction, which included 1 μL primer (3.2 μm), 1 μL BDT, plate and deionized water. The total volume was 5 μL. It was vortexed, centrifuged and then PCR amplificated. It was purified by magnetic beads and detected by 3730XL sequencer (Applied Biosystems). The final peak figure was exported.

3 | RESULTS

We recruited a 32-year-old female proband and her family, in which her mother was also a RP patient. The family pedigree and general conditions including gender and age are shown in Figure 1. Genomic DNA from the participants whose individual IDs were L0001-L0006 were used for WES. Mean coverage reached up to 99.9% of the targeted bases, and 91.9% for 20× sequencing depth and 70.1% for 50× sequencing depth of WES (Tables S1 and S2). We first analyzed the genetic backgrounds of the members of the studied family using principal component analysis (PCA) and found their genetic backgrounds were likely attributed to Han Chinese in Beijing, China (CHB) (Figure S2). After sequence alignment, variant calling and candidate filtering, 37 single nucleotide polymorphisms (SNPs) and 1 deletion from different genes were found in patients with RP (Table S4). To validate the analysis of WES, Sanger sequencing was used to analyze the mutated locus of the variants Ring finger protein 207 (*RNF207*), collagen type XXII alpha 1 chain (*COL22A1*) rs200464636, glycosylphosphatidylinositol anchor attachment 1 (*GPAA1*) rs201424010, recQ like helicase 4 (*RECQL4*) rs757703895, folliculin (*FLCN*) NM_144606: c.G838A: p.E280K, DNA ligase 3 (*LIG3*) rs761808558, NM_207396: c.T425C:p.I142T, were mutated in 2 patients with RP and the proband's daughter (individual ID: L0005), but were not changed in the other 9 healthy donors. Myosin heavy chain 15 (*MYH15*) NM_014981: c.G4462A: p.A1488T, purkinje cell protein 2 (*PCP2*) rs144974437 and coiled-coil domain containing 61 (*CCDC61*) rs777816675 were mutated in 2 patients but not in the healthy donors including the proband's daughter (Tables 1 and S5). It is noteworthy that *POTEJ*, *TUBA3E*, *LRRC37A2* and *RIMBP3* have homologous members in their families so that we failed to design specific primers to distinguish them. However, the other 25 gene variants were not identified by Sanger sequencing in all 12 individuals (Table S5).

4 | DISCUSSION

Relapsing polychondritis is a rare multisystemic inflammatory disease and affects the cartilage of the ears, nose, larynx, tracheobronchial tree and cardiovascular system. Its annual incidence is about 3.5 per million. There is no family occurrence reported at present. We found the occurrence of RP cases in a Chinese family for the first time, which was crucial to explore the relationship between RP and genetic alteration. Previous studies showed that some factors including infectious agents, mechanical or chemical aggression would trigger autoimmune responses, and then affect known target autoantigens involving collagen and matrilin-1 in RP and other possible target autoantigens like cartilage oligomeric matrix protein, and finally lead to cartilage destruction.[7] Moreover, the susceptibility to RP has been reported to be significantly linked with genetic



(B)

Individual ID	Gender	Age	Diagnosis
L0001	♀	32	RP
L0002	♂	58	Healthy
L0003	♀	56	RP
L0004	♀	52	Healthy
L0005	♀	2	Not yet
L0006	♂	29	Healthy
L0007	♂	62	Healthy
L0008	♂	51	Healthy
L0009	♂	39	Healthy
L0010	♂	25	Healthy
L0011	♂	25	Healthy
L0012	♂	53	Healthy

FIGURE 1 Family pedigree of relapsing polychondritis (RP) (A) and general conditions (B). The red arrow denotes the proband. "Not yet" refers to the proband's daughter who was too young to evaluate whether she was affected or not

TABLE 1 Nine gene mutations are found with whole-exome sequencing in patients with relapsing polychondritis and identified by Sanger sequencing

Item	*RNF207*	*COL22A1*	*GPAA1*	*RECQL4*	*FLCN*	L/G3	*MYH1S*	*PCP2*	*CCDC61*
Chr	Chr1	chr8	chr8	chr8	chr17	chr17	chr3	chr19	chr19
Position	6269041	139661991	145138351	145737572	17124884	33310188	108129523	7697658	46518628
REF	T	G	C	C	C	C	C	C	G
ALT	C	C	T	T	T	G	T	T	C
Filter	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS
avsnp144	NA	rs200464636	rs201424010	rs757703895	NA	rs761808558	NA	rs144974437	rs777816675
AAChange_refGene	NM_207396:c.T425C:p.I142T	NM_152888:c.C3364G:p.P1122A	NM_003801:c.C314T:p.T105M	NM_004260:c.G3191A:p.R1064H	NM_144606:c.G838A:p.E280K	NM_002311:c.C164G:p.P55R	NM_014981:c.G4462A:p.A14887	NM_001271830:c.G62A:p.R21Q	NM_001267723:c.G788C:p.R263p
L0001	C	C	T	T	T	G	T	T	C
L0002	T	G	C	C	C	C	C	C	G
L0003	C	C	T	T	T	G	T	T	C
L0004	T	G	C	C	C	C	C	C	G
L0005	C	C	T	T	T	G	C	C	G
L0006	T	G	C	C	C	C	C	C	G
L0007	T	G	C	C	C	C	C	C	G
L0008	T	G	C	C	C	C	C	C	G
L0009	T	G	C	C	C	C	C	C	G
LO010	T	G	C	C	C	C	C	C	G
LO011	T	G	C	C	C	C	C	C	G
LO012	T	G	C	c	C	C	C	C	G

Note: The mutated bases or patients with gene mutations are labeled with red font.

Abbreviations: ALT, alternate base(s); CChr, chromosome; REF, reference base(s); NA, not available.

factors. Lang et al found DR4 antigen frequency was 56.1% in 41 RP patients and 25.5% in healthy controls.[5] The increase was significant ($P < .001$). Zeuner et al showed that susceptibility to RP was significantly associated with HLA-DR4 ($P < .001$) as well.[8] However, further study about the mechanism of RP has been deficient.

WES is an efficient approach in uncovering the Mendelian diseases due to a significant part of their mutations existing in the exons, which is employed to detect the exons in the genome. The effects of exome sequencing on revealing the mutations and identifying disease-causal genes have been demonstrated by previous studies.[9,10] Eighty-five percent of the disease-causing mutations were estimated to locate in coding and functional regions of the genome.[11] Therefore, WES has the potential to uncover the underlying causes of plentiful diseases such as rare, mostly monogenic, genetic disorders as well as predisposing variations in common diseases.[12] Phenotype studies of complex diseases have mainly focused on the candidate gene, linkage and association before using WES. Linkage analysis was used to define the variants in 1000s of Mendelian diseases, but it was not effective in complex diseases because of their genetic and phenotypic heterogeneity or environmental factors, as well as rare diseases and sporadic cases for the difficulty in finding sufficient samples. WES, used since 2007, makes up the deficiency to a certain extent and brings new opportunities to the study of these diseases.[13] Only less than half of these diseases have been determined to result from the allelic variants so far. At present, WES has been applied successfully to define the variants of several rare diseases like rhabdoid glioblastoma tumor, Kabuki syndrome and Schinzel-Giedion syndrome. This approach could be considered as an effective technique to demonstrate the genetic heterogeneity in diseases.[10,14] This is the first study on mechanism of RP by WES at the molecular level. It will provide a deeper understanding of the genetic background of RP, and offer the theoretical basis of developing other useful therapeutic methods.

The gene mutations found by WES were validated by Sanger sequencing. *RNF207*, *COL22A1*, *GPAA1*, *RECQL4*, *FLCN*, *LIG3*, *CCDC61*, *PCP2* and *MYH15* gene mutations were identified in the patients with RP. We also found that *RNF207*, *COL22A1*, *GPAA1*, *RECQL4*, *FLCN and LIG3* were mutated in the proband's daughter as well. The proband's daughter was too young to evaluate whether she was affected or not, but her results will be beneficial for us to further locate the mutated SNP with follow-up based on Sanger sequencing.

COL22A1 gene locates on human chromosome 8q24.2 and encodes Collagen XXII that structurally belongs to the fibril-associated collagens with interrupted triple helices (FACIT) protein family. The protein is deposited in tissue junction involving extrafibrillar matrix in cartilage, the basement membrane zone of the myotendinous junction and the hair follicle, and exhibits a striking restricted localization at these tissue junctions.[15] It suggests that mutation of *COL22A1* gene might lead to variation of Collagen XXII, and its restricted deposition at certain tissue junctions could influence the function of the cartilage involved or induce immune responses by providing new autoantigens. This may contribute to the pathogenesis of RP to some extent. *FLCN* gene locates on human chromosome 17p11.2 and modulates a variety of cell signaling pathways important in growth, proliferation, metabolism, survival, motility, and adhesion as an autosomal dominant tumor suppressor gene.[16,17] *FLCN* loss of hematopoietic cells drives adult hematopoietic stem/progenitor cells (HSPCs) into proliferative exhaustion, resulting in the rapid depletion of HSPCs and all hematopoietic cell lineages and in acute bone marrow failure.[18] Whether *FLCN* takes part in cartilage growth, development, survival or tissue repairment still lacks solid evidence. However, previous findings do highlight the potential cell signal pathways for us to do deeper research. Nguyen et al performed a functional study of *GPAA1* in the cerebellum and skeletal system and demonstrated that most individuals with *GPAA1* mutations presented with global developmental delay, early-onset seizures, hypotonia, cerebellar atrophy and osteopenia.[19] However, functional research on *GPAA1* in RP needs further investigation to see if there are similar effects on cartilage growth and development. A previous study demonstrated that *RECQL4* played a key role in early development such as the skeletal system.[20] *RECQL4* overexpression promoted proliferation activity of osteoblastic cells, while its depletion impeded cell growth.[21] This indicates *RECQL4* expression is crucial in the skeletal system. Whether there are abnormal expressions of *RECQL4* in RP patients and how they influence cell function still needs further study. *MYH15* differentially expressed on inflammatory cells could influence airway inflammation, which contribute to the generation of airway responsiveness.[22] This highly suggests that *MYH15* might play an important role in the pathogenesis of RP through participating in recurrent airway inflammation which leads to permanent destruction of the respiratory tract.

The functions of *RNF207*, *LIG3*, *PCP2* and *CCDC61* are still unknown in the process of inflammation and cartilage formation. The sequences of *POTEJ*, *TUBA3E*, *LRRC37A2* and *RIMBP3* are highly similar to other homologous members of their families, respectively. Therefore, we failed to design specific primers to distinguish them, and we are looking forward to developing more effective methods to study them.

This study demonstrated that coinheritance of multigene mutation may lead to RP predisposition. The candidate genes discovered could be considered as potential targets for further functional studies.

ACKNOWLEDGMENTS

Special thanks to the support from Guangdong Natural Science Funds for Distinguished Young Scholar (Grant No.2014A030306039), High-level personnel of special support program for Technology Innovative Talents and the Top Young of Guangdong Province (Grant No.2015TQ01R516), Distinguished Young Scholar Candidates Program for The Third Affiliated Hospital of Sun Yat-Sen University and Pearl River Nova Program of Guangzhou (Grant No. 201610010005).

CONFLICT OF INTEREST

None declared.

AUTHORS' CONTRIBUTIONS

Study design: Zhiming Lin; sample collection: Junmei Feng, Jun Qi, Xinghua Guo, Qing Lv, Yanli Zhang, Linkai Fang, Xi Zhang, Jieruo Gu, Zhiming Lin; data collection: Lian Gui; data analysis: Xiaoyu Zuo; article writing: Lian Gui, Zhiming Lin.

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REFERENCES

1. Trentham DE, Le CH. Relapsing polychondritis. *Ann Intern Med*. 1998;129(2):114-122.
2. McAdam LP, O'Hanlan MA, Bluestone R, Pearson CM. Relapsing polychondritis: prospective study of 23 patients and a review of the literature. *Medicine*. 1976;55(3):193-215.
3. Pearson CM, Kline HM, Newcomer VD. Relapsing polychondritis. *N Engl J Med*. 1960;263:51-58.
4. Cantarini L, Vitale A, Brizi MG, et al. Diagnosis and classification of relapsing polychondritis. *J Autoimmun*. 2014;48–49:53-59.
5. Lang B, Rothenfusser A, Lanchbury JS, et al. Susceptibility to relapsing polychondritis is associated with HLA-DR4. *Arthritis Rheum*. 1993;36(5):660-664.
6. Terao C, Yoshifuji H, Yamano Y, et al. Genotyping of relapsing polychondritis identified novel susceptibility HLA alleles and distinct genetic characteristics from other rheumatic diseases. *Rheumatology (Oxford)*. 2016;55(9):1686-1692.
7. Arnaud L, Mathian A, Haroche J, Gorochov G, Amoura Z. Pathogenesis of relapsing polychondritis: a 2013 update. *Autoimmun Rev*. 2014;13(2):90-95.
8. Zeuner M, Straub RH, Rauh G, Albert ED, Scholmerich J, Lang B. Relapsing polychondritis: clinical and immunogenetic analysis of 62 patients. *J Rheumatol*. 1997;24(1):96-101.
9. Kuhlenbaumer G, Hullmann J, Appenzeller S. Novel genomic techniques open new avenues in the analysis of monogenic disorders. *Hum Mutat*. 2011;32(2):144-151.
10. Ng SB, Bigham AW, Buckingham KJ, et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nat Genet*. 2010;42(9):790-793.
11. Majewski J, Schwartzentruber J, Lalonde E, Montpetit A, Jabado N. What can exome sequencing do for you? *J Med Genet*. 2011;48(9):580-589.
12. Rabbani B, Tekin M, Mahdieh N. The promise of whole-exome sequencing in medical genetics. *J Hum Genet*. 2014;59(1):5-15.
13. Ku CS, Naidoo N, Pawitan Y. Revisiting Mendelian disorders through exome sequencing. *Hum Genet*. 2011;129(4):351-370.
14. Biesecker LG. Exome sequencing makes medical genomics a reality. *Nat Genet*. 2010;42(1):13-14.
15. Koch M, Schulze J, Hansen U, et al. A novel marker of tissue junctions, collagen XXII. *J Biol Chem*. 2004;279(21):22514-22521.
16. Schmidt LS. Birt-Hogg-Dube syndrome: from gene discovery to molecularly targeted therapies. *Fam Cancer*. 2013;12(3):357-364.
17. Tee AR, Pause A. Birt-Hogg-Dube: tumour suppressor function and signalling dynamics central to folliculin. *Fam Cancer*. 2013;12(3):367-372.
18. Baba M, Toyama H, Sun L, et al. Loss of folliculin disrupts hematopoietic stem cell quiescence and homeostasis resulting in bone marrow failure. *Stem Cells*. 2016;34(4):1068-1082.
19. Nguyen TTM, Murakami Y, Sheridan E, et al. Mutations in GPAA1, encoding a GPI transamidase complex protein, cause developmental delay, epilepsy, cerebellar atrophy, and osteopenia. *Am J Hum Genet*. 2017;101(5):856-865.
20. Mann MB, Hodges CA, Barnes E, Vogel H, Hassold TJ, Luo G. Defective sister-chromatid cohesion, aneuploidy and cancer predisposition in a mouse model of type II Rothmund-Thomson syndrome. *Hum Mol Genet*. 2005;14(6):813-825.
21. Yang J, Murthy S, Winata T, et al. Recql4 haploinsufficiency in mice leads to defects in osteoblast progenitors: Implications for low bone mass phenotype. *Biochem Biophys Res Commun*. 2006;344(1):346-352.
22. Hargreave FE, Dolovich J, O'Byrne PM, Ramsdale EH, Daniel EE. The origin of airway hyperresponsiveness. *J Allergy Clin Immunol*. 1986;78(5 Pt 1):825-832.

SUPPORTING INFORMATION

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

How to cite this article: Feng J, Zuo X, Gui L, et al. Genetic basis of relapsing polychondritis revealed by family-based whole-exome sequencing. *Int J Rheum Dis*. 2020;23:641–646. https://doi.org/10.1111/1756-185X.13809

Received: 21 July 2019 | Accepted: 12 February 2020

DOI: 10.1111/1756-185X.13819

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases | WILEY

Association of anti-cyclic citrullinated peptide antibodies and rheumatoid factor isotypes with HLA-DRB1 shared epitope alleles in Egyptian rheumatoid arthritis patients

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Funding information
This study was funded by the Science and Technology Development Fund (STDF), Egypt, Grant No. 3242.

Abstract

Introduction: The most common genetic risk factor for rheumatoid arthritis (RA) is human leucocyte antigen DRB1 (HLA-DRB1) shared epitope (SE).

Aim: To investigate the relationship between anti-cyclic citrullinated peptide (anti-CCP), rheumatoid factor (RF), immunoglobulin (Ig)G, IgM and IgA and HLA-DRB1 SE among Egyptian patients with RA.

Methods: Serum levels of anti-CCP antibodies and RFIgG, RFIgM, RFIgA were assayed using enzyme-linked immunosorbent assay for 157 Egyptian RA patients and 150 healthy controls attending the outpatient clinics of National Research Center and Kasr El Aini Hospital. HLA-DRB1 genotyping was performed by the DynalAllSetTM polymerase chain reaction (PCR) single specific primer low-resolution typing kits. Amplified PCR product was checked using 3% agarose gel.

Results: HLA-DRB1-SE was found among 129 (82.2%) RA patients and 67 (44.7%) controls (odds ratio [OR] 5.7, CI 3.4-9.6, $P < .0001$). The risk of RA development was higher with the presence of SE two alleles (OR 11.6, $P < .0001$), while the OR for 1 copy SE allele was 4.4 ($P < .0001$). *HLA-DRB1*-SE was significantly associated with positive as well as negative anti-CCP and RF isotypes. The stronger association was with anti-CCP positivity with OR 11 (5.1-23.6), $P < .0001$. Furthermore, the risk of development of positive anti-CCP and RF isotypes was higher with the presence of 2 copies of SE alleles than with 1 copy.

Conclusion: The prevalence of HLA-DRB1-SE is high in Egyptian RA patients. The role of SE in RA patients is most probably related to the development of anti-CCP positive RA rather than the development of anti-CCP positivity.

KEYWORDS

anti-cyclic citrullinated peptide, rheumatoid arthritis, rheumatoid factor isotypes shared epitope

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a polygenic systemic autoimmune disease affecting mainly synovial joints causing their destruction. The pathogenesis of RA is multifactorial, with genetic and environmental risk factors playing important roles. Genetic factors contribute to 40%-60% of the total risk.[1] The strongest genetic association is with human leucocyte antigen-DRB1 (HLA-DRB1) alleles encoding shared epitope (SE). The commonly recognized SE-coding alleles include HLA-DRB1*04:01, *04:04, *04:05, *04:08, *04:09, *01:01, *01:02 and *10:01.[2] The molecular basis for the association of SE with RA is still unclear.

Serologically, rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) have been reported as important diagnostic and prognostic biomarkers for RA. The major RF isotypes are RF immunoglobulin (Ig)G, RFIgM and RFIgA. They have different sensitivities and specificities in RA. Although RFIgM and RFIgG are more sensitive than RFIgA, RFIgA is more specific and associated with more severe disease. Also, it has been reported that their appearance in serum is sequential before diagnosis: first RFIgM, then RFIgA, and finally RFIgG.[3] More recently, antibodies directed against cyclic citrullinated peptide (anti-CCP) have received much attention as they are more specific than RF, they may be detected years before disease onset and are stable over time.[4] However, anti-CCP alone is not a golden diagnostic marker of RA; its combination with RF shows stronger effect in the diagnosis of RA as they complement each other.[5] Autoantibodies may moderate the risk of RA in the context of SE, suggesting that interactions between SE and autoantibodies may play a role in RA development.[6]

Several studies have investigated the association of SE-encoding HLA-DRB1 and anti-CCP and fewer studies have assessed the association with RF isotypes. In Egypt, no study has been done to investigate the association of SE-encoding HLA-DRB1 with anti-CCP and RF. Therefore, this study aimed to determine the prevalence of HLA-DRB1-SE alleles in Egyptian RA patients and to investigate the association of SE-encoding HLA-DRB1 with anti-CCP and RFIgM, RFIgG, RFIgA.

2 | PATIENTS AND METHODS

This study is a case control study. We included 157 RA patients from the rheumatology outpatient clinic of the Medical Services Unit at National Research Center and outpatient rheumatology clinic of Internal Medicine Department at Kasr El Aini Hospital. One hundred and fifty healthy volunteers as controls were selected from healthy people with no history of inflammatory arthritis and with negative family history of RA or any autoimmune disease. RA patients and controls were matched as regards age and gender. They were all Egyptians of the same ethnic origin. Patients and controls were subjected to detailed history-taking, thorough clinical examination including musculoskeletal examination. For each patient, we recorded number of tender and/ or swollen joints. Disease activity was assessed using Disease Activity Score in 28 joints (DAS28).

2.1 | Laboratory tests for RA patients and controls

2.1.1 | Serologic tests

Serum samples were assayed for:

1. RF of IgM, IgG and IgA isotypes quantitatively using commercially available enzyme-linked immunosorbent assay (ELISA: provided by Orgentec Diagnostica GmbH, Mainz, Germany) according to Ernst et al.[7] RF was considered positive above 20 IU/mL
2. Anti-CCP using third-generation assays (Quanta Lite™ CCP3 IgG ELISA; Inova Diagnostics) according to Bizzaro et al[8] Anti-CCP antibody was considered positive above 20 IU/mL.

2.1.2 | HLA-DRB1 typing

For HLA-DRB1 typing and subtyping, genomic DNA was extracted from ethylenediaminetetraacetic acid anticoagulated blood using QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's guidelines. HLA-DRB1 genotyping was performed by the DynalAllSet™ polymerase chain reaction (PCR) single specific primer low-resolution typing kits according to the manufacturer's instructions (Dynal). Amplified PCR product was checked using 3% agarose gel. The following alleles were classified as SE-positive: DRB1 *04:01, *04:04, *04:05, *04:08, 04:09, *01:01, *01:02 and *10:01.[9] Patients carrying 1 or 2 alleles of shared epitope were classified as SE-positive carrier.

2.2 | Statistical methods

Data entry was carried out on Excel spreadsheets, and statistical analysis was done using SPSS software program version 18.0 (SPSS Inc). Data were statistically described in terms of mean ± standard deviation (SD), frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using *t* test for comparison between 2 means. When data were not normally distributed, Mann–Whitney *U* test was used when comparing 2 groups. Chi-square was done for qualitative data and presented by numbers and percentages to assess significant association and odds ratio (OR) was used to assess the strength of association. *P* values were considered statistically significant when $P < .05$ and considered statistically highly significant if $P < .01$.

3 | RESULTS

3.1 | Demographic and clinical characteristics of RA patients and controls

One hundred and fifty-seven RA patients, 21 male and 136 female with mean age 46.2 ± 12.2 years and 150 healthy controls, 27 male

and 123 female with mean age 46.2 ± 12.2 years were enrolled in the study ($P > .05$). Mean duration of the disease was 8.7 ± 8.0 years. Nine patients (5.7%) were smokers. Twenty-eight patients (17.8%) reported positive family history of RA. Seventy-seven patients (49.0%) had extra-articular manifestations. Deformity by clinical examination, at the time of the study, was present in 55 patients (35.3%). Mean disease activity assessed by DAS28 was 5.9 ± 5.1. Patients were treated with disease-modifying anti-rheumatic drugs in the form of methotrexate and/or leflunomide and/or hydroxychloroquine and/or sulfasalazine and/or prednisone in doses of 2.5-7.5 mg/d.

Frequency of positive RF isotypes and anti-CCP in RA patients and controls are presented in Table 1. The risk of RA development was higher with the presence of positive anti-CCP or positive RF IgG than RF IgM or RF IgA.

3.2 | Frequency of HLA-DRB1-SE in RA patients and controls

HLA-DRB1-SE was detected among 129 (82.2%) RA patients and 67 (44.7%) controls (OR 5.71, CI 3.39-9.6, $P < .0001$). Moreover, we observed a gene-dose effect, with the presence of two copies of SE alleles, the risk for RA development increases, as the OR of the association of two copies of SE alleles with RA (using no copies of the SE allele in the healthy control group as the referent) was 11.61 ($P < .0001$), while the OR for one copy SE allele was 4.42 ($P < .0001$).

3.3 | Association between HLA-DRB1-SE and anti-CCP, RF isotypes

The associations of HLA-DRB1-SE with anti-CCP and RF isotypes are presented in Tables 2 and 3. *HLA-DRB1*-SE was significantly associated with positive as well as negative anti-CCP antibodies and RF isotypes. The association with positive antibodies was significantly higher than with negative antibodies. The stronger significant association was detected with anti-CCP positivity. Within the RF isotypes, the RFIgA showed stronger association compared to RFIgG or RFIgM, while the RFIgG and RFIgM showed comparable results. Furthermore, the risk of development of positive anti-CCP and RF isotypes is higher with the presence of two copies SE alleles than with one copy of SE allele (Tables 4 and 5). Interestingly, the presence of only one copy of SE allele is not associated with negative RFIgG RA patients.

4 | DISCUSSION

We demonstrated in the current study a high frequency of HLA-DRB1-SE among the studied population. At least one HLA-DRB1-SE allele was detected, among 82.2% of RA patients and 47.8% of controls. This percentage is comparable to that of Caucasian populations as in northern Irish (81.6%) and in southern Swedish (87.7%).[10,11] On the other hand, lower percentage was reported in a Bangladeshi population, as SE positivity was found in 67.3% of RA patients, and

TABLE 1 Frequency of positive anti-CCP and RF isotypes in RA patients and controls

	RA patients n (%)	Controls n (%)	*P* value	OR (95% CI)
Anti-CCP ≥ 20	89 (56.7)	5 (3.3)	<.0001	37.9 (14.7-97.7)
Anti-CCP < 20	68 (43.3)	145 (96.7)		
RFIgG > 20	124 (79.0)	28 (18.7)	<.0001	16.4 (9.3-28.7)
RFIgG ≤ 20	33 (21.0)	122 (81.3)		
RFIgM > 20	102 (65.0)	21 (14.0)	<.0001	11.4 (6.5-20.1)
RFIgM ≤ 20	55 (35.0)	129 (86.0)		
RFIgA > 20	84 (53.5)	13 (8.7)	<.0001	12.1 (6.3-23.2)
RFIgA ≤ 20	73 (46.5)	137 (91.3)		

Abbreviations: CCP, cyclic citrullinated peptide; CI, confidence interval; Ig, immunoglobulin; OR, odds ratio; RA, rheumatoid arthritis; RF, rheumatoid factor.

TABLE 2 Association of HLA-DRB1-SE with anti-CCP antibodies

	Controls N = 150	Anti-CCP positive RA N = 89		Anti-CCP negative RA N = 68	
HLA-DRB1-SE	n (%)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
Carrier	67 (44.7)	80 (89.9)	11 (5.2-23.6)**	49 (72.1)	3.19 (1.7-5.9)*
Non-carrier	83 (55.3)	9 (10.1)		19 (27.9)	

Abbreviations: CCP, cyclic citrullinated peptide; CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio; RA, rheumatoid arthritis; SE, shared epitope.

*$P < .01$.

**$P < .0001$.

TABLE 3 Association of HLA-DRB1-SE with RFIgG, RFIgM and RFIgA

	Controls N = 150	RFIgG positive RA N = 124		RFIgG negative RA N = 33		RFIgM positive RA N = 102		RFIgM negative RA N = 55		RFIgA positive RA N = 84		RFIgA negative RA N = 73	
HLA-DRB1-SE	**n (%)**	**n (%)**	**OR (95% CI)**	**n (%)**	**OR (95% CI)**	**n (%)**	**OR (95% CI)**	**n (%)**	**OR (95% CI)**	**n (%)**	**OR (95% CI)**	**n (%)**	**OR (95% CI)**
Carrier	67 (44.7)	106 (85.5)	7.3 (4.0-13.2)**	23 (69.7)	2.6 (1.3-6.4)*	88 (86.3)	7.8 (4.1-14.9)**	41 (74.5)	3.6 (1.8 - 7.2)*	75 (89.3)	10.3 (4.8- 22.1)**	54 (74.0)	3.5 (1.9-6.5)*
Non-carrier	83 (55.3)	18 (14.5)		10 (30.3)		14 (13.7)		14 (25.5)		9 (10.7)		19 (26.0)	

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; Ig, immunoglobulin; OR, odds ratio; RA, rheumatoid arthritis; RF, rheumatoid factor; SE, shared epitope.

*$P < .01$.

**$P < .0001$.

TABLE 4 Dose effect of HLA-DRB1-SE on anti-CCP antibodies among RA patients and controls

	Controls N = 150	Anti-CCP positive RA patients N = 89			Anti-CCP negative RA patients N = 68		
HLA-DRB1-SE	**n (%)**	**n (%)**	**OR (95% CI)**	***P* value**	**n (%)**	**OR (95% CI)**	***P* value**
SE+/SE+	12 (8.0)	29 (27.4)	22.3 (8.5-58.3)	<.0001	18 (26.5)	6.6 (2.7- 15.9)	<.0001
SE+/SE−	55 (36.7)	51 (58.3)	8.6 (3.9-18.8)	<.0001	31 (45.6)	2.46 (1.3-4.8)	.008
SE−/SE−	83 (55.3)	9 (14.3)	1		19 (27.9)	1	

Abbreviations: CCP, cyclic citrullinated peptide; CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio; RA, rheumatoid arthritis; SE, shared epitope.

TABLE 5 Dose effect of HLA-DRB1-SE on RFIgG, RFIgM and RFIgA

HLA-DRB1-SE	Controls N = 150	RFIgG positive RA N = 124		RFIgG negative RA N = 33		RFIgM positive RA N = 102		RFIgM negative RA N = 55		RFIgA positive RA N = 84		RFIgA negative RA N = 73	
	n (%)	n (%)	OR (95%CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
SE+/SE+	12 (8.0)	39 (31.5)	15.0 (6.6-34.2)**	8 (24.2)	5.5 (1.8-16.8)*	31 (30.4)	15.3 (6.4-36.7)**	6 (29.1)	7.9 (3.1-20.2)**	28 (33.3)	21.5 (8.2-56.5)**	9 (26.0)	6.9 (2.9-16.6)**
SE+/SE−	55 (36.7)	67 (54.0)	5.8 (3.1-10.9)**	15 (45.5)	2.2 (0.95-5.4)	57 (55.9)	6.1 (3.1-12.1)**	25 (45.5)	2.7 (1.3-5.6)*	47 (56.0)	7.9 (3.6-17.4)**	35 (47.9)	2.7 (1.4-5.4)*
SE−/SE−	83 (55.3)	18 (14.5)	1	10 (30.3)	1	14 (13.7)	1	4 (25.5)	1	9 (10.7)	1	19 (26.0)	1

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; Ig, immunoglobulin; OR, odds ratio; RF, rheumatoid factor; SE, shared epitope.

*$P = .0001$.

**$P < .0001$.

in 32.7% of healthy controls[12] and in African Americans where SE positivity was 42.1% in RA cases and 25.3% in healthy controls.[13]

In the current study, HLA-DRB1-SE was significantly associated with RF isotypes positive RA patients and to lesser extent with negative RF patients. The strongest association was observed with RFIgA which is more specific than RFIgG and RFIgM. The presence of two SE alleles was associated with higher risk of RF positivity with IgM and IgA but not with IgG. Perhaps this may be attributed to the lower specificity of RF IgG isotypes, while IgM and IgA RF are almost exclusively observed in RA patients.[14] The association between SE and RF has not been extensively studied as that of anti-CCP. The detection of RF in other diseases that lack symptoms of arthritis may indicate that RF is indirectly involved in RA pathogenesis. Its presence is considered a physiological response in host defense facilitating the clearance of immune complexes. There is no evidence of being involved in the initial events of the RA disease process. RF is an autoantibody directed to denatured IgG or Fc fragments of denatured IgG. One of the functions of HLA class II molecules is the aberrant transport of cellular misfolded proteins to the cell surface without processing to peptides.[15] HLA class II via association with the peptide-binding groove transports IgG heavy chain (IgGH) to the cell surface which efficiently stimulates antigen-specific B cells.[16] Accordingly, IgGH/HLA class II complexes are specifically recognized by autoantibodies in sera from RF positive RA patients.[15]

We observed association between HLA-DRB1-SE and anti-CCP positive RA patients and to a lesser extent anti-CCP negative RA patients. The association with positive anti-CCP antibodies was stronger than with any of RF isotypes. This is not surprising as anti-CCP is more specific than RF. Anti-CCP is considered a RA disease-specific humoral immune response. Studies assessing the association of HLA-DRB1 with autoantibodies in RA showed conflicting results due to differences in the genetic or environmental background. The association of HLA-DRB1-SE with positive and negative ACPA RA patients was found in Korean RA patients.[17] Similarly, in a large UK study, the association of HLA-DRB1-SE was found with positive and negative ACPA and also with positive and negative RF RA patients but the association was stronger with positive antibodies.[18] However, previous studies in Caucasians and Asians revealed association of HLA-DRB1-SE with anti-CCP positive RA but not with anti-CCP negative RA patients.[19-21] On the other hand, recent studies among south Indian populations, and among Bangladeshi RA patients, no significant difference in frequency of anti-CCP and RF between HLA-DRB1-SE-positive and negative RA patients was found.[12,22] Among Turkish and Saudi RA patients the association was found with positive anti-CCP and not with RF.[23,24]

Like many other studies,[25-27] we found gene-dose effect of SE alleles. The presence of two SE alleles confers a higher risk to develop RA and also to develop positive anti-CCP.

The mechanisms underlying the association of SE and anti-CCP is unclear. The pathogenic effect may be through the presentation of citrullinated peptides which are recognized as non-self by T cells that trigger maturation of B cells synthesizing anti-CCP.[25]

It has been demonstrated that the interaction of DRB1*04:01 protein with citrullinated peptide shows higher affinity than with non-citrullinated peptide.[28] Hill and co-workers[29] reported that this interaction increases peptide–major histocompatibility complex affinity, leading to the activation of CD4± T cells in mice transgenic for 1 of the SE alleles. Accordingly, it has been suggested that SE is associated only with anti-CCP-positive RA. However, our results are in disagreement with this hypothesis; also Charpin and co-workers[30] stated that RA-associated HLA-DR alleles are not mandatory for the production of anti-CCP. It seems that SE plays a role in the development of anti-CCP-positive RA rather than in the development of anti-CCP positivity. Studies have reported that the HLA class II locus is associated with established ACPA positivity in RA patients, but, surprisingly, only to a limited extend with ACPA positivity in healthy subjects, proposing an abnormal humoral response to the citrullinated proteins in RA patients. Therefore, it is likely that the CD4+ T helper cells restricted by the predisposing HLA molecules play a role in the development of ACPA + RA rather than in the development of ACPA positivity.[31,32] Other pathogenetic mechanisms unrelated to citrullinated peptides may play a role in RA development in SE-positive patients. It has been hypothesized that SE-positive DRB1 alleles confer disease susceptibility through a mechanism that involves alteration of the peripheral T-cell repertoire. Moreover, it has been proposed that SE, analogous to certain domains of class I MHC-molecules, acts as a ligand that interacts with cell surface calreticulin and activates innate immune signaling.[33] The effect of SE lacks antigen- or species-specificity as studies reported the association of SE with several other autoimmune diseases and also in experimental disease models.[34]

5 | CONCLUSION

The prevalence of HLA-DRB1-SE is high in Egyptian RA patients. Anti-CCP as well as RFIgG, RFIgM and RFIgA are associated with HLA-DRB1-SE in RA. The role of SE in RA patients is most probably related to the development of anti-CCP positive RA rather than the development of anti-CCP positivity. Further studies are needed to clarify the molecular mechanism for the association of RF with HLA-SE.

CONFLICT OF INTEREST

All authors declare they have no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Khalda Saiid Amr, Eman Abdelmotaleb Bayomi, Hanaa Rasmy Attia, Mona Hamed Ibrahim and Eman Mahmoud Hassan. All authors have contributed significantly and are in agreement with the content of the manuscript.

ETHICAL APPROVAL

The study has been approved by the ethical committee of the National Research Center and has been performed in accordance with the ethical standards of the National Research Center committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

INFORMED CONSENT

Informed consent was obtained from all participants included in the study.

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REFERENCES

1. Lin L, Chen Y, Xiao Z, Huang S, Yang Z. The association of HLA-DRB1 alleles with rheumatoid arthritis in the Chinese Shantou population: a follow-up study. *Biochem Cell Biol*. 2007;85:227-238.
2. Holoshitz J. The rheumatoid arthritis HLA–DRB1 shared epitope. *Curr Opin Rheumatol*. 2010;22(3):293-298.
3. Berglin E, Johansson T, Sundin U, et al. Radiological outcome in rheumatoid arthritis is predicted by presence of antibodies against cyclic citrullinated peptide before and at disease onset, and by IgA-RF at disease onset. *Ann Rheum Dis*. 2006;65(4):453-458.
4. Bos WH, van de Stadt LA, Sohrabian A, Rönnelid J, van Schaardenburg D. Development of anti-citrullinated protein antibody and rheumatoid factor isotypes prior to the onset of rheumatoid arthritis. *Arthritis Res Ther*. 2014;16(2):405-406.
5. Gomez EL, Gun SC, Somnath SD, et al. The prevalence of rheumatoid factor isotypes and anti-cyclic citrullinated peptides in Malaysian rheumatoid arthritis patients. *Int J Rheum Dis*. 2011;14:12-17.
6. Klareskog L, Malmstrom V, Lundberg K, Padyukov L, Alfredsson L. Smoking, citrullination and genetic variability in the immunopathogenesis of rheumatoid arthritis. *Semin Immunol*. 2011;23:92-98.
7. Ernst E, Espersen G, Andersen M, Grunnet N. RF-classes (IgM, IgG, IgA) in a group of highly active RA-patients in relation to disease activity and treatment. *Scand J Rheumatol Suppl*. 1998;75:250-255.
8. Bizzaro N, Mazzanti G, Tonutti E, Villalta D, Tozzoli R. Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid. *Arthritis Clin Chem*. 2011;47:1089-1093.
9. Zanelli E, Breedveld FC, de Vries RR. Hla class II association with rheumatoid arthritis: facts and interpretations. *Hum Immunol*. 2000;61:1254-1261.
10. Griffiths B, Situnayake RD, Clark B, Tennant A, Salmon M, Emery P. Racial origin and its effect on disease expression and HLA-DRB1 types in patients with rheumatoid arthritis: a matched cross-sectional study. *Rheumatology (Oxford)*. 2000;39:857-864.
11. Eberhardt K, Fex E, Johnson U, Wollheim FA. Associations of HLA-DRB and -DQB genes with two and five year outcome in rheumatoid arthritis. *Ann Rheum Dis*. 1996;55:34-39.
12. Begum M, Sattar H, Haq SA, et al. Study on association of human leukocyte antigen-DRB1 alleles in rheumatoid arthritis within Bangladeshi population. *Int J Rheum Dis*. 2018;21:1543-1547.
13. Hughes LB, Morrison D, Kelley JM, et al. The HLA–DRB1 shared epitope is associated with susceptibility to rheumatoid arthritis in African Americans through European genetic admixture. *Arthritis Rheum*. 2008;58(2):349-358.
14. Deane KD, O'Donnell CI, Hueber W, et al. The number of elevated cytokines and chemokines in preclinical seropositive rheumatoid arthritis predicts time to diagnosis in an age-dependent manner. *Arthritis Rheum*. 2010;62(11):3161-3172.

15. Jina H, Arasea N, Hirayasua K, et al. Autoantibodies to IgG/HLA class II complexes are associated with rheumatoid arthritis susceptibility. *Proc Natl Acad Sci U S A*. 2014;111(10):3787-3792.
16. Jiang Y, Arase N, Kohyama M, et al. Transport of misfolded endoplasmic reticulum proteins to the cell surface by MHC class II molecules. *Int Immunol*. 2013;25(4):235-246.
17. Bang SY, Han TU, Choi CB, Sung YK, Bae SC, Kang C. Peptidyl arginine deiminase type IV (PADI4) haplotypes interact with shared epitope regardless of anti-cyclic citrullinated peptide antibody or erosive joint status in rheumatoid arthritis: a case control study. *Arthritis Res Ther*. 2010;12:R115.
18. Mackie SL, Taylor JC, Martin SG, et al. A spectrum of susceptibility to rheumatoid arthritis within HLA-DRB1: stratification by autoantibody status in a large UK population. *Genes Immun*. 2012;13:120-128.
19. Plenge RM, Padyukov L, Remmers EF, et al. Replication of putative candidate-gene associations with rheumatoid arthritis in.4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. *Am J Hum Genet*. 2005;77:1044-1060.
20. Barton A, Thomson W, Ke X, et al. Re-evaluation of putative rheumatoid arthritis susceptibility genes in the post-genome wide association study era and hypothesis of a key pathway underlying susceptibility. *Hum Mol Genet*. 2008;17:2274-2279.
21. Chun-Lai T, Padyukov L, Dhaliwal JS, et al. Shared epitope alleles remain a risk factor for anti-citrullinated proteins antibody (ACPA) – positive rheumatoid arthritis in three asian ethnic groups. *PLoS ONE*. 2011;6:e21069.
22. Mohan VK, Ganesan N, Gopalakrishnan R, Venkatesan V. HLA-DRB1 shared epitope alleles in patients with rheumatoid arthritis: relation to autoantibodies and disease severity in a south Indian population. *Int J Rheum Dis*. 2017;20:1492-1498.
23. Dayan İ, Tıkız C, Taneli F, Ulman C, Ulutaş G, Tüzün Ç. Relationship between cyclic citrullinated peptide antibodies positivity and HLA-DRB1 shared epitope alleles in patients with rheumatoid arthritis in Turkey. *Turk J Rheumatol*. 2010;25:12-18.
24. Alrogy A, Dirar A, Alrogy W, Fakhoury H, Hajeer A. Association of human leukocyte antigen- DRB1 with anti-cyclic citrullinated peptide autoantibodies in Saudi patients with rheumatoid arthritis. *Ann Saudi Med*. 2017;37(1):38-41.
25. Huizinga TWJ, Amos CI, van der Helm-van Mil AHM, et al. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. *Arthritis Rheum*. 2005;52(11):3433-3438.
26. van der Helm-van Mil AHM, Verpoort KN, Breedveld FC, Huizinga TWJ, Toes REM, de Vries RRP. The HLA–DRB1 shared epitope alleles are primarily a risk factor for anti–cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. *Arthritis Rheum*. 2006;54(4):1117-1121.
27. Ohmura K, Terao C, Maruya E, et al. Anti-citrullinated peptide antibody-negative RA is a genetically distinct subset: a definitive study using only bone-erosive ACPA-negative rheumatoid Arthritis. *Rheumatology*. 2010;49:2298-2304.
28. Scally SW, Petersen J, Law SC, et al. A molecular basis for the association of the HLADRB1 locus, citrullination, and rheumatoid arthritis. *J Exp Med*. 2013;210:2569-2582.
29. Hill JA, Southwood S, Sette A, Jevnikar AM, Bell DA, Cairns E. Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. *J Immunol*. 2013;171:538-541.
30. Charpin C, Balandraud N, Guis S, et al. HLA-DRB1*0404 is strongly associated with high titers of anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis. *Clin Exp Rheumatol*. 2008;26:627-631.
31. van de Stadt LA, de Koning MH, van de Stadt RJ, et al. Development of the anti-citrullinated protein antibody repertoire prior to the onset of rheumatoid arthritis. *Arthritis Rheum*. 2011;63:3226-3233.
32. Suwannalai P, van de Stadt LA, Radner H, et al. Avidity maturation of anti-citrullinated protein antibodies in rheumatoid arthritis. *Arthritis Rheum*. 2012;64:1323-1328.
33. Ling S, Cheng A, Pumpens P, Michalak M, Holoshitz J. Identification of the rheumatoid arthritis shared epitope binding site on calreticulin. *PLoS ONE*. 2010;5:e11703.
34. de Almeida DE, Ling S, Holoshitz J. New insights into the functional role of the rheumatoid arthritis shared epitope. *FEBS Lett*. 2011;585(23):3619-3626.

How to cite this article: Raslan HM, Attia HR, Hamed Ibrahim M, et al. Association of anti-cyclic citrullinated peptide antibodies and rheumatoid factor isotypes with HLA-DRB1 shared epitope alleles in Egyptian rheumatoid arthritis patients. *Int J Rheum Dis*. 2020;23:647–653. https://doi.org/10.1111/1756-185X.13819

Received: 30 September 2019 | Revised: 15 January 2020 | Accepted: 3 February 2020

DOI: 10.1111/1756-185X.13810

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases | WILEY

Increased CD200 levels in peripheral blood mononuclear cells of patients with primary Sjögren's syndrome

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Funding information
This study was supported by the National Natural Science Foundation of China (Grant No. 8167060222 and 81671556).

Abstract

Objectives: Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease with an unknown etiology. CD200 is associated with many autoimmune diseases, but little is known about its role in pSS. This study aims to correlate the expression of CD200 with pSS and evaluate its significance.

Methods: Plasma CD200, CD200R, and interleukin (IL)-17 levels were measured and analyzed by enzyme-linked immunosorbent assay. Messenger RNA levels of CD200 and CD200R in peripheral blood mononuclear cells (PBMCs) were quantified by quantitative real-time polymerase chain reaction (RT-qPCR). Following pretreatment of CD200-Fc, the protein levels of IL-17A were measured in PBMCs from patients and healthy controls.

Results: Results showed that, compared to CD200 in healthy controls, the relative levels in PBMCs from pSS were greater than 2-fold. In addition, CD200 levels in plasma positively correlated with IL-17 levels, as well as between plasma CD200 and pSS activity indexes (including immunoglobulin G and European League Against Rheumatism SS Disease Activity Index). While CD200R levels were significantly decreased in pSS patients, no correlation could be found. Furthermore, the protein level of IL-17 decreased after pretreatment of CD200-Fc in PBMCs from pSS patients.

Conclusion: Our results suggested that the CD200/CD200R pathway is involved in pSS pathogenesis. It is hypothesized that regulation of IL-17 expression affects Th17 differentiation. This newly discovered pathway could give rise to a novel targeted therapy for pSS.

KEYWORDS

CD200, IL-17, peripheral blood mononuclear cell, primary Sjögren's syndrome

1 | INTRODUCTION

Primary Sjögren's syndrome (pSS) is a clinically chronic autoimmune disease, characterized by the dysfunction of exocrine glands, which results in dry eyes and mouth.[1] The etiology of pSS is currently unclear, but previous studies have suggested that the human immune system could produce auto-antibodies that target and damage exocrine glands, which play a central role in the development of pSS.[1,2] Sustained perturbations of the immune system are harmful to the host and eventually result in disease conditions such as rheumatoid arthritis (RA),[3,4] systemic lupus erythematosus (SLE)[5,6] and cancer.[7]

Recent studies have revealed that a key mediator of interleukin (IL)-17/Th17 (T-helper cell 17) could significantly contribute to pSS pathogenesis, which is characterized by abnormal cytokine

Liu and Zeng are contributed equally to this work.

production.[8-10] IL-17 is one of the six members of the IL-17 cytokine family.[11] Higher IL-17 expression has been observed in many autoimmune diseases, such as SLE and primary biliary cirrhosis. After induction of pSS, wild type mice showed a significant reduction in saliva flow rate while IL-17-deficient mice remained unaffected,[12-14] suggesting IL-17 is a leading contributor to the pSS pathological process.

CD200 (also known as OX2) was discovered by Barclay in 1981.[15] It acts as a leukocyte differentiation antigen and type-1 transmembrane cell-surface glycoprotein containing 2 immunoglobulin superfamily domains. CD200 plays a role in inducing cell signaling through the CD200 receptors (CD200R), which are expressed by a variety of cells including active B cells, T cells, dendritic cells (DC), monocytes/macrophages, and myeloid-derived cells.[16-18] Akman-Karakaş et al[19] demonstrated that serum-soluble CD200 level was higher in autoimmune and inflammatory skin disorders compared to healthy controls where CD200 played a role in maintaining proinflammatory reactions or immune tolerance. In addition, recent studies showed that the downregulation of CD200R1 in RA patients rectified the Th17/Treg (T-regulator) imbalance.[20,21] However, the function of the CD200/CD200R pathway in patients with pSS and the relationship with IL-17/Th17 have not yet been established.

To better understand the role of the CD200/CD200R pathway in pSS, we analyzed the expression of CD200/CD200R in patients and correlated it with disease progression. Furthermore, we investigated the relationships between CD200, CD200R, and IL-17 expressions in pSS.

2 | MATERIALS AND METHODS

2.1 | Patients and controls

Thirty-two patients with pSS admitted to Changhai Hospital from June 2016 to March 2018 were enrolled in the study. The diagnosis of pSS was based on the 2002 revised international standard classification of pSS.[22] Patients who had history of diabetes, hypertension, or infectious diseases were excluded. Their average age was 54.91 ± 2.54 years, and the proportion of females was nearly 90%. Twenty-eight healthy individuals (25 female and three male; average age: 49.54 ± 1.79 years) who received a health check-up during the same period in Changhai Hospital were included as controls. Table 1 shows the main characteristics of the two groups, and there were no statistical differences in age and gender between them ($P > .05$). We followed seven new-onset patients after effective treatment of glucocorticoid and/or immunosuppressant drugs. The criterion for assessing response to pSS treatments was conformed with previous reports.[23-25]

This study was approved by the Ethics Committee of Changhai Hospital, Shanghai, China. Written informed consents were obtained from all participating subjects.

TABLE 1 Primer sequences for quantitative real-time polymerase chain reaction

Gene	Primer sequences (5′-3′)
CD200	F: TGCCCAGGAAGCCCTCATT
	R: AGGCTGGATCACCACCCCAT
CD200R	F: TGAGACCAAGGAAACCAACTG
	R: CCACGGTACGAATCTGAAGG
β-actin	F: AGATCAAGATCATTGCTCCTCCT
	R: ACGCAGCTCAGTAACAGTCC-3

Note: Abbreviations: F, forward; R: reverse.

3 | EXPERIMENTAL PROCEDURES

3.1 | Separation and treatment of peripheral blood mononuclear cells (PBMCs)

Peripheral blood mononuclear cells were separated from blood samples of the 32 pSS patients and the 28 healthy controls (Table 2). Briefly, blood without anticoagulant was centrifuged at 2500 *g* for 10 minutes, and the plasma was collected into an Eppendorf tube and stored at −80℃ for subsequent studies. The remainder cells were diluted by phosphate-buffered saline and slowly poured into the tube containing the Ficoll-Hypaque density gradient solution

TABLE 2 Clinical characteristics of pSS patients and healthy controls

Items	pSS Patients (N = 32)	Healthy controls (N = 28)	*P*
Age, years	54.91 ± 2.53	49.55 ± 1.79	.143
Gender (male/female)	29/3	25/3	.796
C-reactive protein, mg/L	24.58 ± 6.07	—	—
Erythrocyte sedimentation rate, mm/h	64.19 ± 7.33	—	—
C3, g/L	0.93 ± 0.06	—	—
C4, g/L	0.16 ± 0.01	—	—
Serum immunoglobulin G, g/L	22.63 ± 1.25	—	—
Antinuclear antibodies, +/−	26/6	—	—
Anti-SSA, +/−	25/7	—	—
Anti-SSB, +/−	16/16	—	—
ESSDAI score	5 (0-11)	—	—
Extraglandular manifestations			
Interstitial lung disease	19	—	—
Primary biliary cirrhosis	1	—	—
Renal tubular acidosis	2	—	—

Note: Abbreviations: ESSDAI, European League Against Rheumatism SS Disease Activity Index; pSS, primary Sjögren's syndrome.

(Sigma-Aldrich), and then centrifuged at 400 *g* for 30 minutes and washed twice to obtain PBMCs.

In vitro, PBMCs (6×10^5/well) from pSS patients and healthy controls were pre-treated with CD200Fc (300 ng/mL, R&D Systems) or immunoglobulin G (IgG) Fc (used as control, R&D Systems) for 24 h, after which the proteins of the cells were collected for Western blot analysis.

3.2 | Quantitative real-time polymerase chain reaction (qRT-PCR)

Under no ribonuclease, total RNA was separated from PBMCs using the Trizol kit (ThermoFisher Scientific), and was then reverse-transcribed into complementary DNA (cDNA) by the cDNA Synthesis Kit (Takara). qRT-PCR was performed through the SYBR Green Master Mix on Roche LightCycler® 480II (Roche). All samples were repeated in triplicates. The reference gene was β-actin and target genes were CD200 and CD200R. Primer sequences are listed in Table 1.

3.3 | Enzyme-linked immunosorbent assay (ELISA)

ELISA Kits (Abcam) were used to test the protein levels of CD200, CD200R, and IL-17 for the patients according to the protocols provided by the manufacturer. The assays were repeated three times.

3.4 | Western blot

The standard Western blotting procedure was performed as follows: lysates of PBMCs were prepared using radioimmunoprecipitation assay lysis buffer, and then separated by 10% sodium dodecyl polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes, and subsequently blocked by 5% milk for 1 hour. The membranes were incubated with primary antibodies including IL-17A (Abcam) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (CMCTAG) at 4°C overnight, followed by incubation with the target secondary antibodies for 1.5 hours at room temperature. The target proteins were then visualized using the ECL system (Amersham Imager 600; GE). GAPDH was used as internal control.

3.5 | Statistical analyses

All analyses were performed using the SPSS software (version 23.0; IBM). Categorical variables were expressed as counts (percentages) and compared using the χ^2 test. The normality of continuous variables was checked using the Kolmogorov-Smirnov test, and variables with normal distributions were presented as mean ± SD and compared by unpaired *t* test. The correlation of CD200/CD200R and other cytokines mentioned above was measured by the Pearson correlative coefficient. Statistical analyses were conducted at a significance level of *P* value <.05 for all analyses.

4 | RESULTS

4.1 | Increased messenger RNA and protein CD200 levels and decreased CD200R in patients with pSS

We evaluated the messenger RNA (mRNA) levels of CD200 and CD200R in PBMCs through qRT-PCR, and protein levels in plasma samples through ELISA. As illustrated in Figure 1A,B, the mRNA and plasma levels of CD200 in patients with pSS were significantly higher than those in healthy controls. In addition, the protein levels of CD200 in pSS patients with extraglandular manifestations (EGMs) were significantly higher compared with those without EGMs (Figure 1C). mRNA and protein levels of CD200R in patients with pSS were significantly lower than those in healthy controls (Figure 1D,E), yet there was no significant difference between patients with EGMs and without EGMs (Figure 1F).

4.2 | Expression of plasma CD200 was positively correlated with clinical parameters in patients with pSS

Figure 2A shows there was a positive correlation between the plasma CD200 concentration and the IgG level (R = 0.4822, P = .0052). CD200 levels were also in significantly positive correlation with European League Against Rheumatism SS Disease Activity Index (R = 0.3673, P = .0387) (Figure 2B). Moreover, we found that the serum CD200 levels in pSS patients with interstitial lung disease (ILD) were higher than those without ILD (Figure 2C), and there was a significant positive correlation between CD200 and IgG levels in pSS patients with ILD (Figure 2D). There was no significant positive correlation in pSS patients without ILD (Figure 2E). Moreover, there was no correlation between the CD200R level and clinical features (data not shown).

4.3 | Expression of plasma CD200 was positively associated with IL-17, and pretreatment of CD200Fc reduced the expression of IL-17A in PBMCs

We also examined IL-17 levels in the peripheral blood of the subjects, and the expression of IL-17 in pSS patient samples was higher than those of the healthy controls (Figure 3A). As illustrated in Figure 3B, the plasma CD200 levels of patients with pSS were markedly positively correlated with IL-17 levels. After pretreatment with CD200 Fc, the expression of IL-17 of PBMCs derived from pSS patients was significantly decreased (Figure 3C). In addition, we followed up on 7 new-onset patients after effective treatment with glucocorticoid and/or immunosuppressant drugs, and their plasma levels of CD200

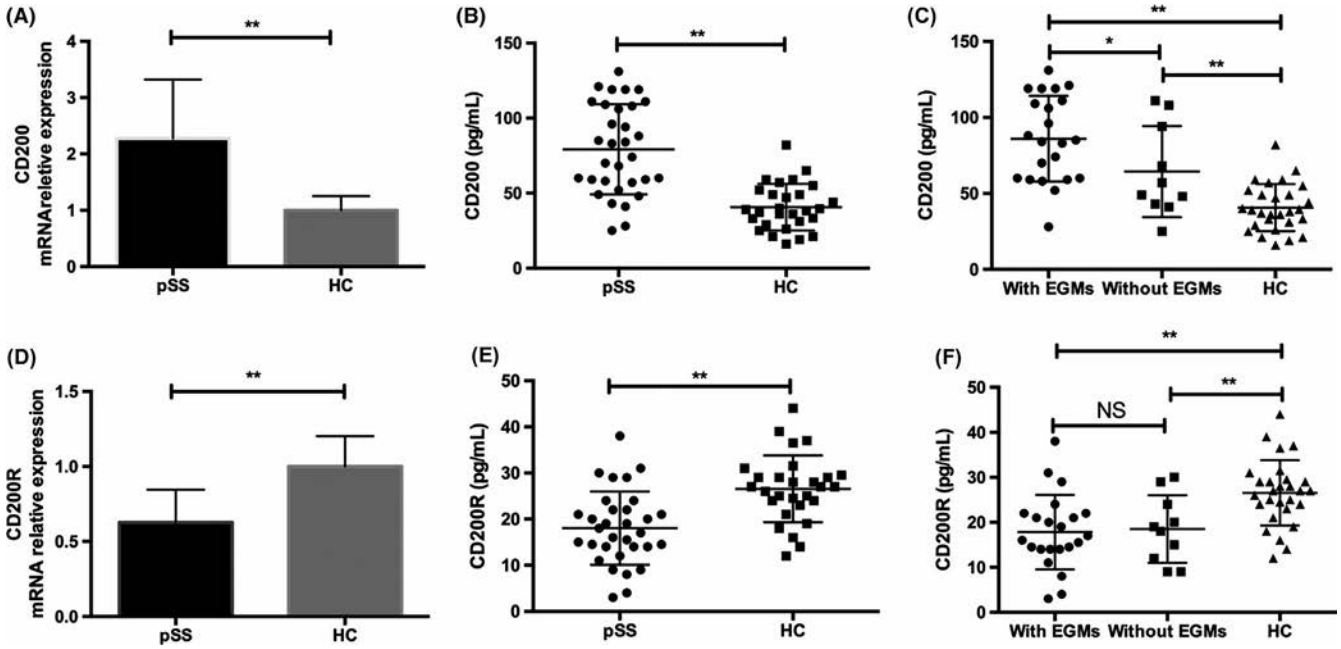

FIGURE 1 Increased messenger RNA (mRNA) and protein CD200 levels and decreased CD200R in patients with primary Sjögren's syndrome (pSS). A, Comparison of relative mRNA expression levels of CD200 between patients with pSS and healthy controls (HC). B, Plasma expressions of CD200 and (C) comparison of plasma expressions of CD200 in patients with extraglandular manifestations (EGMs), without EGM, and in HC. D-F, Comparisons of (D) relative mRNA expression levels of CD200R between patients with pSS and HCs, (E) plasma CD200R expressions, and (F) plasma expressions of CD200R in patients with EGM, without EGM and HCs. $^{*}P < .05$, $^{**}P < .01$

and IL-17 significantly decreased after effective therapy. The expression of CD200R was increased with no statistical difference (Figure 4A-C).

5 | DISCUSSION

In the present study, we demonstrated that the mRNA levels of CD200 and IL-17A were significantly increased and that of CD200R was decreased in PBMCs from patients with pSS. We also found that the plasma CD200 and IL-17 levels were higher and that of CD200R and were lower in patients with pSS. Moreover, the plasma expression of CD200 positively correlated with disease activity and IL-17A levels. To our best knowledge, we have unprecedentedly reported that the aberrant expression of CD200/CD200R pathway was associated with IL-17 and disease activity in pSS patients.

The CD200/CD200R pathway plays an important role in immunomodulatory effects, especially in immune tolerance and the differentiation of cells. CD200 is highly expressed in myeloid cells such as macrophages, DCs, and lymphoid cells.[18] Valente et al showed that the expression of CD200R1 in an experimental autoimmune encephalomyelitis model was significantly raised, and the CD200/CD200R1 system was a potential therapeutic target in multiple sclerosis.[26] The expressions of CD200 in circulation of autoimmune and inflammatory skin diseases such as psoriasis vulgaris and pemphigus vulgaris are higher than healthy controls.[19] Moreover, a recent report demonstrated that the number of $CD200^{+}$ cells and the level of soluble CD200 were raised in patients with SLE.[27]

Our results suggested that the elevated plasma level of CD200 in pSS patients and the level of CD200 proteins positively correlated with disease activity. We also found elevated expression of CD200 in pSS patients with ILD compared to patients without ILD. The reason might be the involvement of CD200 in the control and resolution of inflammation. CD200 is expressed by airway epithelium and is critically involved in pulmonary immunoregulation,[28] and elevated levels of CD200 during inflammation could induce the inflammatory cascade. Recently, research reported that CD200R activation is followed by decreased expression of major histocompatibility complex (MHC) II and reduction in synthesis of proinflammatory cytokines.[29] In pSS salivary glands, epithelial cells have also been found to express MHC I and II, as well as adhesion and costimulatory molecules critical for activation and regulation of naïve T cells.[30] Therefore, the imbalance of the CD200/CD200R axis might be involved in T cell activation and regulation in pSS.

IL-17 has been acknowledged as a prime representative of the new-generation proinflammatory cytokines for Th17 cells, a newly defined $CD4^{+}$ Th cells subset.[31,32] IL-17-producing T cells play a role in pSS pathogenesis.[33,34] Until now, many researchers have revealed that the expressions of IL-17 and IL-17R are increased in salivary glands and serum from pSS patients.[35,36] Lin et al demonstrated that immunized IL-17 knock-out mice were completely resistant to SS induction.[13] Yet, the mechanism of IL-17 in pSS remains unknown. The present study showed that the plasma CD200 level of patients with pSS was positively related to IL-17, which provides potential new insights on the pathogenesis mechanisms of IL-17 in pSS.

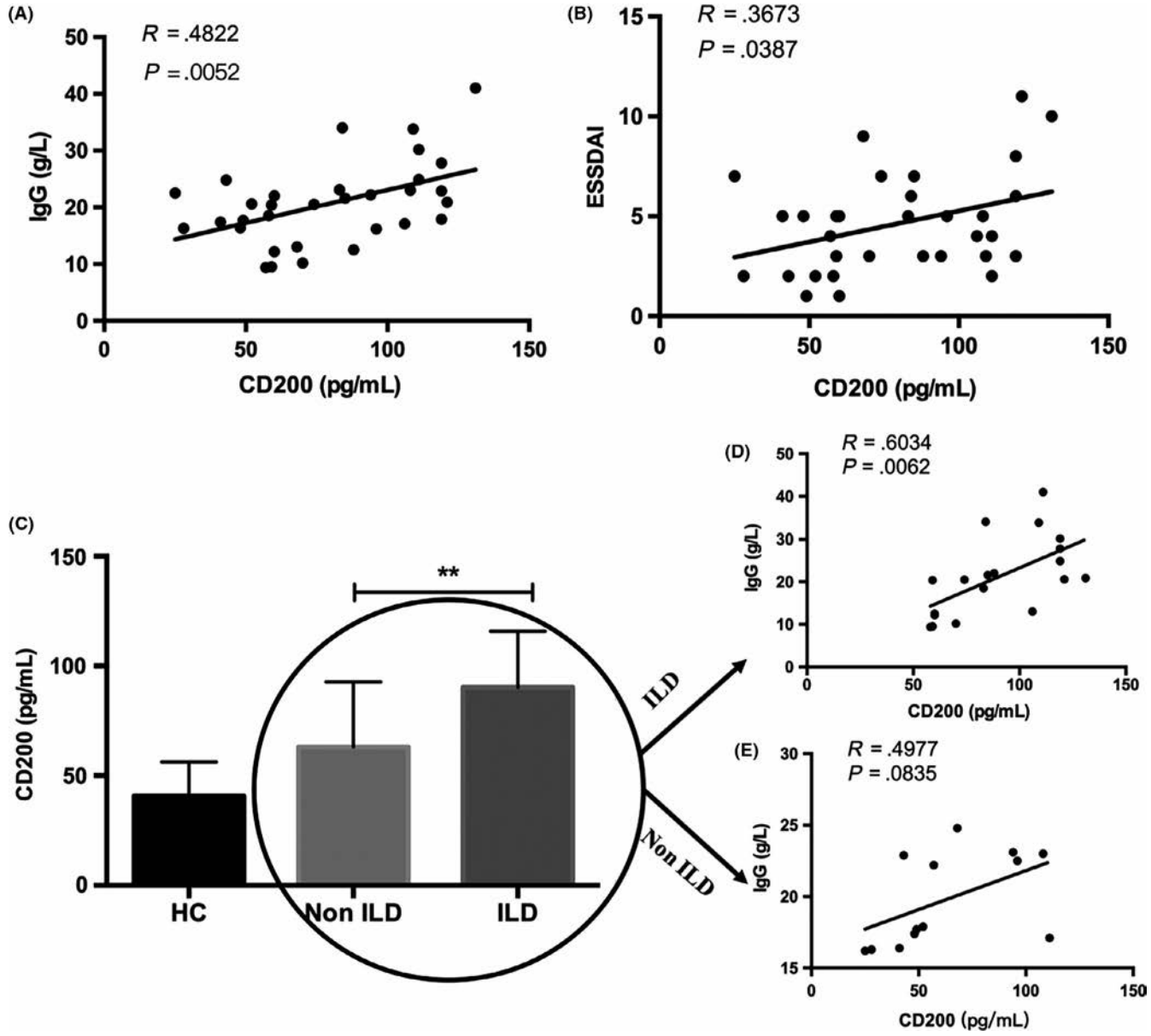

FIGURE 2 Expression of plasma CD200 was positive correlated with clinical parameters in patients with primary Sjögren's syndrome (pSS). A, The correlations between the concentrations of CD200 and immunoglobulin G (IgG), and (B) European League Against Rheumatism SS Disease Activity Index (ESSDAI). C, Comparison of plasma CD200 expression in patients with interstitial lung disease (ILD) and non-ILD. D, The correlations between concentrations of CD200 and IgG in plasma in patients with ILD, and (E) non-ILD

Recent studies have shown that in vitro CD200 Fc could inhibit the differentiation of $CD4^+$ T cells into Th17 cells in RA patients.[20,21] They also reported that CD200 Fc down-regulated the CC chemokine receptor 6 (CCR6) expression and inhibited CC chemokine ligand 20 (CCL20)/CCR-driven Th17 chemotaxis in RA. Moreover, CD200 Fc but not anti-CD200R1 reduced the percentage of Th17 cells in SLE patients.[27] These reports suggested that CD200 plays a crucial role in $CD4^+$ T cell differentiation into Th17 cells. Our results showed that CD200 was positively correlated with IL-17 in plasma from pSS patients, suggesting that the IL-17 level may be affected by CD200. We further found that pretreatment of CD200 Fc could reduce the expression of IL-17 of PBMCs derived from pSS patients. The reason might be that the CD200/CD200 axis is unbalanced in pSS because of reduction of degradation rate of CD200, and increased expression of CD200 could regulate the immune functions through inhibiting Th17 differentiation in pSS.

Further, we followed up on 7 new-onset patients after effective treatment with glucocorticoid and/or immunosuppressant drugs. Treatment of pSS was determined by symptoms and EGM. Based on our findings, glucocorticoids and/or immunosuppressant drugs should perhaps be added for treating patients with organ involvement.[23,25] After effective therapy, the levels of CD200 and IL-17 were significantly decreased. This could be explained by the aberrant CD200 expression by apoptotic cells, which decreases proinflammatory cytokine production, contributing to reduced clearance of apoptotic fragments.[27] Thus, the CD200/

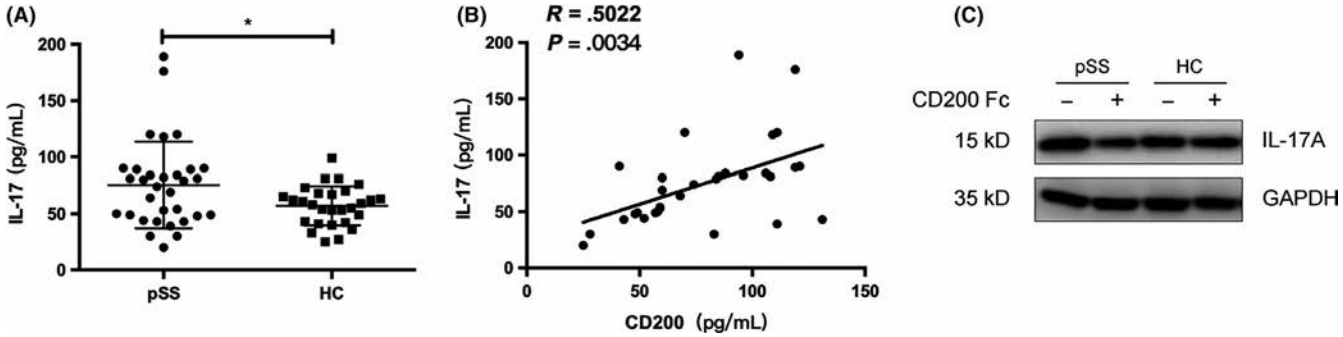

FIGURE 3 Positive correction between plasma CD200 and interleukin (IL)-17, and CD200 reduces IL-17A protein expression after pretreatment of CD200 Fc. A, Comparison of plasma expression levels of IL-17 between patients with primary Sjögren's syndrome (pSS) and healthy controls (HCs). B, Correction between plasma CD200 and IL-17 in pSS patients. C, The change of IL-17 protein level in peripheral blood mononuclear cells from pSS patients and HCs after CD200 Fc. GAPDH, glyceraldehyde-3-phosphate dehydrogenase

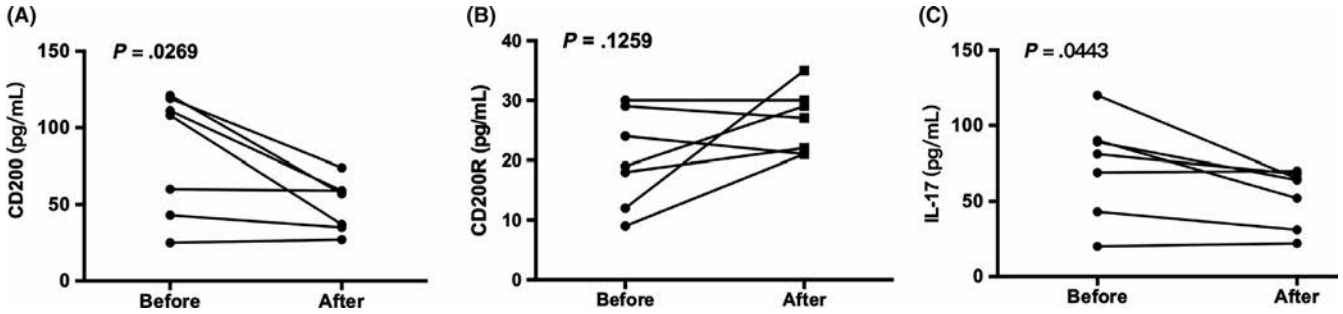

FIGURE 4 The cytokine levels change in 7 new-onset patients with primary Sjögren's syndrome (pSS) after effective treatment. A-C, The change of plasma CD200, CD200R and interleukin (IL)-17 concentrations in new-onset patients with pSS after effective treatment

CD200R inhibitory axis becomes imbalanced, and a crucial role for glucocorticoid is needed to regulate the number of T cells and double-positive T cells. These cells are highly sensitive to glucocorticoid-induce apoptosis,[37,38] leading to promoted clearance of apoptotic fragments through glucocorticoid treatment. As such, effective therapies can improve the CD200/CD200R homeostasis and keep the axis balanced under inflammatory conditions. Moreover, glucocorticoids modifying the Th balance affect the predominance of the different Th cell subsets.[39] Decreased levels of IL-17 may be caused by the lowered number of Th17 cells after glucocorticoid treatment.

Nevertheless, there are still several limitations in our present study. First, it belongs to a single-center study and includes only a small number of cases. Second, as most of our patients had not been systematically treated or previous therapies were ineffective, their serum levels of C-reactive protein were high, potentially magnifying the positive correlation between the CD200 levels in pSS patients versus health controls. Third, the specific mechanism of CD200 affecting IL-17 expression and Th17 differentiation in pSS was not fully elaborated.

In conclusion, the CD200/CD200R pathway is involved in pSS pathogenesis probably by regulation of IL-17 expression, and this could be a potential target for therapy in pSS.

CONFLICT OF INTEREST

The authors are unaware of any conflicts of interest.

AUTHOR CONTRIBUTIONS

Ting-Ting Liu, Xiang-Peng Zeng and Ming-Li Gu participated in the data acquisition and manuscript drafting. An-Mei Deng contributed to the conception, design, and data interpretation, as well as revised the manuscript for important intellectual content.

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REFERENCES

1. Fox RI. Sjogren's syndrome. *Lancet*. 2005;366(9482):321-331.
2. Odani T, Chiorini JA. Targeting primary Sjogren's syndrome. *Mod Rheumatol*. 2019;29(1):70-86.
3. Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature*. 2003;423(6937):356-361.
4. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet*. 2016;388(10055):2023-2038.
5. Mizui M, Tsokos GC. Targeting regulatory T cells to treat patients with systemic lupus erythematosus. *Front Immunol*. 2018;9:786.
6. Tsokos GC, Lo MS, Costa Reis P, Sullivan KE. New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat Rev Rheumatol*. 2016;12(12):716-730.
7. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science*. 2015;348(6230):56-61.
8. Bikker A, van Woerkom JM, Kruize AA, et al. Clinical efficacy of leflunomide in primary Sjogren's syndrome is associated with regulation of T-cell activity and upregulation of IL-7 receptor alpha expression. *Ann Rheum Dis*. 2012;71(12):1934-1941.

9. Fei Y, Zhang W, Lin D, et al. Clinical parameter and Th17 related to lymphocytes infiltrating degree of labial salivary gland in primary Sjogren's syndrome. *Clin Rheumatol*. 2014;33(4):523-529.
10. Sun Y, Wang Y, Chen S, et al. Expression of galphaq is decreased in lymphocytes from primary Sjogren's syndrome patients and related to increased IL-17A expression. *J Immunol Res*. 2018;2018:8212641.
11. Gaffen SL. Structure and signalling in the IL-17 receptor family. *Nat Rev Immunol*. 2009;9(8):556-567.
12. Harada K, Shimoda S, Sato Y, Isse K, Ikeda H, Nakanuma Y. Periductal interleukin-17 production in association with biliary innate immunity contributes to the pathogenesis of cholangiopathy in primary biliary cirrhosis. *Clin Exp Immunol*. 2009;157(2):261-270.
13. Lin X, Rui K, Deng J, et al. Th17 cells play a critical role in the development of experimental Sjogren's syndrome. *Ann Rheum Dis*. 2015;74(6):1302-1310.
14. Oke V, Brauner S, Larsson A, et al. IFN-lambda1 with Th17 axis cytokines and IFN-alpha define different subsets in systemic lupus erythematosus (SLE). *Arthritis Res Ther*. 2017;19(1):139.
15. Barclay AN. Different reticular elements in rat lymphoid tissue identified by localization of Ia, Thy-1 and MRC OX 2 antigens. *Immunology*. 1981;44(4):727-736.
16. Yu K, Chen Z, Gorczynski R. Effect of CD200 and CD200R1 expression within tissue grafts on increased graft survival in allogeneic recipients. *Immunol Lett*. 2013;149(1–2):1-8.
17. Oria M, Figueira RL, Scorletti F, et al. CD200-CD200R imbalance correlates with microglia and pro-inflammatory activation in rat spinal cords exposed to amniotic fluid in retinoic acid-induced spina bifida. *Sci Rep*. 2018;8(1):10638.
18. Holmannová D, Koláčková M, Kondělková K, Kuneš P, Krejsek J, Andrýs C. CD200/CD200R paired potent inhibitory molecules regulating immune and inflammatory responses; Part I: CD200/CD200R structure, activation, and function. *Acta Medica (Hradec Kralove)*. 2012;55(1):12-17.
19. Akman-Karakas A, Yalcin AD, Koc S, et al. There might be a role for CD200 in the pathogenesis of autoimmune and inflammatory skin disorders. *Med Sci Monit*. 2013;19:888-891.
20. Ren Y, Yang BO, Yin Y, et al. Aberrant CD200/CD200R1 expression and its potential role in Th17 cell differentiation, chemotaxis and osteoclastogenesis in rheumatoid arthritis. *Rheumatology (Oxford)*. 2015;54(4):712-721.
21. Gao S, Hao B, Yang XF, Chen WQ. Decreased CD200R expression on monocyte-derived macrophages correlates with Th17/Treg imbalance and disease activity in rheumatoid arthritis patients. *Inflamm Res*. 2014;63(6):441-450.
22. Vitali C, Bombardieri S, Jonsson R, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis*. 2002;61(6):554-558.
23. Stefanski AL, Tomiak C, Pleyer U, et al. The diagnosis and treatment of Sjogren's syndrome. *Dtsch Arztebl Int*. 2017;114(20):354-361.
24. Both T, Dalm VA, van Hagen PM, van Daele PL. Reviewing primary Sjogren's syndrome: beyond the dryness - From pathophysiology to diagnosis and treatment. *Int J Med Sci*. 2017;14(3):191-200.
25. Saraux A, Pers JO, Devauchelle-Pensec V. Treatment of primary Sjogren syndrome. *Nat Rev Rheumatol*. 2016;12(8):456-471.
26. Valente T, Serratosa J, Perpina U, Saura J, Sola C. Alterations in CD200-CD200R1 system during EAE already manifest at presymptomatic stages. *Front Cell Neurosci*. 2017;11:129.
27. Li Y, Zhao L-D, Tong L-S, et al. Aberrant CD200/CD200R1 expression and function in systemic lupus erythematosus contributes to abnormal T-cell responsiveness and dendritic cell activity. *Arthritis Res Ther*. 2012;14(3):R123.
28. Lauzon-Joset JF, Marsolais D, Tardif-Pellerin E, Patoine D, Bissonnette EY. CD200 in asthma. *Int J Biochem Cell Biol*. 2019;112:141-144.
29. Liu Y, Bando Y, Vargas-Lowy D, et al. CD200R1 agonist attenuates mechanisms of chronic disease in a murine model of multiple sclerosis. *J Neurosci*. 2010;30(6):2025-2038.
30. Goules AV, Tzioufas AG. Primary Sjogren's syndrome: clinical phenotypes, outcome and the development of biomarkers. *Immunol Res*. 2017;65(1):331-344.
31. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmun Rev*. 2014;13(6):668-677.
32. Hoe E, Anderson J, Nathanielsz J, et al. The contrasting roles of Th17 immunity in human health and disease. *Microbiol Immunol*. 2017;61(2):49-56.
33. Matsui K, Sano HT. Helper 17 cells in primary Sjogren's syndrome. *J Clin Med*. 2017;6(7):65.
34. Alunno A, Carubbi F, Bistoni O, et al. T Regulatory and T helper 17 cells in primary Sjogren's syndrome: facts and perspectives. *Mediators Inflamm*. 2015;2015:243723.
35. Szodoray P, Horvath IF, Papp G, et al. The immunoregulatory role of vitamins A, D and E in patients with primary Sjogren's syndrome. *Rheumatology (Oxford)*. 2010;49(2):211-217.
36. Zhang R, Sun T, Song L, Zuo D, Xiao W. Increased levels of serum galectin-3 in patients with primary Sjogren's syndrome: associated with interstitial lung disease. *Cytokine*. 2014;69(2):289-293.
37. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol Cell Endocrinol*. 2011;335(1):2-13.
38. Purton JF, Monk JA, Liddicoat DR, et al. Expression of the glucocorticoid receptor from the 1A promoter correlates with T lymphocyte sensitivity to glucocorticoid-induced cell death. *J Immunol*. 2004;173(6):3816-3824.
39. Cindy S, Lisa E, Timo G, Frank B. Glucocorticoids—All-rounders tackling the versatile players of the immune system. *Front Immunol*. 2019;10:1744.

How to cite this article: Liu T-T, Zeng X-P, Gu M-L, Deng A-M. Increased CD200 levels in peripheral blood mononuclear cells of patients with primary Sjögren's syndrome. *Int J Rheum Dis*. 2020;23:654–660. https://doi.org/10.1111/1756-185X.13810

Received: 18 July 2019 | Revised: 23 December 2019 | Accepted: 6 February 2020

DOI: 10.1111/1756-185X.13811

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases | WILEY

The burden of subclinical intra-articular inflammation in gout

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Funding information
This work was supported by University of Western Australia seeding grant awarded to Helen Keen.

Abstract

Objective: To assess the burden of subclinical intra-articular inflammation using ultrasound in people with gout.

Methods: A pilot, prospective longitudinal cohort of 28 participants with gout were examined twice, once during a gout flare (n = 25) and then during an inter-critical phase (n = 27). At each visit, a 52 joint count was done followed by ultrasound examination for detection of intra-articular power Doppler (PD) signal. Clinically active joints were defined as tender and swollen. Data was collected on patient reported gout pain - visual analog scale (VAS) (painVAS), physician global VAS (physicianVAS), Health Assessment Questionnaire (HAQ), serum uric acid, erythrocyte sedimentation rate (ESR), and high-sensitivity C-reactive protein (HsCRP).

Results: At the flare visit, participants had a median of 1 clinically active joint (interquartile range [IQR] 1-2), and a median of 5 joints with a PD score ≥ 2 (IQR 4-10, $P < .001$). At the inter-critical visit, participants reported an median of 0 clinically active joints (IQR 0-0), and a median of 4 joints with a PD score ≥ 2 (IQR 3-7, $P < .001$). Physician VAS (5.69 vs 3.40, $P < .001$), painVAS (6 vs 0, $P < .001$), HAQ (0.75 vs 0.12, $P = .032$), and ESR (29 vs 13.5 mm/h, $P = .02$) were higher at the acute visit, but HsCRP levels were similar (8.88 vs 5.15 mg/L, $P = .062$).

Conclusion: This pilot study established the presence of subclinical intra-articular inflammation in gout at both acute and inter-critical phases. Despite the apparent resolution of symptoms after an acute flare, a relatively high proportion of joints had subclinical inflammation in the inter-critical visit. The long-term implications of untreated subclinical joint inflammation are not clear.

KEYWORDS
biomarkers, gout, inflammation, ultrasonography

1 | INTRODUCTION

Gout is a prevalent inflammatory arthropathy in the community affecting 3.8%-5.8% of the adult population and is rising.[1,2] Gout is the result of persistent serum levels of uric acid above the saturation point (hyperuricemia), which leads to the deposition of monosodium urate (MSU) crystals in articular and peri-articular tissues.[3] The build-up of MSU crystals causes an activation of the nucleotide-binding oligomerization domain, leucine-rich repeat and pryrin domain 3 inflammasome response, which releases interleukin (IL)-1β and IL-18 in an attempt to attract macrophages to remove the birefringent crystals.[4]

Poorly treated gout can cause joint damage, nephrolithiasis and is associated with increased morbidity and mortality.[5] The

etiopathogenesis and pathophysiology of gout is relatively well understood, and clinicians have access to effective treatments, such as allopurinol or febuxostat.[6] Despite the advances in understanding about the disease, the availability of effective therapies, and guidelines on treatment targets published, gout remains sub-optimally treated with both non-pharmacological and pharmacological therapies.[7] As a consequence of the inadequate application of treat-to-target approaches, patients experience sub-optimal disease-specific and health-related outcomes.[7]

Current therapeutic strategies focus on suppressing serum uric acid (SUA) levels.[8] However, as a biomarker, a reduction in SUA has not yet conclusively been shown to be related to a reduction in the number of clinical flares or improved long-term outcomes for patients.[8,9] Because outcomes from gout are likely mediated through an inflammatory response to MSU deposition,[10] ultrasound (US) may have a role in predicting long-term outcomes in gout. US has been validated in detecting intra-articular joint inflammation, even when subclinical, which means that the joint was not found to be swollen or tender by a clinician.[8,11] These findings suggest that US may have a role as a biomarker in gout.

Several studies have demonstrated subclinical synovitis in gout,[12,13] but none have addressed the extent of joint involvement through systematic evaluation of 66 joints, or examined the joints longitudinally over time to determine the natural history of subclinical inflammation. As such, the significance of this subclinical inflammation is uncertain. It may predict short-term outcomes (such as clinical flares) or long-term outcomes, such as joint damage or cardiovascular risk, and thus it may have therapeutic implications.

This study aimed to better understand the burden of subclinical synovitis, and its short-term natural history, through a thorough exploration of the presence of US detected synovitis in 66 joints of people with gout, over time.

Key messages

- Subclinical intra-articular inflammation is common and is present both in the acute and inter-critical phases of gout.
- Persistence of subclinical intra-articular inflammation was found in the inter-critical visit.

2 | METHODS

In this pilot study, 28 participants were recruited from 2 tertiary hospitals in Perth (Royal Perth Hospital and Sir Charles Gairdner Hospital), who met either the American College of Rheumatology (ACR)[14] or European League Against Rheumatism (EULAR)[15] Classification Criteria for Gout. The inclusion criteria included a diagnosis of gout based on clinical presentation according to contemporary EULAR or ACR criteria.[14,15] Patients were excluded if they had a concurrent inflammatory joint disorder, such as rheumatoid arthritis (RA). The study was conducted in accordance with the Declaration of Helsinki and was approved by the local Human Research Ethics Committee (HREC no.: 2009/020). Informed consent was obtained from all patients before study enrolment.

Participants attended 2 study visits, once during a flare of gout and once during the inter-critical period. The flare visit occurred during a period of acute gout as defined by the presence of any of the following criteria: swelling of an affected joint, redness of an affected joint, and marked tenderness of an affected joint determined through clinical examination and maximum pain within 4-12 hours as reported by the participant.[16] The inter-critical visit was during a period of inter-critical gout, defined as the absence of symptoms of acute gout for at least 4 weeks prior to the study visit. Participants were recruited during either the flare or inter-critical period.

2.1 | Clinical evaluation

Clinical evaluation documented the patient's relevant medical history, and a physician global visual analog scale (VAS). Patient reported outcomes included a gout-related pain VAS, a gout severity VAS, a global health VAS, and Health Assessment Questionnaire (HAQ).[17] A total of 52 joints were examined in each patient by a doctor (rheumatology fellow); a joint was considered clinically active if it was both tender and swollen at the time of examination. Laboratory tests conducted included full blood picture, urea and electrolytes, high-sensitivity C-reactive protein (HsCRP), erythrocyte sedimentation rate (ESR), and serum urate levels.

2.1.1 | US assessment

The US examination was performed during both study visits on an Esaote mylab 70 XVG, with linear array probes 6-12 and 10-18 MHz. Each patient underwent power Doppler (PD) US examination of 52 peripheral joints by a physician experienced in rheumatological ultrasonography who was blinded to the clinical status, laboratory and (any available) radiographic findings. The joints examined included bilateral shoulders, elbows, wrists, metacarpophalangeals (MCP) 1-5, 1st interphalangeal (IP) (hand), proximal interphalangeals (PIP) 2-5, distal interphalangeals (DIP) (hand) 2-5, knees, ankles, midfoot, metatarsophalangeals (MTP) 1-5, and 1st IP (foot). Bilateral US examination of all joints included longitudinal and cross-sectional images according to published guidelines.[18] Joints were assessed by intra-articular PD signal on a 0-3 semi-quantitative scale.

For this study, a joint was considered inflamed if the intra-articular PD signal was ≥2. Low levels of PD signal can be detected in the setting of osteoarthritis (OA), which is common in adult populations; however, scores of ≥2 are uncommonly attributable to OA, and likely to be representative of inflammation secondary to gout in the context of a cohort with a clinical diagnosis of gout.[19,20]

The reliability of this sonographer (HK) in detecting PD signal has been reported in multiple publications.[19-23]

2.2 | Statistical analysis

This observational study to generate pilot data from which larger studies could be powered and local HREC regulations limit pilot studies to 30 patients. Data are described as count and proportion (%) or as a measure of central tendency, that is, mean ± standard deviation or median and interquartile range (IQR). Comparative statistics include related Wilcoxon signed rank and Mann-Whitney *U* tests as all data were treated as non-parametrically distributed given the small group size (N < 30). We compared the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of clinically determined joint inflammation compared to PD US scores ≥ 2. Subsequently, we undertook a post hoc analysis in the subset of those with uric acid levels (≤0.36 mmol/L) below reported therapeutic targets[6] at both visits; we compared patient and physician reported outcomes, clinically active joints, active joints determined by PD ≥ 2, and HsCRP scores at the acute and inter-critical visits. Data were entered and analyzed on IBM SPSS Version 24. A *P* value < .05 was regarded as statistically significant.

3 | RESULTS

3.1 | Demographic details

Twenty-eight participants with gout with a mean age of 63.9 years and predominantly male (n = 25, 89.3%) were recruited from May 2010 to January 2012. The participants' demographic characteristics are presented in Tables 1 and 2. Twenty-five participants attended the acute visit, 27 attended the inter-critical visit, with data available for 24 participants who attended both visits.

Participants reporting having had gout for an average of 4.3 (IQR 2.44-10.89; range 0.47-31.24) years, and reported an average of 4 gout flares per annum, lasting for on average 7 days.

TABLE 1 Demographics and clinical characteristics of the study cohort (N = 28)

Age, y	63.86 ± 16.59
Ethnicity	
Caucasian	24 (85.7%)
Asian	4 (14.3%)
BMI, kg/m^2	27.28 ± 5.20
Duration of gout, y	4.3 (IQR 2.4-10.9; range 0.47-31.24)
Duration of attacks, d	7 (IQR 3-7; range: 0-30)
One day, number of participants (%)	2 (7.14%)
Two days	3 (10.7%)
Three days	4 (14.3%)
Seven days	16 (57.1%)
Fourteen days	1 (3.57%)
Thirty days	2 (7.14%)
Frequency of attacks, per annum	4 (IQR 2-12; range: 1-52)
Weekly, number of participants (%)	1 (3.57%)
Monthly	10 (35.7%)
Quarterly	9 (32.1%)
Six monthly	5 (17.9%)
Yearly	3 (10.7%)
MSU crystals in synovial fluid, number of participants (%)	19 (67.9%)

Abbreviations: BMI, body mass index; IQR, interquartile range; MSU, monosodium urate.

3.2 | Clinical, ultrasound, laboratory and patient reported outcome findings at each visit

3.2.1 | Acute visit

The acute visit represented a period of active disease which was supported by higher physician and patient reported health outcome measures, increased clinically active joints, and raised inflammatory markers. The clinical and ultrasound characteristics are presented in Tables 2 and 3.

Of the 25 participants attending the flare visit, there were a total of 66 clinically active joints found, and participants reported an average of 1 clinically active joint(s) (IQR 1-2; range 0-15). Out of these, 82.5% (n = 47/66) (82.5%) joints were deemed subclinically active with US detected (PD ≥ 2) in inflammation. However, utilizing US to assess intra-articular PD signal determined that 203 joints had active inflammation, as defined by PD ≥ 2 (imaging at the acute visit is shown in Figure 1 and the results of this imaging modality are presented in Table 3). Assessment with PD ≥ 2 revealed a median of 5 active joints (IQR 4-10; range 0-23). This is in stark contrast to the findings on clinical examination which missed this subclinical inflammation in the majority of joints including shoulders, ankles, elbows, wrists, MCP, MTP and PIP joints (Table 3). Furthermore, the clinicians' ability to detect any signs of disease activity, including redness, tenderness or swelling, compared to the hypothetical gold standard of US PD ≥ 2 resulted in a sensitivity, specificity, PPV, NPV and accuracy of 33.33%, 96.11%, 60.58%, 88.95%, and 86.58%, respectively; whereas, comparing clinically active joints, that is, simultaneously swollen and tender, against US PD ≥ 2 resulted in a sensitivity, specificity, PPV, NPV and accuracy of 28.66%, 98.64%, 77.59%, 89.40%, and 88.79%, respectively.

3.2.2 | Inter-critical visit

The inter-critical visit occurred at average 10 weeks from the acute visit (IQR 6-26; range 4-62). At the inter-critical visit, very few (4 joints in n = 3 participants) clinically active joints were identified,

TABLE 2 Clinical findings, patient reported outcome measures and laboratory results in acute and inter-critical visits

Variable	Acute visit N = 25 Median (IQR)	Inter-critical visit N = 27 Median (IQR)	Related samples Wilcoxon signed rank test
Physician global VAS	5.69 (4.00-6.90)	3.40 (1.30-4.30)	<.001
Patient reported gout pain VAS	6.00 (3.20-7.60)	0.00 (0.00-0.60)	<.001
Patient reported gout severity VAS	6.40 (3.60-7.90)	5.20 (3.60-7.00)	.230
Patient reported global health VAS	4.60 (2.80-5.70)	3.50 (2.00-5.20)	.137
Health Assessment Questionnaire	0.75 (0.37-1.63)	0.12 (0.00-1.13)	.032
Number of clinically active joints	1 (1-2)	0 (0-0)	<.001
Number of with intra-articular PD ≥ 2	5 (4-10) range 0-23	4 (3, 7) range 0-18	.114
ESR, mm/h	29.00 (18.00-48.50)	13.5 (6.0-33.0)	.020
Serum uric acid, mmol/L	0.44 (0.37-0.50)	0.38 (0.30-0.48)	.065
HsCRP, mg/L	8.88 (5.35-37.20)	5.15 (1.39-8.46)	.062
Serum creatinine, μmol/L	107.00 (82.0-156.0)	103.00 (77.0-176.0)	.082

Abbreviations: ESR, erythrocyte sedimentation rate; HsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; PD, power Doppler; VAS, visual analog scale.

The normal range for ESR is 1-20 mm/h, HsCRP is < 0.10 mg/L for our laboratory.

and participants also demonstrated better physician and patient reported health outcomes, along with improved biomarkers of inflammation. The patient reported outcome measures and biochemical markers at the inter-critical visit are presented in Table 2.

Across all patients, a total of 4 (7.4%) clinically active joints were identified by a rheumatologist; whereas, US detected 146 inflamed or active joints (median of 4 joints with PD score of ≥ 2; IQR 3-7; range 0-18) during the inter-critical visit. The most common location of joint inflammation on US were 1st MTP, wrist, and 2nd MCP (Table 3). The ultrasound examination findings of intra-articular PD signal at the inter-critical visit are shown in Figure 2. Clinical examination missed US detected inflammation in the majority of joints including MTP, MCP, wrist, elbow, knee and ankle joints (Table 3).

There was a significant discrepancy in the number of joints deemed clinically active compared with detection of inflammation with US. At the inter-critical visit, the clinicians' ability to detect any symptoms compared to the hypothetical gold standard of US PD score of ≥2 resulted in a sensitivity, specificity, PPV, NPV and accuracy of 9.79%, 97.41%, 31.11%, 90.05%, and 88.08%, respectively; whereas, comparing clinically active joints, that is, simultaneously swollen and tender, against US PD ≥ 2 resulted in a sensitivity, specificity, PPV, NPV and accuracy of 1.40%, 99.83%, 50.00%, 89.46%, and 89.34%, respectively.

3.2.3 | Comparison between visits

The acute visit featured significantly more clinically active joints compared to the inter-critical visit ($P < .001$) (Table 2). Despite the increase in clinically determined joint inflammation at the acute visit, the absolute number of inflamed joints detected with PD signal far surpassed the clinicians' ability to detect intra-articular inflammation.

There was a statistically significant drop in clinically active joints from the acute to inter-critical visits (1 vs 0 joints, $P < .001$). However, this finding was not mirrored with US, where the reduction of the median number of inflamed joints with PD score of ≥2 across the acute and inter-critical visits did not reach statistical significance (5 vs 4 joints, $P = .114$) (Table 2).

Beyond the assessment of inflamed joints count, the average physician global VAS (5.69 vs 3.40 points, $P < .001$), patient reported gout pain (6 vs 0 points, $P < .001$) and HAQ (0.75 vs 0.12 points, $P = .032$) scores were higher at the acute visit. These

TABLE 3 Clinical and US findings at visits

Joints examined	Flare visit		Inter-critical visit	
	Clinically active, ie, tender and swollen joint n (%) or median (IQR)	US detected PD signal (score ≥ 2) n (%) or median (IQR)	Clinically active, ie, tender and swollen joint n (%) or median (IQR)	US detected PD signal (score ≥ 2) n (%) or median (IQR)
Shoulder	0	3 (5.56)	0	1 (1.85)
Elbow	2 (3.70)	10 (18.51)	0	7 (12.96)
Wrist	1 (1.85)	17 (31.48)	0	17 (31.48)
MCP involvement	2 (8.0) 4 (IQR 2-6) range 2-6	12 (48.0) 3.5 (IQR 1-4.5) range 1-10	1 (3.7) 2 (IQR 2-2) range 2-2	15 (55.6) 2 (IQR 1-3) range 1-9
MCP 1	2 (3.70)	8 (14.81)	0	5 (9.25)
MCP 2	3 (5.66)	16 (30.19)	1 (1.88)	17 (32.07)
MCP 3	3 (5.56)	11 (20.37)	1 (1.85)	9 (16.66)
MCP 4	0	8 (14.81)	0	2 (3.70)
MCP 5	0	6 (11.11)	0	5 (9.26)
IP + PIP involvement	1 (4.0) 4 (IQR 4-4) range 4-4	6 (24.0) 2.5 (IQR 1-5) range 1-5	0 (0.0) 0 (IQR 0-0) range 0-0	5 (18.5) 1 (IQR 1-1) range 1-2
IP 1	0	3 (5.56)	0	2 (3.70)
PIP 2	2 (5.66)	6 (11.32)	0	1 (1.88)
PIP 3	2 (3.70)	4 (7.41)	0	2 (3.70)
PIP 4	0	2 (3.70)	0	1 (1.85)
PIP 5	0	3 (5.56)	0	0
DIP involvement	0 (0.0) 0 (IQR 0-0) range 0-0	3 (12.0) 1 (IQR 1-2) range 1-2	0 (0.0) 0 (IQR 0-0) range 0-0	3 (11.1) 1 (IQR 1-2) range 1-2
2nd DIP	0	2 (3.77)	0	3 (5.66)
3rd DIP	0	2 (3.70)	0	1 (1.85)
4th DIP	0	0	0	0
5th DIP	0	0	0	0
Joint counts of the lower limb				
Knee	6 (11.11)	16 (29.63)	0	14 (25.92)
Ankle	9 (18.52)	19 (35.19)	2 (3.70)	8 (14.81)
Mid foot	5 (9.25)	14 (25.93)	0	8 (14.81)
MTP involvement	8 (32.0) 1,5 (IQR 1-3.5) range 1-10	18 (72.0) 2.5 (IQR 1-3) range 1-6	0 (0.0) 0 (IQR 0-0) range 0-0	16 (59.3) 2 (IQR 1-2.5) range 1-6
MTP 1	9 (17.65)	30 (55.55)	0	21 (41.17)
MTP 2	4 (7.41)	8 (14.81)	0	6 (11.11)
MTP 3	4 (7.41)	6 (11.11)	0	5 (9.25)
MTP 4	3 (5.56)	3 (5.56)	0	3 (5.56)
MTP5	3 (5.56)	4 (7.40)	0	5 (9.25)
1st IP	0 (0)	6 (13.04)	0	3 (7.7)

Note: The maximum number possible value at the flare visit is 50 and the inter-critical visit is 54 (ie, 25 attended the flare visit and 27 participants attended the inter-critical visit); however, some joints were un-assessable due to large tophi or amputation, and some participants were missing joints.

Abbreviations: DIP, distal interphalangeal joint; IP, interphalangeal joint; MCP, metacarpophalangeal joint; MTP, metatarsophalangeal joint; PIP, proximal interphalangeal joint.

findings were accompanied by increased ESR (29 vs 13.5 mm/h, $P = .02$) at the acute visit. However, HsCRP, serum creatinine, and SUA (0.44 vs 0.38 mmol/L, $P = .065$) levels were equivalent across visits (Table 2).

A post hoc analysis of 5 participants who had achieved a SUA at therapeutic target (≤0.36 mmol/L) at both visits, reported similar clinically (2 vs 1 joints, $P = .34$) or US determined (6 vs 8 joints, $P = .64$) inflamed joints, and global and gout-specific health reported

outcomes (Table 4). However, Hs-CRP trended higher, albeit non-significantly, at the flare (9.26 vs 1.52 mg/L, P = .21) and inter-critical visit (5.26 vs 0.23 mg/L, P = .31) in those who were unable to sustain SUA levels at or below the therapeutic target.

4 | DISCUSSION

This pilot study establishes the presence of subclinical joint inflammation detected by US in both the acute and inter-critical phases of gout in a prospective longitudinal cohort.

In the inter-critical phase, subclinical intra-articular inflammation on US was commonly found in our study. The median number of clinically active joints per participant during the inter-critical visit was 0, yet US detected intra-articular inflammation found a median of 4 inflamed joints per participant. The degree of US detected inflammation is surprising, given the lack of clinically active joints, but this result is consistent with published literature demonstrating the ability of US to detect subclinical inflammation in both gout and other arthritidies.[12,24] Complementing the imaging results, the HsCRP was elevated at the inter-critical visit, and not significantly lower than at the flare visit. This is also consistent with a previous study demonstrating elevated biochemical markers of inflammation in inter-critical gout[10] of uncertain etiology. However, our findings would suggest that the previously unexplained elevated HsCRP in the inter-critical period may be driven by subclinical joint inflammation, which in gout is known to be driven by intra-articular uric acid deposition.[13]

While the presence of subclinical inflammation has previously been demonstrated in the inter-critical phase of gout,[12,25] this study is novel in that participants in the study were systematically followed prospectively and longitudinally through the acute and inter-critical phases. As might be expected, many biochemical, clinical and ultrasonographic outcomes fell significantly during the inter-critical period; however, the HsCRP, the patient reported global health VAS and patient reported gout severity VAS did not fall significantly from the acute visit. Most interestingly, while the median number of joints with intra-articular PD score of ≥2 fell during the inter-critical period, this fall did not reach statistical significance. This finding reminds us that even in a cohort of relatively short disease duration, gout is a chronic disease. The traditional concept of gout being phasic, moving through a period of acute intermittent arthritis, to chronic gout arthritis may be misleading, and that it might better be considered chronic even in its early stages.[26]

The management of gout focuses on suppressing SUA to "target". In this study, 5 participants had a uric acid level within the target range at both visits; however, this subgroup did not demonstrate lower levels of US detected intra-articular inflammation during the inter-critical period, although it is uncertain regarding the historical duration of uric acid suppression to target levels in this cohort. This lack of relationship to US detected inflammation is consistent with a previous publication in which the presence of US detected inflammation and urate deposition in people with gout

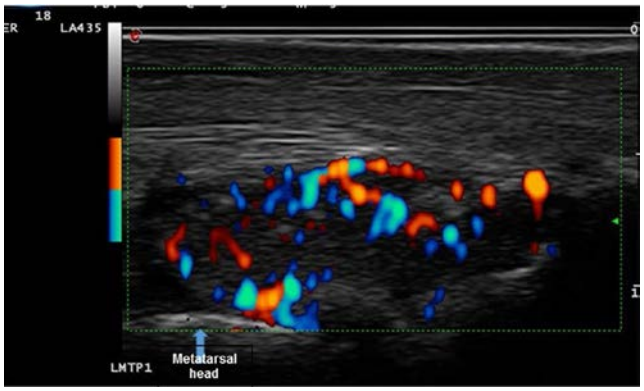

FIGURE 1 Acute visit examination of 1st metatarsophalangeal joint showing an intra-articular power Doppler grade 3 but the joint was not tender or swollen

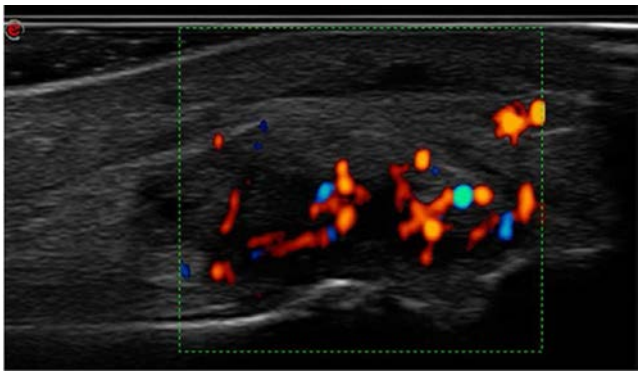

FIGURE 2 Inter-critical visit examination of 1st metatarsophalangeal joint showing power Doppler grade 3 and the joint was tender but not swollen

was not related to serum urate in a cross-sectional sampling.[27] In the current study, it may be hypothesized that with time, and prolonged suppression of serum urid acid, subclinical joint inflammation may also be suppressed. Perhaps imaging inflammation, as a surrogate of uric acid deposition, may be a better indicator of total body burden of urate load than spot or short-term serial serum urate measurements.

The main finding in this study is the persistence of subacute and subclinical joint inflammation, of uncertain relevance to outcomes. Further studies should investigate the relationship between subclinical inflammation and intermediate to long-term outcomes, including clinical flares, joint damage, and cardiovascular outcomes.

4.1 | Study limitations

This study has limitations. The sample size was small, only 24 participants completed both acute and inter-critical visits. As this is a pilot study, local ethics limited the number of participants in the study.

It is possible that this study underestimated US detected intra-articular inflammation as only those joints with a PD score of ≥2 were included as inflamed on ultrasound. The threshold for inflammation

TABLE 4 Comparing clinical and ultrasound findings, patient reported outcomes and laboratory findings of people with target SUA (n = 5) at both visits against those with higher SUA (n = 18)

	Target uric acid level achieved at both visits		Mann-Whitney *U* test
	No (n = 18)	Yes (n = 5)	
Variable	Median (IQR) range	Median (IQR) range	*P* value
Flare visit			
Physician global assessment, 0-10 cm	6.2 (4.0-7.5) 3.1-9.0	6.6 (5.0-6.8) 0.6-6.9	.62
Patient reported gout pain, 0-10 cm	6.4 (3.5-8.5) 1.1-10.0	5.5 (2.5-7.0) 1.7-7.9	1.00
Patient reported gout severity, 0-10 cm	6.7 (5.1-8.4) 0.7-10.0	3.0 (2.4-7.7) 0.7-7.9	1.00
Patient reported global health, 0-10 cm	4.7 (2.2-5.8) 1.1-9.0	3.5 (3.4-5.7) 2.8-6.3	1.00
HAQ, log transformed	0.81 (0.25-1.88) 0.00-2.75	1.13 (0.88-1.63) 0.75-1.63	.64
Clinically active joints	2 (1-2) 0-15	1 (0-1) 0-2	.34
Number of joints with PD ≥ 2	6 (4-11) 0-23	8 (5-10) 2-22	.64
HsCRP, mg/L	9.26 (5.76-26.30) 1.51-71.30	1.52 (0.85-3.36) 0.85-3.36	.21
Inter-critical visit			
Physician global assessment, 0-10 cm	3.45 (0.5-4.4) 0.0-5.4	2.7 (1.4-3.1) 0.1-4.0	.34
Patient reported gout pain, 0-10 cm	0 (0-2) 0-7	0.0 (0.0-0.0) 0.0-0.6	.61
Patient reported gout severity, 0-10 cm	5.1 (3.6-7.0) 0.4-10.0	5.2 (1.5-6.7) 1.1-9.6	.64
Patient reported global health, 0-10 cm	4.2 (2.6-5.2) 0.1-7.9	0.8 (0.3-2.8) 0.1-5.4	.32
HAQ, log transformed	0.06 (0.00-0.88) 0.00-1.88	1.25 (0.63-1.50) 0.13-2.75	.16
Clinically active joints	0 (0-0) 0-2	0 (0-0) 0-0	1.00
Number of joints with PD ≥ 2	5 (3-9) 0-17	3 (0-4) 0-6	.32
HsCRP, mg/L	5.62 (1.39-18.60) 0.40-37.30	0.23 (0.23-0.23) 0.23-0.23	.31

Abbreviations: HAQ, Health Assessment Questionnaire; HsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; SUA, serum uric acid.

was deliberately set high, to exclude changes that might be a result of OA, given the age of the cohort and the literature shows that OA has only low levels of synovitis.[20,28]

We did not examine other features of US detected uric acid deposition like the double contour sign or tophaceous deposits. It may be useful to assess these; given that the SUA in this study did not reflect joint inflammation, which is likely driven by intra-articular deposits of uric acid, US may better reflect the total body burden of uric acid than a SUA level. However, the hypothesis of this study was to focus on inflammation, it was not expected that intra-articular inflammation would be so prevalent, especially in the inter-critical phase, and at the time of this study design there were no internationally recognized consensus definitions of these pathologies. Given the inflammation is likely directly related to urate, this deserves further investigation, and definitions of US detectable uric acid deposition in joints now exist.

5 | CONCLUSIONS

Ultrasound detected subclinical joint inflammation is present and is common both in the acute flares and in the inter-critical periods of gout, and not necessarily reflective of SUA.

The significance of this subclinical inflammation is uncertain. Ultrasonography may be a useful tool to better understand gout and may have implications on the clinical management of this common condition; going forward, larger studies are needed to address potential implications of subclinical synovitis.

ACKNOWLEDGEMENTS

We are grateful for the use of Esaote MyLab 70 XVG Ultrasound Machine under the Royal Perth Hospital Medical Research Foundation Infrastructure scheme.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

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REFERENCES

1. Winnard D, Wright C, Taylor WJ, et al. National prevalence of gout derived from administrative health data in Aotearoa New Zealand. *Rheumatology*. 2012;51(5):901-909.
2. Klemp P, Stansfield SA, Castle B, Robertson MC. Gout is on the increase in New Zealand. *Ann Rheum Dis*. 1997;56(1):22-26.
3. Richette P, Bardin T. Gout. *Lancet*. 2010;375(9711):318-328.
4. Baroja-Mazo A, Martín-Sánchez F, Gomez AI, et al. The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. *Nat Immunol*. 2014;15(8):738.
5. Smith E, Díaz-Torné C, Perez-Ruiz F, March L. Epidemiology of gout: an update. *Best Pract Res Clin Rheumatol*. 2010;24(6):811-827.
6. Khanna D, FitzGerald JD, Khanna PP, et al. 2012 American College of Rheumatology Guidelines for Management of Gout Part I: Systematic Non-pharmacologic and Pharmacologic Therapeutic Approaches to Hyperuricemia. *Arthritis Care Res (Hoboken)*. 2012;64(10):1431-1446.
7. Aung T, Myung G, FitzGerald JD. Treatment approaches and adherence to urate-lowering therapy for patients with gout. *Patient Prefer Adherence*. 2017;11:795.
8. Stamp LK, Zhu X, Dalbeth N, Jordan S, Edwards NL, Taylor W. Serum urate as a soluble biomarker in chronic gout—Evidence that serum urate fulfills the OMERACT validation criteria for soluble biomarkers. *Seminars Arthritis Rheum*. 2011;40(6):483–500.
9. Stamp L, Morillon MB, Taylor WJ,, et al. Serum urate as surrogate endpoint for flares in people with gout: a systematic review and meta-regression analysis. *Seminars Arthritis Rheum*. 2018;48(2):293-301.
10. Grainger R, McLaughlin RJ, Harrison AA, Harper JL. Hyperuricaemia elevates circulating CCL2 levels and primes monocyte trafficking in subjects with inter-critical gout. *Rheumatology*. 2013;52(6):1018-1021.
11. Nguyen H, Ruyssen-Witrand A, Gandjbakhch F, Constantin A, Foltz V, Cantagrel A. Prevalence of ultrasound-detected residual synovitis and risk of relapse and structural progression in rheumatoid arthritis patients in clinical remission: a systematic review and meta-analysis. *Rheumatology*. 2014;53(11):2110-2118.
12. Schueller-Weidekamm C, Schueller G, Aringer M, Weber M, Kainberger F. Impact of sonography in gouty arthritis: comparison with conventional radiography, clinical examination, and laboratory findings. *Eur J Radiol*. 2007;62(3):437-443.
13. Inaba S, Sautin Y, Garcia GE, Johnson RJ. What can asymptomatic hyperuricaemia and systemic inflammation in the absence of gout tell us? *Rheumatology*. 2013;52(6):963-965.
14. Wallace SL, Robinson H, Masi AT, Decker JL, Mccarty DJ, Yü T-F. Preliminary criteria for the classification of the acute arthritis of primary gout. *Arthritis Rheum*. 1977;20:895-900.
15. Zhang W, Doherty M, Pascual E, et al. EULAR evidence based recommendations for gout. Part I: diagnosis. Report of a task force of the standing committee for international clinical studies including therapeutics (ESCISIT). *Ann Rheum Dis*. 2006;65(10):1301-1311.
16. Grainger RHA,Taylor WJ. Preliminary identification of potential items for a definition of 'Gout Flare' using Delphi methodology. *Arthritis Rheum*. 2005;52(Suppl 9):S105.
17. Taylor WJ, Schumacher HR, Baraf HS, et al. A modified Delphi exercise to determine the extent of consensus with OMERACT outcome domains for studies of acute and chronic gout. *Ann Rheum Dis*. 2008;67(6):888-891.
18. Backhaus M, Burmester G, Gerber T, et al. Guidelines for musculoskeletal ultrasound in rheumatology. *Ann Rheum Dis*. 2001;60(7):641-649.
19. Keen HI, Wakefield RJ, Grainger AJ, Hensor E, Emery P, Conaghan PG. An ultrasonographic study of osteoarthritis of the hand: synovitis and its relationship to structural pathology and symptoms. *Arthritis Care Res (Hoboken)*. 2008;59(12):1756-1763.
20. Keen HI, Redmond A, Wakefield RJ, et al. An ultrasonographic study of metatarsophalangeal joint pain: synovitis, structural pathology and their relationship to symptoms and function. *Ann Rheum Dis*. 2011;70(12):2140-2143.
21. Keen HI, Wakefield RJ, Grainger AJ, Hensor EM, Emery P, Conaghan PG. Can ultrasonography improve on radiographic assessment in osteoarthritis of the hands? A comparison between radiographic and ultrasonographic detected pathology. *Ann Rheum Dis*. 2008;67(8):1116-1120.
22. Saleem B, Keen H, Goeb V, et al. Patients with RA in remission on TNF blockers: when and in whom can TNF blocker therapy be stopped? *Ann Rheum Dis*. 2010;69(9):1636-1642.
23. Nam J, Villeneuve E, Hensor E, et al. Remission induction comparing infliximab and high-dose intravenous steroid, followed by treat-to-target: a double-blind, randomised, controlled trial in new-onset, treatment-naive, rheumatoid arthritis (the IDEA study). *Ann Rheum Dis*. 2014;73(1):75-85.
24. Filippucci E, Meenagh G, Delle Sedie A, et al. Ultrasound imaging for the rheumatologist XXXVI. Sonographic assessment of the foot in gout patients. *Clin Exp Rheumatol*. 2010;29(6):901-905.
25. Wright SA, Filippucci E, McVeigh C, et al. High-resolution ultrasonography of the first metatarsal phalangeal joint in gout: a controlled study. *Ann Rheum Dis*. 2007;66(7):859-864.
26. Dalbeth N, Stamp L. Hyperuricaemia and gout: time for a new staging system? *Ann Rheum Dis*. 2014;73(9):1598-1600.
27. Roddy E, Menon A, Hall A, Datta P, Packham J. Polyarticular sonographic assessment of gout: a hospital-based cross-sectional study. *Joint Bone Spine*. 2013;80(3):295-300.
28. Padovano I, Costantino F, Breban M, D'Agostino MA. Prevalence of ultrasound synovial inflammatory findings in healthy subjects. *Ann Rheum Dis*. 2016;75(10):1819-1823.

How to cite this article: Chowalloor P, Raymond WD, Cheah P, Keen H. The burden of subclinical intra-articular inflammation in gout. *Int J Rheum Dis*. 2020;23:661–668. https://doi.org/10.1111/1756-185X.13811

Received: 10 December 2019 | Accepted: 6 February 2020

DOI: 10.1111/1756-185X.13812

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases | WILEY

Translation, validation and cross-cultural adaptation of the mouth handicap in systemic sclerosis questionnaire into the Turkish language

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Abstract

Aim: The aim of this study was to translate and adapt the Mouth Handicap in Systemic Sclerosis (MHISS) Questionnaire into the Turkish language and evaluate its validity and reliability in Turkish systemic sclerosis (SSc) patients.

Method: The MHISS was translated according to Beaton guidelines. Patients being diagnosed with SSc, being between 18-65 years old and receiving no treatment between test-retest assessments were included to study. Test-retest reliability was evaluated, comparing the results of two administrations, with Spearman's correlation. Internal consistency was assessed by Cronbach's α. Validity of the questionnaire was assessed by comparison with mouth opening, total scores of Disabilities of Arm, Shoulder and Hand Questionnaire (DASH) and Health Assessment Questionnaire (HAQ). Construct validity was tested by factor analysis.

Results: Forty-five SSc patients were included in the study. The Turkish version of the MHISS (MHISS-T) met set criteria of reliability and validity. Internal consistency (Cronbach's α = 0.863) and test-retest reliability were excellent (r = .88). The correlations between MHISS-T and inter-incisor distance, MHISS-T and HAQ and MHISS-T and DASH were negatively and statistically significant (r = −0.739, P < .001), very good and statistically significant (r = .664, P < .001), good and statistically significant (r = .570, P < .001), respectively. Regarding factor analysis, MHISS-T has three subscales.

Conclusion: Our results demonstrated that the Turkish version of the MHISS-T has excellent test-retest reliability and very good validity. As a result of this study we determined that MHISS-T is a valid and reliable instrument to measure mouth disabilities in Turkish-speaking SSc patients.

KEYWORDS

disability, mouth, questionnaire, systemic sclerosis, Turkish version

1 | INTRODUCTION

Systemic sclerosis (SSc) or scleroderma is a multisystemic connective tissue disease characterized by excessive collagen deposition, vascular hyper-reactivity and microvascular obliteration. It leads to retraction and atrophy in various tissues like skin, tendons, joints and vessels.[1]

Skin is one of the most affected tissues; it becomes harder and thicker by infiltration of dermis with collagen. Skin and especially face involvement is responsible for oral complications and results in esthetic changes and reduction of self-confidence. The face becomes amimic, wrinkles disappear, vertical furrows develop around the mouth. Telangiectasia, sicca syndrome, reduction of mouth width and opening besides osteolysis of mandibula and fibrosis of soft tissues are also seen in these patients.[2] Limited range of mouth opening, along with other symptoms such as dry mouth, can lead to difficulties with oral hygiene and eating. Such facial involvements also make the patient's self-image impaired, limit expression and cause disability and decrease in health-related quality of life. Thus, outcome measures reliably assessing handicaps are mandatory to properly evaluate patients and to follow-up disease evolution and treatment efficacy.[2]

Global disability in SSc patients is usually measured by the Health Assessment Questionnaire (HAQ). However, more body region-specific disability scales can be used like Cochin Hand Function Scale.[2] These specific scales are useful to clinicians in assessing the impact of certain locations of the disease and the efficacy of treatment. Therefore, due to significant oral disability, a mouth-specific self-assessment tool was developed for these patients. Mouth Handicap in Systemic Sclerosis (MHISS) scale is the first tool available to quantify the handicap associated with mouth disability in SSc patients. Although mouth disability seems to have less weight than hand disability in total disability, the MHISS score explained up to 36% of the variance of the HAQ score, which highlights the need to specifically assess disability involving the mouth in patients with SSc when evaluating treatment efficacy.[2]

To sum up, MHISS is a validated tool for measuring the handicap associated with mouth disability and the quality of life, that explores problems not assessed by HAQ and the Short Form-36.[2] Therefore, cross-cultural adaptation of the scale is needed to use in different languages. The aim of our study was to validate the Turkish version of MHISS (MHISS-T) and to assess its reliability and validity.

2 | MATERIALS AND METHODS

Permission was received from the author who had developed the MHISS questionnaire. The study was approved by the Local Ethics Commission (2017-411) and conducted between January 2018 and June 2019.

2.1 | Translation procedure

The translation and cross-cultural adaptation of the MHISS were performed according to the Beaton guidelines.[3] Two translations from English to Turkish were done by two native Turkish speakers who were also fluent in English. To achieve better conceptual equivalence between the original version and translation of the questionnaire, one of the translators had knowledge about the purpose of the study. The other translator was uninformed about the study for the purpose of gathering unexpected meanings from the original version of the questionnaire. Both Turkish translations were synthesized by these translators. Then the Turkish translation was retranslated back to English by two independent professional bilingual translators who were completely blind to the original version of the questionnaire. A committee, consisting of 4 translators, a Turkish linguist and a methodologist, investigated both English translations and compared with the original version of the MHISS to check for inconsistencies and suitability to Turkish culture. After the committee agreed that both English and Turkish versions of the MHISS were equivalent, the last stage of the cross-cultural adaptation process was performed. The comprehensibility of the MHISS-T was tested on 20 patients with SSc and 20 healthy individuals. Participants reported they could understand all questions well and the final version of the MHISS-T was created.

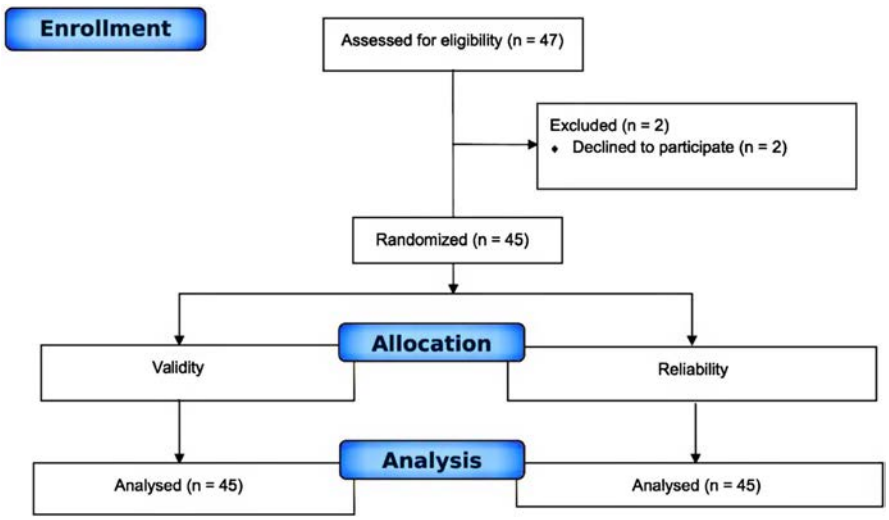

FIGURE 1 Flow diagram of the patients

2.2 | Patients

The study included 45 patients (44 female, one male; mean age 46.06 ± 12.06 years; range 20-65) with SSc disease (Figure 1). Patients were referred to the physiotherapy and rehabilitation outpatient department. The inclusion criteria were: (a) being diagnosed with SSc; (b) being between 18-65 years old; and (c) receiving no treatment between test-retest assessments. The exclusion criteria was being illiterate in Turkish. All participants read and signed the written informed consent form. Demographic characteristics of participants were recorded. All participants filled in the following questionnaires: Turkish version of the Health Assessment Questionnaire (HAQ) and Disabilities of Arm, Shoulder and Hand (DASH) Questionnaire. They also completed the MHISS-T questionnaire for a second time with a 1 week interval with the aim of determining the test-retest reliability of the questionnaire.

2.3 | The DASH

The DASH was developed as an outcomes measurement for use in patients with upper extremity complaints. The DASH is a generic questionnaire to assess upper extremity disorders. The DASH comprises of 37 questions which evaluate upper extremity functions from patients' perceptions. The DASH produces scores between 0 and 100. High DASH score is a demonstration of severe disability.[4] The Turkish version and cross-cultural adaptation of DASH were performed by Duger et al.[5]

2.4 | The HAQ-DI

The HAQ contains 20 questions assessing physical disabilities over the past 1 week in 8 categories of daily living activities: dressing and grooming, rising, eating, walking, hygiene, reach, grip, and activities. Each question is scored on a 4-point rating scale which ranges from 0 (without any difficulty) to 3 (unable to do). Moreover, the HAQ consist of four sections which are about use of devices, aids and needing help from another person when performing daily living activities in any of the eight categories. The total disability HAQ score (HAQ-DI) can be calculated by determining the highest score in each category. Then the average of the category scores are taken. Consequently, scores of the HAQ-DI can take values between 0 and 3. Higher HAQ-DI values reflect more disability.[6] The Turkish version and cross-cultural adaptation of the HAQ-DI were done by Kucukdeveci et al.[7]

TABLE 1 Factor analysis of the Turkish version of the mouth handicap in systemic sclerosis

	Reduced mouth opening	Sicca syndrome	Esthetic concerns
Item 1	**0.848***	0.154	0.113
Item 2	0.087	**0.918***	0.160
Item 3	**0.882***	0.064	0.093
Item 4	**0.835***	0.014	−0.051
Item 5	**0.829***	0.120	0.198
Item 6	**0.891***	0.187	0.296
Item 7	0.160	**0.853***	0.013
Item 8	0.138	**0.840***	0.113
Item 9	0.078	**0.843***	0.009
Item 10	0.025	**0.754***	−0.123
Item 11	0.118	0.080	**0.903***
Item 12	0.219	−0.018*	**0.881***

Both bold and * incicates items loaded highly.

2.5 | The MHISS

The MHISS is composed of 12 questions. All questions inquire about the frequency of the complaints and symptoms. The scores range from 0 (never) to 4 (always). MHISS includes three domains: mouth opening (questions 1, 3, 4-6), mouth dryness (questions 2, 7-10) and esthetic concerns (questions 11, 12). Total MHISS score ranges from 0 to 48. Higher scores reflect more limitations about mouth functions.[2]

Apart from the questionnaires, maximal mouth opening was assessed by using a tape measure. For this assessment, the inter-incisor distance was measured and noted in mm.

2.6 | Statistical analysis

Statistical analyses were executed using the Statistical Package for the Social Sciences (SPSS) 22.0 software. Statistical data were expressed as mean ± standard deviations (±SD), or as interquartile ranges or medians, in accordance with distributions of data. Test-retest and internal consistency analyses were conducted to determine the reliability of the MHISS-T questionnaire. Test-retest reliability was analyzed by using Spearman's ρ correlation coefficients. The internal consistency of MHISS-T was assessed by using Cronbach's α coefficient. The construct validity of MHISS-T was assessed by correlating total scores with total scores of DASH and HAQ-DI questionnaires, maximal mouth opening distance by using Spearman's ρ correlation coefficients. Spearman's ρ correlation coefficients ranging between 0.81 and 1.00 were considered excellent, while 0.61 and 0.80; 0.41 and 0.60; 0.21 and 0.40; and 0 and 0.20

TABLE 2 Internal consistency analysis of the Turkish version of the Mouth Handicap in Systemic Sclerosis, Cronbach's α coefficients

	Item	Cronbach's α value
Reduced mouth opening	5	0.920
Sicca syndrome	5	0.905
Aesthetic concerns	2	0.782
Total score	12	0.863

TABLE 3 Test-retest reliability of the Turkish version of the Mouth Handicap in Systemic Sclerosis and its subscales

		Reduced mouth opening (Second)	Sicca syndrome (Second)	Esthetic concerns (Second)
Reduced mouth opening (First)	Spearman correlation	**0.696****	0.150	0.248
	Sig. (2-tailed) (**)	0.000	0.324	0.101
Sicca syndrome (First)	Spearman correlation	0.234	**0.914****	0.086
	Sig. (2-tailed) (**)	0.121	0.000	0.577
Esthetic concerns (First)	Spearman correlation	0.130	0.147	**0.823****
	Sig. (2-tailed) (**)	0.393	0.334	0.000

Both bold and ** incicates $p < .01$.

were accepted as very good, good, weak, and bad, respectively.[8] Factor analysis was also done to test construct validity. Kaiser–Meyer–Olkin KMO) and Bartlett tests were used for factor analysis. Statistical significance was accepted as $P < .05$.

3 | RESULTS

Forty-four of the 45 patients were female. The mean age for all patients was 46.06 ± 12.06 years. First, KMO test was used to analyze the adequacy of the sample. The value of KMO test was 0.757, which shows the adequacy of the sample size. The significance of the square test statistic acquired as a result of Bartlett sphericity value is a demonstration that the data come from a highly variable normal distribution. The Bartlett sphericity value was significant (Chi-square = 375.338, $P < .001$). MHISS-T constantly showed three different dimensions (Table 1). The total Cronbach's α value for the MHISS-T was 0.863. This value demonstrated that MHISS-T had excellent internal consistency. The Cronbach's α values were 0.920 for reduced mouth opening, 0.905 for sicca syndrome and 0.782 for esthetic concerns subscales (Table 2). The test-retest reliability for the MHISS-T was excellent (rho = 0.888 $P < .001$). The test-retest scores of the MHISS-T subscales were found as 0.696 for reduced mouth opening, 0.914 for sicca syndrome and 0.823 for the aesthetic concerns (Table 3). The Spearman's correlations between the total scores of the MHISS-T scale and inter-incisor distance were negatively and statistically significant ($r = -.739$, $P < .001$). The correlations between the total scores of the MHISS-T scale and the total scores of HAQ-T were very good and statistically significant ($r = .664$, $P < .001$). The correlations between the total scores of the MHISS-T scale and the total scores of DASH-T were good and statistically significant ($r = .570$, $P < .001$) (Table 4).

TABLE 4 Correlations between the Turkish version of the Mouth Handicap in Systemic Sclerosis (MHISS-T) and other outcome measures

	MHISS-T Spearman correlation (N = 40)	
	r	*P*
Inter-incisor distance	−.739	<.001
HAQ-T	.664	<.001
DASH-T	.570	<.001

Abbreviations: DASH-T, Turkish version of the Disabilities of Arm, Shoulder and Hand Questionnaire; HAQ-T, Turkish version of the Health Assessment Questionnaire.

4 | DISCUSSION

The purpose of the study was to translate, validate and adapt the MHISS into the Turkish language. Turkish translation and cultural adaptation of the MHISS questionnaire was performed following a systematic standardized approach. Our results demonstrated that the MHISS-T questionnaire is a valid and reliable instrument to evaluate disability involving the mouth in patients with SSc. As far as we know, MHISS is the first outcome measure which was designed for SSc patients to assess the handicap and the disorder of oral health-related quality of life.

In this study, the mean MHISS-T total score was 22.4 ± 10.72, higher than the scores of the other versions of MHISS. The total scores for original, Dutch and Italian versions of MHISS were 18.8 ± 10.2, 20.3 ± 9.7, 17.65 ± 5.20, respectively.[2,9,10] Our results indicated that Turkish patients with SSc have more mouth disability than French, Dutch and Italian people with SSc.

In the original version, Mouthon et al assessed construct validity of MHISS and their study showed that MHISS has weak correlation with mouth opening, Cochin Hand Function Scale and HAQ (Spearman's ρ = 0.34, 0.37 and 0.33, respectively).[2] In the Dutch version, Schouffoer et al also assessed the construct validity of MHISS and they reported there were moderate correlations between MHISS and HAQ and maximal mouth opening (Spearman's ρ = 0.599, −0.518, respectively).[9] Lastly, in the Italian version by Bongi et al evaluated the construct validity of MHISS and their study demonstrated there was a weak correlation between MHISS and mouth opening (Spearman's ρ = −0.386) and they stated that MHISS was not significantly related to HAQ.[10] In our study, MHISS-T showed very good correlation with HAQ-T and inter-incisor distance (Spearman's ρ = 0.664 and −0.739, respectively) and good correlation with DASH-T (Spearman's ρ = 0.570). Since they are specific to different locations, the moderate correlation between MHIS-T and DASH-T is not surprising. In MHISS, some questions directly inquire about mouth opening. Therefore, very good correlation between inter-incisor distance and MHISS-T score is also not surprising. HAQ

includes 20 questions about daily living activities in 8 different categories. Considering subtypes of SSc, when compared to the other studies, most of the patients in our study had diffuse subtype of SSc (97.7%). Thus, not only their mouths and distal parts of extremities but also their proximal parts of extremities and internal organs were involved. It might be a reason for the very good correlation between MHISS-T and HAQ-T.

In the original version, Mouthon et al reported three factors for MHISS questionnaire and they explained 62.44% of the variance. The first factor (items 1, 3, 4, 5 and 6) indicates handicap induced by reduced mouth opening; the second factor, (items 2, 7, 8, 9 and 10) indicates handicap induced by sicca syndrome and the third factor (items, 11 and 12) indicates esthetic concerns.[2] In the Dutch and Italian versions, Schouffoer et al and Bongi et al reported the same factors.[9,10] In our study, we also found the same factors after we had done the factor analysis and they explained 77.37% of the variance.

The reliability of MHISS-T was assessed with test-retest reliability analysis and Cronbach's α coefficient. In the original version, test-retest reliability analysis demonstrated excellent reliability and it gave an intraclass correlation coefficient (ICC) of 0.96.[2] In the Dutch version, the ICC was 0.94, demonstrating excellent reliability.[9] In the Italian version, MHISS also had an excellent reliability: the ICC was 0.93.[10] In our study, test-retest reliability was evaluated by using Spearman's ρ correlation coefficient. The test-retest reliability of MHISS-T was excellent with a Spearman's ρ correlation coefficient of 0.888. The high correlation coefficient indicates the stability of the measurement obtained from the questionnaire.

The internal consistency was assessed using Cronbach's α coefficient value. In the Dutch version, Cronbach's α was 0.88 for the total MHISS. It was 0.86 for the subscale which is about mouth opening and 0.79 for the subscale that is about mouth dryness.[9] In the Italian version, Bongi et al reported that Cronbach's α was 0.99 and MHISS showed excellent reliability.[10] In the original version, Mouthon et al did not report any Cronbach's α coefficient value.[2] In this study, the Cronbach's α coefficient was 0.863 for the questionnaire. It was 0.92 for the first subscale which is about mouth opening, 0.905 for the second subscale that is about sicca syndrome and 0.782 for the third subscale which is about esthetic concerns. These results indicate that the answers to the questions are consistent. When Cronbach's α coefficient is between 0.70 and 0.95, the internal consistency is considered to be significant.[11]

A limitation of this study was that responsiveness, one of the important psychometric considerations for outcome measurements, was not checked. Responsiveness of the MHISS-T can be analyzed in a future study.

In conclusion, validation and adaptation of the questionnaires in different languages play an important role in standardizing assessment and follow-up of patients over different countries. MHISS is a patient-reported outcome measurement which is especially developed to assess mouth handicap in SSc. It is short and easy to be filled in. Our results indicated that the Turkish version of the MHISS questionnaire is a valid and reliable instrument for assessing mouth handicap in Turkish SSc patients.

AUTHOR CONTRIBUTIONS

Nurten Gizem Tore: design of the study, interpretation of the results and writing the manuscript. Fulden Sari, Hamit Kucuk: data collection. Zeynep Tuna: data analysis. Deran Oskay, Seminur Haznedaroglu: interpretation of the results and reviewing the manuscript.

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REFERENCES

1. Servettaz A, Agard C, Tamby MC, et al. Systemic sclerosis: pathophysiology of a multifaceted disease. *Presse Med*. 2006;35(12):1903-1915.
2. Mouthon L, Rannou F, Bérezné A, et al. Development and validation of a scale for mouth handicap in systemic sclerosis: the Mouth Handicap in Systemic Sclerosis scale. *Ann Rheum Dis*. 2007;66(12):1651-1655.
3. Beaton DE, Bombardier C, Guillemin F, et al. Guidelines for the process of cross-cultural adaptation of self-report measures. *Spine*. 2000;25(24):3186-3191.
4. Hudak PL, Amadio PC, Bombardier C, et al. Development of an upper extremity outcome measure: the DASH (disabilities of the arm, shoulder, and head). *Am J Ind Med*. 1996;29(6):602-608.
5. Duger T, Yakut E, Oksuz C, et al. Reliability and validity of the Turkish version of the Disabilities of the Arm, Shoulder and Hand (DASH) Questionnaire. *Fizyoterapi Rehabil*. 2006;17(3):99.
6. Bruce B, Fries JF. The health assessment questionnaire (HAQ). *Clin Exp Rheumatol*. 2005;23(5):14.
7. Kucukdeveci AA, Sahin H, Ataman S, et al. Issues in cross-cultural validity: Example from the adaptation, reliability, and validity testing of a Turkish version of the Stanford Health Assessment Questionnaire. *Arthritis Care Res (Hoboken)*. 2004;51(1):14-19.
8. Feise RJ, Menke JM. Functional rating index: a new valid and reliable instrument to measure the magnitude of clinical change in spinal conditions. *Spine*. 2001;26(1):78-87.
9. Schouffoer AA, Strijbos E, Schuerwegh AJM, et al. Translation, cross-cultural adaptation, and validation of the Mouth Handicap in Systemic Sclerosis questionnaire (MHISS) into the Dutch language. *Clin Rheumatol*. 2013;32(11):1649-1655.
10. Bongi SM, Del Rosso A, Miniati I, et al. The Italian version of the Mouth Handicap in Systemic Sclerosis scale (MHISS) is valid, reliable and useful in assessing oral health-related quality of life (OHRQoL) in systemic sclerosis (SSc) patients. *Rheumatol Int*. 2012;32(9):2785-2790.
11. Zinbarg RE, Revelle W, Yovel I, et al. Cronbach's α, Revelle's β, and McDonald's ω H: their relations with each other and two alternative conceptualizations of reliability. *Psychometrika*. 2005;70(1):123-133.

How to cite this article: Tore NG, Sarì F, Tunà Z, Kucuk H, Haznedaroglu S, Oskay D. Translation, validation and cross-cultural adaptation of the mouth handicap in systemic sclerosis questionnaire into the Turkish language. *Int J Rheum Dis*. 2020;23:669–673. https://doi.org/10.1111/1756-185X.13812

Received: 11 December 2019 | Revised: 7 February 2020 | Accepted: 19 February 2020

DOI: 10.1111/1756-185X.13822

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases | WILEY

Single nucleotide polymorphisms of the *HIF1A* gene are associated with susceptibility to pulmonary arterial hypertension in systemic sclerosis and contribute to SSc-PAH disease severity

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Funding information
This study was supported by a scleroderma research grant from the Ministry of Health, Labor, and Welfare in Japan and a Contracted Research Grant of Actelion Pharmaceuticals Japan.

Abstract

Aim: Hypoxia-inducible factor (HIF)1α is induced by endothelial cells under hypoxic conditions, suggesting that HIF1α may be involved in vascular impairment in patients with systemic sclerosis (SSc). The purpose of this study was to evaluate whether single nucleotide polymorphisms (SNPs) of the *HIF1A* gene are associated with susceptibility to SSc and its complications, including pulmonary arterial hypertension (PAH).

Method: This study involved 182 Japanese SSc patients (discovery cohort) and 178 healthy controls. Four SNPs (rs11549465, rs11549467, rs1957757, and rs12434438) of the *HIF1A* gene were genotyped using specific TaqMan probes. We also employed another SSc cohort (N = 135) to validate the significant results of SNPs found in the discovery SSc cohort.

Results: The frequencies of the four SNPs did not show any significant differences between the SSc and healthy control groups. The AA genotype at rs12434438 was significantly higher in SSc patients with PAH than in those without PAH (P = .012). These results were validated using another SSc cohort (N = 135, P = .006). Moreover, the AA genotype was significantly associated with the severity of PAH.

Conclusion: Although *HIF1A* gene polymorphisms were not associated with susceptibility to SSc, the AA genotype at rs12434438 was associated with a subset of SSc patients with severe PAH, suggesting that the rs12434438 SNP may contribute to the development of PAH with SSc.

KEYWORDS

gene polymorphism, HIF1α, pulmonary arterial hypertension, systemic sclerosis

1 | INTRODUCTION

Systemic sclerosis (SSc) is a multisystem disease characterized by fibrosis in the skin and internal organs, endothelial injury and immune dysregulation. Its pathogenesis remains poorly understood. SSc is thought to develop due to the association of multiple genetic factors and/or environmental effects. Several reports describe genetic disease susceptibility loci.

Case-control candidate gene studies have identified several robust SSc susceptibility loci, *IRF5*,[1] *STAT4*,[2] *BANK1*[3] and *BLK*,[4] in pathways involved in immune regulation. Genome-wide association studies identified the major histocompatibility complex as the strongest susceptibility locus associated with SSc.[5-7] In addition, associations of SSc clinical and autoantibody subgroups with *IRF8*, *GRB10*, *SOX5*, *HLA-DQB1*, *HLA-DPA1/B1* and *NOTCH4* have been reported.[8]

Case-control candidate gene studies also identified an association of SSc complications with genetic loci. Single nucleotide polymorphisms (SNPs) of *CTGF*,[9] *HGF*,[10] *IRAK1*,[11] *IRF5*,[1,12,13] *MMP-12*[14] and *SP-B*[15] are associated with interstitial lung disease in SSc. These SNPs are associated with distinct de novo interstitial lung disease.[16] The *IL23R*,[17] *KCNA5*,[18] *TLR2*,[19] *TNAIP3*[20] and *UPAR*[21] genes have been associated with pulmonary arterial hypertension (PAH), while *HLA-DRB1*04:07* and *HLA-DRB1*13:04* were associated with scleroderma renal crisis.[22]

Peripheral circulatory failures with vascular lesions occur in the first step of SSc and are a key pathophysiology. Reduced blood flow in the tissues leads to ischemia and sequential development of tissue fibrosis. Vascular lesions of the lung and kidney are associated with the development of PAH and renal hypertension (scleroderma renal crisis), respectively. Tissues with circulatory failure suffer a low-oxygen state. When the low-oxygen state persists, tissues are able to trigger an adaptive response to hypoxic conditions to cope with these threatening conditions. Hypoxia-inducible factor (HIF) is a transcriptional factor that plays a central role in the adaptive response to low-oxygen stress.[23] Under low-oxygen conditions, HIF is stabilized by decreased activity of prolyl hydroxylases.[24] Then, the stabilized HIF translocates into the nucleus. HIF1α forms a heterodimer with HIF1β and binds to the hypoxia-response element.[25] More than 100 HIF1-downstream genes have been identified, including *EPO*, *VEGF*, and vasoreactive proteins.[26] Cell biology studies have revealed that the upregulation of *VEGF* expression is associated with the accumulation of HIF1α in the skin of SSc patients.[27]

There are many reviewed studies describing the association between *HIF1A* polymorphisms and autoimmune diseases and dermatological diseases.[28-32] SNPs of the *HIF1A* gene may regulate the expression and stability of both HIF1α messenger RNA (mRNA) and protein. The association of *HIF1A* gene polymorphisms with SSC in a French European Caucasian population has been reported. The A/G and/or GG genotype of the rs12434438 SNP was significantly more prevalent in SSc patients than in controls.[33] All DNA samples collected in this study were from European Caucasians. Ethnicity is strongly associated with disease susceptibility. We believe that an association study of *HIF1A* gene polymorphisms with susceptibility to SSc in a Japanese population should exist. In particular, because HIF1α is involved in vascular injuries in the hypoxic atmosphere, another purpose of this study is to determine whether the polymorphisms of the *HIF1A* gene might be an indicator for vasculopathy complications in SSc.

2 | METHODS AND MATERIALS

2.1 | Enrollment of patients

Our first cohort consisted of 182 SSc patients diagnosed based on 2013 classification criteria[34] for SSc and 177 healthy controls. These patients were enrolled consecutively in the Rheumatology Outpatient Clinic of Tokyo Women's Medical University from January 2014 to December 2016. Patients and controls were matched for age and gender, and all participants were Japanese. Enrollment in this study and genotyping were performed with informed consent. In the validation cohort, DNA samples were collected from 135 patients with SSc who were consecutively enrolled from 2010 to 2013 and who were different from the patients of the first cohort. The study design was approved by the Tokyo Women's Medical University Gene Ethics Committee (approval No. 185B). SSc patients were classified with diffuse cutaneous type or limited cutaneous type disease according to the classification scheme of LeRoy et al.[35] The following clinical data were collected: age, gender, duration of disease, skin score of the modified Rodnan total skin thickness score (mRTSS), and complications (digital ulcer, interstitial lung disease, gastroesophageal reflux disease, arthritis, and pulmonary arterial hypertension). Immunological tests for anti-Scl70, anti-centromere protein (CENP), anti-U1 ribonucleoprotein (anti-U1RNP), and anti-Sjögren's syndrome A (anti-SS-A) antibodies were conducted.

Pulmonary arterial hypertension was diagnosed when the pulmonary artery wedge pressure (PAWP) was <15 mm Hg and the mean pulmonary artery pressure (mPAP) was more than 25 mm Hg.[36] Patients with pulmonary hypotension with severe pulmonary fibrosis and chronic thromboembolic pulmonary hypotension were excluded. The clinical severity of PAH was estimated using the World Health Organization functional classification (WHOFC).

2.2 | Preparation of genomic DNA

DNA was isolated from peripheral blood white blood cells from SSc patients and matched controls. Four SNPs (rs11549465, rs11549467, rs1957757, and rs12434438) located in the intron of the *HIF1A* gene were genotyped using TaqMan probes. Each primer used for genotyping is shown in Table 1.

2.3 | Statistics

Significance tests for deviation from Hardy-Weinberg equilibrium of these SNPs were performed using Fisher's exact test. The genotype relative risk between the cases and controls was investigated using odds ratios (OR). Power analysis was performed with G*PowerV3.1.7.[37]

TABLE 1 Primer list

	Functional consequence	Global MAF (1000 genome)	
rs11549465	Intron variant	T = 0.3259/1632	GTTACGTTCCTTCGATCAGTTGTCA[C/T]CATTAGAAAGCAGTTCCGCAAGCCC
rs11549467	Intron variant	A = 0.0090/45	GTTGTCACCATTAGAAAGCAGTTCC[A/G]CAAGCCCTGAAAGCGCAAGTCCTCA
rs1957757	Intron variant	T = 0.3259/1632	TCTGGACACCCCGACCCTTTTGGTA[C/T]AGAATATAACCTATGTAATTCTCTA
rs12434438	Intron variant	G = 0.4219/2113	TATACCATACCTTTTTCCTGAAAGC[A/G]GTTTTGCCTTTCCTTTGTCTCTAGT

Abbreviation: MAF, minor allele frequency.

3 | RESULTS

3.1 | Clinical features in patients in the first cohort

The clinical characteristics of SSc patients in the first cohort are described in Table 2. The mean disease duration of SSc was 12.4 ± 10.2 years. The diffuse type was found in 58% of patients, and the limited type was found in 42%. The average mRTSS was 15 ± 16 points. In terms of treatment, a vasodilator was used in 65% of patients, immunosuppressive drugs including steroid and cyclophosphamide were used in 45%, and a proton pomp inhibitor was administered in 35% of patients.

3.2 | Frequencies of each SNP

Table 3 summarizes the frequencies of the four SNPs (rs11549465 C/T, rs11549467 A/G, rs1957757 C/T, and rs12434438 A/G) in patients with SSc and healthy controls, which were in Hardy-Weinberg equilibrium for both groups.

There were no differences in the frequencies of these four SNPs between SSc patients and healthy controls.

In addition, SNP association analysis of the four SNPs with clinical manifestations of SSc was carried out. The results of the association of the rs12434438 SNP genotype with disease phenotypes are shown in Table 4. There was a significant correlation between PAH complications and the genotype of rs12434438 (P = .012, Table 4). Unfortunately, after Bonferroni correction, the P value changed to .144, which indicated that the association was not significant. We did not find any correlation of clinical features with the genotype of the other three SNPs (data not shown). To confirm the results of the rs12434438 SNP, we performed genotyping using a "validation cohort" of patients with SSc (N = 135). As shown in Table 5, the results of the validation cohort indicated a significant difference between patients with and without PAH (P = .006). Moreover, we investigated the association of the rs12434438 SNP with PAH severity. As shown in Table 6, the AA genotype was significantly correlated with PAH complications (P = .00065). As a result of an association study in PAH patients with WHOFC classes I + II and WHOFC classes III + IV disease, we confirmed that the AA genotype was a risk factor for complications of PAH and that the OR showed that the AA genotype correlated with the severity of PAH with SSc; the OR (95% confidence interval) was 2.9 (1.3-6.4) in WHOFC classes I + II disease and 8.5 (1.1-6.6) in WHOFC classes III + IV disease.

TABLE 2 Clinical characteristics of patients in the first cohort (N = 182)

Clinical characteristics	
Age, y	57.5 ± 18.3
Duration of disease, y	12.4 ± 10.2
Female, n	167
Lc type, n	78
mRTSS	15 ± 16
DU, n	25
ILD, n	95
GERD, n	120
Arthritis, n	54
PAH, n	27
Anti-Scl70, n	58
Anti-CENP, n	47
Anti-U1RNP, n	32
Anti-SSA, n	30

Abbreviations: CENP, centromere protein; DU, digital ulcer; GERD, gastroesophageal reflex disease; ILD, interstitial lung disease; Lc, limited cutaneous; mRTSS, modified Rodnan total skin thickness score; PAH, pulmonary arterial hypertension; RNP, ribonucleoprotein; SSA, Sjögren's syndrome A.

4 | DISCUSSION

We demonstrated that the *HIF1A* gene is a risk factor for developing PAH in patients with SSc. Recently, many studies have identified genes associated with the risk of autoimmune disease, including SSc and PAH. In terms of the *HIF1A* gene, Wipff et al[33] reported that the frequency of the rs12434438 G allele was significantly higher in French SSc patients than in controls, and the heterozygous rs12434438 A/G genotype was associated with the limited type and positive anti-centromere antibodies. In the present study, our findings were not consistent with that previous study. An association between four SNPs, including rs12434438, of the *HIF1A* gene and susceptibility to SSc was not observed in a Japanese SSc cohort. These controversial results can be explained by differences in ethnicity and race. Interestingly, data from Hap

TABLE 3 Summary of allele and genotype of four SNPs of *HIF1A* gene in SSc patients (first cohort) and HC

		Genotype				Allele			
		n (%)	n (%)	n (%)	*P*	n (%)	n (%)	*P*	OR (95% CI)
rs11549465		CC	CT	TT		C	T		
HC	(N = 177)	159 (89.8)	17 (9.6)	1 (0.6)	NS	335 (94.6)	19 (5.4)	NS	1.23 (0.63-2.44)
SSc	(N = 182)	167 (91.8)	14 (7.7)	1 (0.5)		348 (95.6)	16 (4.4)		
rs11549467		AA	GA	GG		A	G		
HC	(N = 174)	1 (0.6)	16 (9.2)	157 (90.2)	NS	18 (5.2)	330 (94.8)	NS	1.05 (0.54-2.05)
SSc	(N = 182)	0 (0)	18 (9.9)	164 (90.1)		18 (4.9)	346 (95.1)		
rs1957757		CC	CT	TT		C	T		
HC	(N = 178)	131 (73.7)	42 (23.5)	5 (2.8)	NS	304 (85.4)	52 (14.6)	NS	1.05 (0.69-1.59)
SSc	(N = 182)	132 (72.5)	49 (26.9)	1 (0.5)		313 (86.0)	51 (14.0)		
rs12434438		AA	GA	GG		A	G		
HC	(N = 178)	114 (64)	57 (32)	7 (3.9)	NS	285 (80.1)	71 (19.9)	NS	1.10 (0.76-1.60)
SSc	(N = 182)	120 (65.9)	57 (31.3)	5 (2.7)		297 (81.6)	67 (18.4)		

Abbreviations: CI, confidence interval; HC, healthy controls; NS, not significant; OR, odds ratio; SSc, systemic sclerosis.

TABLE 4 Association of allele and genotype of rs12434438 SNP with clinical features in SSc patients in the first cohort (N = 182)

		Genotype				Allele		
		AA	GA	GG		A	G	
		n (%)	n (%)	n (%)	*P*	n (%)	n (%)	*P*
SSc patients	(N = 182)	120 (65.9)	57 (31.3)	5 (2.7)	NS	297 (81.6)	67 (18.4)	NS
Lc type	(N = 78)	53 (67.9)	24 (30.8)	1 (1.3)	NS	130 (83.3)	26 (16.7)	NS
Anti-RNP	(N = 33)	21 (63.6)	11 (33.3)	1 (3.0)	NS	53 (80.3)	13 (19.7)	NS
Anti-CENP	(N = 48)	34 (70.8)	14 (29.2)	0 (0.0)	NS	82 (85.4)	14 (14.6)	NS
Digital ulcer	(N = 25)	15 (60.0)	10 (40.0)	0 (0)	NS	40 (80.0)	10 (20.0)	NS
PAH	(N = 27)	22 (81.5)	3 (11.1)	2 (7.4)	0.012	47 (87)	7 (13)	NS
ILD	(N = 95)	65 (68.4)	26 (27.4)	4 (4.2)	NS	156 (82.1)	34 (17.9)	NS
Arthritis	(N = 54)	35 (64.8)	19 (35.2)	0 (0.0)	NS	89 (82.4)	19 (17.6)	NS
Myositis	(N = 21)	18 (85.7)	3 (14.3)	0 (0.0)	NS	39 (92.9)	3 (7.1)	NS
Renal crisis	(N = 19)	13 (68.4)	5 (26.3)	1 (5.3)	NS	31 (81.6)	7 (18.4)	NS
GERD	(N = 120)	81 (67.5)	36 (30.0)	3 (2.5)	NS	198 (82.5)	42 (17.5)	NS
Myocarditis	(N = 4)	3 (75.0)	1 (25)	0 (0.0)	NS	7 (87.5)	1 (12.5)	NS

Abbreviations: CENP, centromere; GERD, gastroesophageal reflux disease; ILD, interstitial lung disease; Lc, limited cutaneous; NS, not significant; PAH, pulmonary arterial hypertension; RNP, ribonucleoprotein; SSc, systemic sclerosis.

Map-JPT and Hap Map-CEU show that the frequencies of the rs12434438 AA genotype are lower and the frequencies of the rs12434438 GA and GG genotype are higher in the Japanese population than in central Europeans. If the genotype frequency in the control population is adjusted to that of the Japanese population, the significance of the rs12434438 G allele for SSc development in the French population might disappear. These observations suggest that the contribution of rs12434438 to SSc development can be different among races.

In terms of PAH in SSc, the association of several SNPs has been reported. *IL23R*,[17] *KCNA5*,[18] *TLR2*,[19] *TNAIP3*[20] and *UPAR*[21] were associated not only with susceptibility to SSc development but also PAH in SSc. These findings suggested a close relationship between genes involved in inflammation and fibrosis and PAH in SSc. Distler et al[32] reported that hypoxia directly contributed to the progression of fibrosis in SSc by in vivo analyses. Moreover, skin biopsies from SSc patients strongly expressed vascular endothelial growth factor (VEGF) and HIF1α.[27] Increased HIF1α in SSc fibroblasts can be a key mechanism for the transcriptional regulation of VEGF.[27] HIF1α and VEGF play important roles in vascular complications in patients with SSc, including Raynaud's phenomenon, digital ulcers, and PAH.

Pulmonary arterial hypertension is one of the major manifestations directly associated with mortality in patients with SSc.[38] In the past 2 decades, the strategy for the treatment of PAH in SSc has improved due to specific PAH drugs, including endothelin receptor

TABLE 5 Genotype of rs12434438 SNP in SSc patients with and without PAH

	Discovery cohort (N = 182)				Validation cohort (N = 135)			
	AA	GA	GG	*P*	AA	GA	GG	*P*
PAH (+)	22	3	2	.012	26	3	1	.006
PAH (–)	98	54	3		59	38	8	
Total	120	57	5		85	41	9	

Note: P value was estimated by Fisher's exact test.

Abbreviations: PAH, pulmonary arterial hypertension; SNP, single nucleotide polymorphism; SSc, systemic sclerosis.

TABLE 6 Association of rs12434438 SNP and disease severity of PAH in SSc patients combining the discovery cohort with the validation cohort (N = 57)

	Absence of PAH	All of PAH	PAH (WHOFC I + II)	PAH (WHOFC III + IV)
Genotype	n	n	n	n
AA	157	48	35	13
GA + GG	103	9	8	1
P value		.0007[a]	.0097[b]	.020[c]
Odds ratio (95% CI)		3.5 (1.7-7.4)	2.9 (1.3-6.4)	8.5 (1.1-66)
Power		0.97	0.84	0.78

Note: P value was estimated by Fisher's exact test. Power analysis was performed with G*PowerV3.1.7.

Abbreviations: CI, confidence interval; PAH, pulmonary arterial hypertension; SNP, single nucleotide polymorphism; SSc, systemic sclerosis; WHOFC, World Health Organization functional class.

[a]The difference between all patients with PAH and patients without PAH is shown.

[b]The difference between patients with PAH (WHOFC I + II) and patients without PAH is shown.

[c]The difference between patients with PAH (WHOFC III + IV) and patients without PAH is shown.

antagonists, phosphodiesterase 5 inhibitors, and prostanoids.[38] However, compared to that of idiopathic PAH, the mortality of SSc-associated PAH is currently worse. Early diagnosis of PAH might contribute to improvement of survival in patients with SSc. At the 6th World Symposium on Pulmonary Hypertension, the clinical classification of precapillary pulmonary hypotension was proposed as follows: mean pulmonary artery pressure (mPAP) ≥21 mm Hg, PAWP ≤15 mm Hg, and pulmonary vascular resistance (PVR) ≥3 Wood units (WU).[36] Recently, several studies were published concerning precapillary PAH with SSc, and observations from those studies suggested that the function of the right ventricle was impaired.[39,40] In the future, starting the treatment for SSc-precapillary PAH (mPAP ≥21) earlier would improve the prognosis. Analysis of predictive factors for early detection of PAH will be important for early treatment of PAH. The *HIF1A* gene SNP may be a candidate predictive factor for PAH in patients with SSc. In the present study, our findings contribute to the early diagnosis of PAH in SSc, resulting in improved mortality.

In the discovery cohort (N = 182) and the validation cohort (N = 135), the number of patients who had manifest PAH (mean PAP ≥25 mm Hg) was 27 and 30, respectively, and the number of patients with borderline PAH (21 ≤ mean PAP ≤ 24) was 5 and 4, respectively. The genotypes of the SNP at rs12434438 in these nine patients were as follows: 5 AA, 3 GA, and 1 GG. In a combined cohort including the discovery and the validation cohorts, the numbers of AA and AG + GG were 152 and 99, respectively, in patients without PAH and 53 and 13, respectively, in patients with precapillary PAH (mean PAP ≥21 mm Hg, PVR ≥3 WU). The *P* value was .0024 by Fisher's exact test. The correlation in patients with manifest PAH (mean PAP ≥25 mm Hg) was similar to that in patients with precapillary PAH (mean PAP ≥21 mm Hg).

The P582S SNP at rs11549465 leads to increased transcriptional activity of *HIF1A*.[41] In our study, an association of rs11549465 with SSc development or any phenotypes was not observed. The rs12434438 SNP is located in intron 6. This SNP might be involved in the transcriptional activity of the *HIF1A* gene, but its precise function has not been clarified. The overexpression of HIF1A in hepatocellular carcinoma is associated with tumor angiogenesis, invasion, metastasis, treatment resistance and poor prognosis. The genotype AA + AG > GG of the rs12434438 SNP of the *HIF1A* gene was statistically significantly associated with survival.[42] These findings suggest that the AA genotype may be associated with endothelial injuries and may inhibit cancer metastasis through blood vessels. Although further genetic and cell biology studies are required to understand the role of the rs12434438 SNP of the *HIF1A* gene, the AA genotype of the rs12434438 SNP may be involved in endothelial impairment in SSc, affecting PAH severity.

The limitations in the present study are the following: (a) a small population of SSc patients enrolled in this study; (b) the genetic

analyses in only a center in Japan; and (c) unknown biological functions of the *HIF1A* gene by polymorphism (rs12434438). In the future, we will validate different samples in multiple centers in Japan and analyze the function of HIF1α expressed by different gene polymorphisms.

In conclusion, PAH is the complication responsible for the highest mortality in patients with SSc. In the past 2 decades, many therapeutic methods, such as endothelin receptor antagonists and phosphodiesterase 5 inhibitors, have been established for the treatment of PAH with SSc. However, because mortality may still be high, the discovery of predicative markers for PAH will provide us with a means for the early detection of PAH. In the present study, the AA genotype of the *HIF1A* gene was found to be a candidate predictive genetic marker for developing PAH in patients with SSc.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

K. Takagi and Y. Kawaguchi participated in the design of this study. M. Kawamoto contributed to genotyping and analyses of gene polymorphism data. K. Takagi, T. Higuchi, A. Tochimoto, M. Harigai, and Y. Kawaguchi collected the clinical and genomic DNA data of SSc patients and healthy controls. K. Takagi, M. Kawamoto, and Y. Kawaguchi contributed to statistical analyses. K. Takagi, M. Harigai, and Y. Kawaguchi drafted the manuscript.

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REFERENCES

1. Dieudé P, Guedj M, Wipff J, et al. Association between the IRF5 rs2004640 functional polymorphism and systemic sclerosis: a new perspective for pulmonary fibrosis. *Arthritis Rheum*. 2009;60(1):225-233.
2. Rueda B, Broen J, Simeon C, et al. The STAT4 gene influences the genetic predisposition to systemic sclerosis phenotype. *Hum Mol Genet*. 2009;18(11):2071-2077.
3. Rueda B, Gourh P, Broen J, et al. BANK1 functional variants are associated with susceptibility to diffuse systemic sclerosis in Caucasians. *Ann Rheum Dis*. 2010;69(4):700-705.
4. Gourh P, Agarwal SK, Martin E, et al. Association of the C8orf13-BLK region with systemic sclerosis in North-American and European populations. *J Autoimmun*. 2010;34(2):155-162.
5. Radstake TR, Gorlova O, Rueda B, et al. Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. *Nat Genet*. 2010;42(5):426-429.
6. Zhou X, Lee JE, Arnett FC, et al. HLA-DPB1 and DPB2 are genetic loci for systemic sclerosis: a genome-wide association study in Koreans with replication in North Americans. *Arthritis Rheum*. 2009;60(12):3807-3814.
7. Allanore Y, Saad M, Dieudé P, et al. Genome-wide scan identifies TNIP1, PSORS1C1, and RHOB as novel risk loci for systemic sclerosis. *PLoS Genet*. 2011;7(7):e1002091.
8. Gorlova O, Martin JE, Rueda B, et al. Identification of novel genetic markers associated with clinical phenotypes of systemic sclerosis through a genome-wide association strategy. *PLoS Genet*. 2011;7(7):e1002178.
9. Fonseca C, Lindahl GE, Ponticos M, et al. A polymorphism in the CTGF promoter region associated with systemic sclerosis. *N Engl J Med*. 2007;357(12):1210-1220.
10. Hoshino K, Satoh T, Kawaguchi Y, Kuwana M. Association of hepatocyte growth factor promoter polymorphism with severity of interstitial lung disease in Japanese patients with systemic sclerosis. *Arthritis Rheum*. 2011;63(8):2465-2472.
11. Dieudé P, Bouaziz M, Guedj M, et al. Evidence of the contribution of the X chromosome to systemic sclerosis susceptibility: association with the functional IRAK1 196Phe/532Ser haplotype. *Arthritis Rheum*. 2011;63(12):3979-3987.
12. Sharif R, Mayes MD, Tan FK, et al. IRF5 polymorphism predicts prognosis in patients with systemic sclerosis. *Ann Rheum Dis*. 2012;71(7):1197-1202.
13. Dieude P, Dawidowicz K, Guedj M, et al. Phenotype-haplotype correlation of IRF5 in systemic sclerosis: role of 2 haplotypes in disease severity. *J Rheumatol*. 2010;37(5):987-992.
14. Manetti M, Ibba-Manneschi L, Fatini C, et al. Association of a functional polymorphism in the matrix metalloproteinase-12 promoter region with systemic sclerosis in an Italian population. *J Rheumatol*. 2010;37(9):1852-1857.
15. Sumita Y, Sugiura T, Kawaguchi Y, et al. Genetic polymorphisms in the surfactant proteins in systemic sclerosis in Japanese: T/T genotype at 1580 C/T (Thr131Ile) in the SP-B gene reduces the risk of interstitial lung disease. *Rheumatology (Oxford)*. 2008;47(3):289-291.
16. Wu M, Assassi S, Salazar GA, et al. Genetic susceptibility loci of idiopathic interstitial pneumonia do not represent risk for systemic sclerosis: a case control study in Caucasian patients. *Arthritis Res Ther*. 2016;18:20.
17. Agarwal SK, Gourh P, Shete S, et al. Association of interleukin 23 receptor polymorphisms with anti-topoisomerase-I positivity and pulmonary hypertension in systemic sclerosis. *J Rheumatol*. 2009;36(12):2715-2723.
18. Wipff J, Dieudé P, Guedj M, et al. Association of a KCNA5 gene polymorphism with systemic sclerosis-associated pulmonary arterial hypertension in the European Caucasian population. *Arthritis Rheum*. 2010;62(10):3093-3100.
19. Broen JC, Bossini-Castillo L, van Bon L, et al. A rare polymorphism in the gene for Toll-like receptor 2 is associated with systemic sclerosis phenotype and increases the production of inflammatory mediators. *Arthritis Rheum*. 2012;64(1):264-271.
20. Dieudé P, Guedj M, Wipff J, et al. Association of the TNFAIP3 rs5029939 variant with systemic sclerosis in the European Caucasian population. *Ann Rheum Dis*. 2010;69(11):1958-1964.
21. Manetti M, Allanore Y, Revillod L, et al. A genetic variation located in the promoter region of the UPAR (CD87) gene is associated with the vascular complications of systemic sclerosis. *Arthritis Rheum*. 2011;63(1):247-256.
22. Nguyen B, Mayes MD, Arnett FC, et al. HLA-DRB1*0407 and *1304 are risk factors for scleroderma renal crisis. *Arthritis Rheum*. 2011;63(2):530-534.
23. Semenza GL. Hypoxia-inducible factor 1: master regulator of O2 homeostasis. *Curr Opin Genet Dev*. 1998;8:588-594.
24. Tennant DA, Frezza C, MacKenzie ED, et al. Reactivating HIF prolyl hydroxylases under hypoxia results in metabolic catastrophe and cell death. *Oncogene*. 2009;28:4009-4021.
25. Masoud GN, Li W. HIF-1α pathway: role, regulation and intervention for cancer therapy. *Acta Pharm Sin B*. 2015;5:378-389.
26. ChenY ZB, Zhu Y, Zhao H, Ma C. HIF-1-VEGF-Notch mediates angiogenesis in temporomandibular joint osteoarthritis. *Am J Transl Res*. 2019;11:2969-2982.
27. Ioannou M, Pyrpasopoulou A, Simos G, et al. Upregulation of VEGF expression is associated with accumulation of HIF-1α in the skin of naïve scleroderma patients. *Mod Rheumatol*. 2013;23:1245-1248.

28. Bahadori B, Uitz E, Mayer A, et al. Polymorphisms of the hypoxia-inducible factor 1 gene and peripheral artery disease. *Vasc Med*. 2010;15:371-374.
29. Saravani M, Rokni M, Mehrbani M, et al. The evaluation of VEGF and HIF-1α gene polymorphisms and multiple sclerosis susceptibility. *J Gene Med*. 2019;21:e3132.
30. Paradowska-Gorycka A, Stypinska B, Pawlik A, Haladyj E, Romanowska-Prochnicka K, Olesinska M. HIF-1α gene polymorphisms and its protein level in patients with rheumatoid arthritis: a case-control study. *Inflamm Res*. 2018;67:423-433.
31. Zhao W, Wu C, Li LJ, et al. RNAi silencing of HIF-1α ameliorates lupus development in MRL/lpr mice. *Inflammation*. 2018;41:1717-1730.
32. Distler JH, Jungel A, Pileckyte M, et al. Hypoxia-induced increase in the production of extracellular matrix proteins in systemic sclerosis. *Arthritis Rheum*. 2007;56:4203-4215.
33. Wipff J, Dieude P, Avouac J, et al. Association of hypoxia-inducible factor 1A (HIF1A) gene polymorphisms with systemic sclerosis in a French European Caucasian population. *Scand J Rheumatol*. 2009;38(4):291-294.
34. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum*. 2013;65:2737-2747.
35. LeRoy EC, Krieg T, Black C, et al. Scleroderma (systemic sclerosis): classification, subsets, and pathogenesis. *J Rheumatol*. 1988;15:202-205.
36. Simonneau G, Montani D, Celermajer DS, et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J*. 2019;53:1801913.
37. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods*. 2009;41:1149-1160.
38. Denton CP, Khanna D. Systemic sclerosis. *Lancet*. 2017;390:1685-1699.
39. Coghlan JG, Wolf M, Distler O, et al. Incidence of pulmonary hypertension and determining factors in patients with systemic sclerosis. *Eur Respir J*. 2018;51:1701197.
40. Nagel C, Marra AM, Benjamin N, et al. Reduce right ventricular output reserve in patients with systemic sclerosis and mildly elevated pulmonary artery pressure. *Arthritis Rheumatol*. 2019;71:805-816.
41. Tanimoto K. Genetics of the hypoxia-inducible factors in human cancers. *Exp Cell Res*. 2017;356(2):166-172.
42. Faloppi L, Casadei Gardini A, Masi G, et al. Angiogenesis polymorphisms profile in the prediction of clinical outcome of advanced HCC patients receiving sorafenib: combined analysis of VEGF and HIF-1α - final results of the ALICE-2 study. *J Clin Oncol*. 2016;34(suppl):280-280.

How to cite this article: Takagi K, Kawamoto M, Higuchi T, Tochimoto A, Harigai M, Kawaguchi Y. Single nucleotide polymorphisms of the *HIF1A* gene are associated with susceptibility to pulmonary arterial hypertension in systemic sclerosis and contribute to SSc-PAH disease severity. *Int J Rheum Dis*. 2020;23:674–680. https://doi.org/10.1111/1756-185X.13822

Received: 15 October 2019 | Revised: 26 January 2020 | Accepted: 24 February 2020

DOI: 10.1111/1756-185X.13824

ORIGINAL ARTICLE

International Journal of Rheumatic Diseases | WILEY

Ultrasonography involvement of carotid, upper and lower limb arteries in a large cohort of systemic sclerosis patients

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Abstract

Objectives: Data on macrovascular involvement in systemic sclerosis (SSc) are still debatable. The aim of this study was to estimate its prevalence and possible determinants in a large cohort.

Methods: One hundred and fifty-five outpatients with SSc were enrolled. Data about disease characteristics and cardiovascular risk factors were collected and patients underwent ecocolor Doppler ultrasonography of arteries of the neck and lower (LL) and upper (UL) limbs.

Results: Mean age was 57.9 ± 14.5 years and most were female (88.4%) with a limited subset (63.2%). Mean disease duration was 11.4 ± 8.1 years. Twenty-three (14.8%) had hypertension, 7 (4.8%) diabetes, 64 (41.3%) hypercholesterolemia and 63 (40.6%) were active/past smokers. Seventy-nine (49%) patients had plaques at carotids, 49 (32.9%) at LL and 7 (4.9%) at UL. In multivariate analysis, patients with carotid plaques had more often a limited pattern (P = .001), patients with distal LL plaques pulmonary arterial hypertension (P = .006) and patients with proximal LL plaques lower diffusing capacity for carbon monoxide adjusted to hemoglobin and its ratio to alveolar volume (P = .004). In patients with UL plaques traditional cardiovascular risk factors were not more common, while forced vital capacity was lower (P = .023). Finally, upper limb and proximal LL plaques were as common in early disease patients as in longstanding ones, although the former were younger.

Conclusions: This study shows that macrovascular involvement is quite common in SSc and that some disease characteristics linked to microvascular involvement are associated with atherosclerotic plaques, which can be present even in early disease. Our study suggests that a complete evaluation of macrocirculation is mandatory for rheumatologists treating SSc patients.

KEYWORDS

macrovascular involvement, systemic sclerosis, ultrasonography

1 | INTRODUCTION

Skin and visceral microvasculopathy is a typical characteristic of systemic sclerosis (SSc), together with abnormal widespread deposition of collagen and other proteins of extracellular matrix, as shown by some threatening and severe clinical manifestations such as digital ulcers, pulmonary artery hypertension (PAH) and scleroderma renal crisis.[1,2]

Whether macrovasculopathy affects scleroderma patients has been the object of some studies leading to contrasting results. A possible reason is the great heterogeneity between studies, as underlined by a meta-analysis published in 2011.[3] The same paper showed that carotid intima-media thickness (cIMT) was found higher in SSc than controls in 6 out of 14 studies.[3] In those showing higher cIMT, differences with controls were found comparable to those shown in other diseases characterized by an increased cardiovascular risk such as rheumatoid arthritis (0.09 mm),[4] diabetes mellitus (0.13 mm)[5] and familial hypercholesterolemia (0.12 mm),[6] so the burden of carotid atherosclerosis in SSc may be of some relevance, although still debatable.

Also, data on peripheral artery disease at lower limbs (LL) were discordant. Ho et al[7] found evidence of atherosclerosis in 9 out of 53 SSc cases (17%) compared with no controls, whereas Bartoli et al[8] and Nordin et al[9] did not show any difference in ankle-brachial pressure index (ABPI) between patients and healthy subjects.

Some studies underlined the involvement of ulnar artery in SSc. In previous studies, Doppler examination showed that the diameter of ulnar artery was narrower in 20 SSc patients compared to 20 controls[10] and an occlusion of ulnar artery was found in 17 out of 79 SSc patients.[11]

The aim of the present study was to assess macrovascular involvement in a large and well-defined cohort of SSc patients by contemporaneously evaluating, by Doppler ultrasonography, 3 different arterial districts, that is carotid arteries and arteries of upper limbs (UL) and LL; in addition, we evaluated a great number of clinical features in order to study if any of them may be useful to select those SSc patients at increased atherosclerotic risk.

2 | PATIENTS AND METHODS

For the present study we enrolled 155 consecutive outpatients affected by SSc followed at the Rheumatology Unit of Verona, Roma and Modena. All patients fulfilled the American College of Rheumatology/European League Against Rheumatism classification criteria for SSc.[12] The distinction between limited and diffuse cutaneous SSc was made according to LeRoy et al[13] criteria. Skin involvement was assessed by modified Rodnan skin score (mRSS).[14] Antinuclear antibodies (ANA) and anticentromere antibodies (ACA) were tested by indirect immunofluorescence on HEp-2 cells, and anti-Scl70 antibodies were searched by enzyme-linked immunosorbent assay method.

Laboratory evaluation also included erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), creatinine with estimated glomerular filtration rate (eGFR), total, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol, triglycerides, glucose and homocysteine levels. Each patient underwent pulmonary function tests with diffusing capacity for carbon monoxide adjusted to hemoglobin (DLCO) and ratio of DLCO to alveolar volume (DLCO/AV). At the same time body mass index (BMI) was calculated and disease severity score was assessed, as proposed by Medsger et al[15] The diagnosis of interstitial lung disease (ILD) and PAH was based upon lung high-resolution computed tomography and right heart catheterization, respectively. Digital ulcers were defined as ischemic ulcers located at the digit tip. Evaluation of the cardiovascular risk was made in agreement with the European Low Risk Chart proposed by the European Society of Cardiology (ESC),[16] which considers the following parameters: gender, age, systolic blood pressure, total cholesterol value and smoking habits.

All patients underwent Doppler ultrasonography (DUS) of the carotid arteries and of the UL and LL arteries. The following arteries were analyzed: common carotid, internal and external carotid, subclavian artery, humeral artery, ulnar artery, radial artery, common femoral artery, profunda femoral artery, superficial femoral artery, popliteal artery, anterior and posterior tibial artery. cIMT measurements were carried out after a 15-minutes resting interval and no intravenous vasodilators were given the day of the examination and during the 3 previous days. Patients underwent ultrasound measurement of cIMT at both common carotid arteries on the distal wall. cIMT was examined by a skilled operator using a high-resolution linear probe (7.5 MHz) by means of automatic ultrasound detection of cIMT (Esaote MyLab 30 Gold—QIMT). Common carotids were examined at standard angles bilaterally: 1 cm proximally to the bulb, a segment of 2 cm was selected with the cursor of the system and IMT was automatically calculated. Median IMT for each common carotid artery was calculated and expressed in centimeters.

Plaques were defined as a localized increase of vessel wall profile of more than 1.5 mm. Stenoses were calculated in accordance with the Consensus Panel Gray-Scale and Doppler Ultrasound Criteria for Diagnosis reported by Grant et al.[17]

A written informed consent was obtained from all the participants in the study. The protocol study was approved by the local Ethical Committee (protocol no. 55946 for Verona University, protocol no. 3822/14 for Catholic University of the Sacred Heart of Rome, protocol no. 10693 of 25/05/2016 for Modena and Reggio Emilia University).

2.1 | Statistics

Continuous variables were expressed as mean ± standard deviation if they were normally distributed and as median with interquartile range if they were not normally distributed. Categorical variables were expressed as percentage. Comparisons between groups were performed using *t* test, Mann-Whitney or Chi-square tests/Fisher's test, as appropriate. The determinants of macrovascular involvement were explored with logistic multivariate regression. Receiver

TABLE 1 Demographic and the clinical data of the 155 patients affected by systemic sclerosis

Age[a]	57.9 (14.5)
Disease duration, y[b]	11.4 (8.1)
Time from onset of Raynaud's phenomenon, y[a]	16.4 (11.7)
BMI, kg/m^2[a]	24.0 (4.1)
Pack-year[b]	0.0 (7.5)
FVC, predicted (%)[a]	102 (22)
DLCO, predicted (%)[a]	67 (22)
DLCO/AV, predicted (%)[a]	74 (22)
mRSS[b]	7 (7)
Disease severity score (15)[b]	5 (3)
Creatinine, mg/dL[a]	0.8 (0.2)
eGFR using CKD-EPI formula, mL/min/1.73 m^2[a]	88 (21)
Total cholesterol, mg/dL[a]	188 (34)
HDL cholesterol, mg/dL[a]	60 (18)
LDL cholesterol, mg/dL[a]	108 (30)
Triglycerides, mg/dL[a]	103 (46)
Glucose, mg/dL[a]	85 (14)
Homocysteine, μmol/L[a]	14.5 (7.9)
IMT, cm[a]	0.09 (0.05)
ESR, mm/h[a]	24 (16)
CRP, mg/L[b]	3 (0)
ESC score[b]	1 (2)
Female[c]	137 (88.4)
Smoker[c]	
Never	92 (59.4)
Previous	47 (30.3)
Active	16 (10.3)
Endothelin receptor antagonists[c]	20 (13.2)
Iloprost	143 (92.9)
Current immunosuppressants[c]	48 (31.6)
Previous immunosuppressants[c]	57 (36.8)
HCQ[c]	31 (20.0)
Current steroid treatment	30 (19.7)
Autoantibodies[c]	
Scl70	59 (38.1)
ACA	70 (45.2)
ANA	26 (16.8)
Cutaneous pattern[c]	
Limited	98 (63.2)
Diffuse	57 (36.8)
ILD[c]	53 (34.2)
PAH[c]	7 (4.6)
Active ischemic digital ulcers[c]	12 (7.7)
Previous ischemic digital ulcers[c]	48 (31.0)
Scleroderma renal crisis[c]	1 (0.7)
Statin[c]	19 (13.2)
Hypercholesterolemia[c]	64 (41.3)
Blood hypertension[c]	23 (14.8)
Diabetes mellitus[c]	7 (4.8)

Abbreviations: ACA, anticentromere antibodies; ANA, antinuclear antibodies; BMI, body mass index; CDK-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRP, C-reactive protein; DLCO, diffusion lung for carbon monoxide; eGFR, estimated glomerular filtration rate; ESC, European Society of Cardiology; ESR, erythrocyte sedimentation rate; FVC, forced vital capacity; HCQ, hydroxychloroquine; HDL, high density lipoproteins; ILD, interstitial lung disease; IMT, intima-media thickness; LDL, low density lipoprotein; mRSS, modified Rodnan skin score; PAH, pulmonary artery hypertension; RCS, Raynaud's condition score; VA, alveolar volume; VAS, visual analog scale.

[a]Values expressed as mean (SD).

[b]Values expressed as median (interquartile range).

[c]Values expressed as absolute number (%).

operating characteristic analysis was run to evaluate the performance of ESC score in predicting subclinical atherosclerosis. A P value < .05 was considered significant. Statistical analysis was performed by SPSS 17.0 (SPSS Inc).

3 | RESULTS

3.1 | SSc cohort

The study population was composed of 155 subjects, 18 male and 137 female, of which 95 (69.3%) were in menopause status. Mean age was 57.9 ± 14.5 years; the disease duration was 11.4 ± 8.1 years and the time from onset of Raynaud's phenomena was 16.4 ± 11.7 years. The main clinical characteristics are reported in Table 1.

3.2 | Carotid atherosclerosis

Seventy-five patients (49%) had carotid plaques. The artery stenosis was <50% in the majority of subjects (51 cases); 4 and 2 patients showed a hemodynamically significant stenosis comprised between 50% and 60% and between 60% and 70%, respectively. The mean value of IMT was 0.09 mm.

In univariate analysis we found the following differences: scleroderma patients with plaques were older (P < .001), more frequently had a limited cutaneous pattern of disease (P = .030) and hypertension (P = .032), showed higher values of glucose (P = .005), disease severity score (P = .009), homocysteine (P = .014) and ESR (P = .001) and lower values of eGFR (P = .002). Current immunosuppressive therapy was negatively associated with the presence of carotid plaques (P = .017). In terms of autoantibodies, ACAs were more frequent in patients with plaques (58% vs 42%, P = .045). These data are shown in Table 2.

TABLE 2 Differences between patients with plaques at carotid or upper limb arteries and those without

	Carotid plaques			Upper limb plaques		
	No	Yes	*P*	No	Yes	*P*
Age[a]	52.2 (14.2)	63.5 (12.3)	**<.001**	60.2 (21.0)	52.5 (26.0)	ns
Disease duration, y[b]	9 (10.3)	12 (9)	ns	10.0 (10.0)	14.0 (11.0)	ns
BMI, kg/m^{2}[a]	23.4 (4.1)	24.6 (4.1)	0.067	24.0 (6.1)	20.3 (8.2)	ns
FVC, predicted (%)[a]	103 (21)	100 (24)	ns	104 (26)	87 (49)	**.021**
DLCO, predicted (%)[a]	68 (21)	65 (23)	ns	66 (27)	48 (25)	**.023**
DLCO/AV, predicted (%)[a]	74 (21)	74 (24)	ns	77 (29)	56 (43)	**.022**
mRSS[b]	7 (8)	8 (7)	ns	7 (7)	8 (11)	ns
Disease severity score, 15[b]	5 (2.5)	6 (4)	**.009**	5 (3)	p	**.043**
Creatinine, mg/dL[a]	0.7 (0.1)	0.8 (0.3)	ns	0.72 (0.19)	0.63 (0.2)	ns
eGFR using CKD-EPI formula, mL/min/1.73 m^{2}[a]	95 (20)	82 (20)	**.002**	89 (18)		ns
Total cholesterol, mg/dL[a]	189 (36)	186 (33)	ns	187 (44)	177 (37)	ns
HDL cholesterol, mg/dL[a]	61 (20)	59 (15)	ns	58 (21)	52 (20)	ns
LDL cholesterol, mg/dL[a]	109 (30)	107 (31)	ns	104 (43)	95 (23)	ns
Triglycerides, mg/dL[a]	98 (32)	107 (53)	ns	93 (56)	77 (64)	ns
Glucose, mg/dL[a]	82 (13)	88 (14)	**.005**	84 (16)	88 (39)	ns
Homocysteine, µmol/L[a]	12.8 (6.4)	16.4 (8.8)	**.014**	12.4 (6.4)	9.8 (–)	ns
ESR, mm/h[a]	19 (13)	28 (17)	**.001**	18 (22)	35 (46)	ns
CRP, mg/L[b]	3 (1)	3 (1)	ns	3.0 (0.0)	2.0 (4.6)	ns
ESC score[b]	1 (2)	2 (1)	.054	1 (2.0)		ns
Female[c]	73 (93.6)	63 (84.0)	.059	126 (88.1)	7 (100.0)	ns
Smoker[c]						
Never	50 (64.1)	42 (56.0)	ns	86 (60.1)	4 (57.1)	ns
Previous	20 (25.6)	26 (34.7)		43 (30.1)	2 (28.6)	
Active	8 (10.3)	7 (9.3)		14 (9.8)	1 (14.3)	
Endothelin receptor antagonists[c]	11 (14.5)	9 (12.0)	ns	17 (11.9)	3 (42.9)	**.050**
Iloprost	72 (93.5)	69 (92.0)	ns	132 (93.0)	7 (100.0)	ns
Current immunosuppressants[c]	31 (40.8)	17 (22.7)	**.017**	46 (67.8)	5 (71.4)	ns
Previous immunosuppressants[c]	32 (41.0)	24 (32.0)	ns	53 (37.1)	3 (42.9)	ns
HCQ[c]	16 (20.5)	15 (20.0)	ns	30 (21.0)	0 (0.0)	ns
Current steroid treatment	12 (15.8)	18 (24.0)	ns	28 (19.6)	1 (14.3)	ns
Autoantibodies[c]						
Scl70	34 (43.6)	24 (32.0)	ns	53 (37.1)	4 (57.1)	ns
ACA	29 (37.2)	40 (53.3)	ns	65 (45.5)	2 (28.6)	
ANA	15 (19.2)	11 (14.7)	ns	25 (17.5)	1 (14.3)	
Cutaneous pattern[c]						
Limited	43 (55.1)	54 (72.0)	**.030**	91 (63.6)	4 (57.1)	ns
Diffuse	35 (44.9)	21 (28.0)		52 (36.4)	3 (42.9)	
ILD[c]	30 (38.5)	22 (29.3)	ns	47 (32.9)	3 (42.9)	ns
PAH[c]	2 (2.7)	5 (6.8)	ns	7 (5.0)	0 (0.0)	ns
Active ischemic digital ulcers[c]	7 (9.0)	5 (6.7)	ns	9 (6.3)	3 (42.9)	**.012**
Previous ischemic digital ulcers[c]	28 (35.9)	19 (25.3)	ns	41 (28.7)	6 (85.7)	**.004**
Scleroderma renal crisis[c]	0 (0.0)	1 (1.4)	ns	1 (0.7)	0 (0.0)	ns
Statin[c]	9 (11.5)	14 (18.7)	ns	20 (14.0)	2 (28.6)	ns

(Continues)

TABLE 2 (Continued)

	Carotid plaques			Upper limb plaques		
	No	Yes	*P*	No	Yes	*P*
Hypercholesterolemia[c]	30 (38.5)	27 (36.0)	ns	52 (36.4)	4 (57.1)	ns
Blood hypertension[c]	7 (9.0)	16 (21.3)	**.032**	22 (15.4)	0 (0.0)	ns
Diabetes mellitus[c]	2 (2.6)	5 (6.7)	ns	7 (4.9)	0 (0.0)	ns

Abbreviations: ACA, anticentromere antibodies; ANA, antinuclear antibodies; BMI, body mass index; CDK-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRP, C-reactive protein; DLCO, diffusion lung for carbon monoxide; eGFR, estimated glomerular filtration rate; ESC, European Society of Cardiology; ESR, erythrocyte sedimentation rate; FVC, forced vital capacity; HCQ, hydroxychloroquine; HDL, high density lipoproteins; ILD, interstitial lung disease; IMT, intima-media thickness; LDL, low density lipoprotein; mRSS, modified Rodnan skin score; PAH, pulmonary artery hypertension; RCS, Raynaud's condition score; VA, alveolar volume; VAS, visual analog scale.

[a]Values expressed as mean (SD).

[b]Values expressed as median (interquartile range).

[c]Values expressed as absolute number (%).

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

We performed 2 multivariate models (Table 4). The first one considered all the variables with a $P < .1$ in univariate analysis (except ESC) and showed that older age ($P = .001$), higher disease severity scores ($P = .034$) and limited cutaneous pattern ($P = .001$) were significantly associated with carotid plaques. The second model considered the variables with a $P < .1$ in the first one with the addition of ESC score and diabetes mellitus and confirmed the significance of cutaneous pattern ($P = .001$) and ESR ($P = .010$) with only a trend for ESC ($P = .074$).

3.3 | LL artery involvement

The data were collected in 140 patients. Forty-nine patients (32.9%) had plaques at the LL arteries. The artery stenosis was <50% in the majority of subjects (32 cases), but 1 patient had a hemodynamically significant stenosis between 50% and 60% and 1 between 80% and 90%. Moreover, occlusion of the anterior tibial artery was found in three cases.

We divided LL involvement in proximal or distal accordingly to the localization of plaques, that is, proximal (43, 30.7%) or distal (14, 9.3%) to the popliteal artery.

Patients with proximal LLs were older ($P = .007$) than patients with no plaques, more frequently of male gender ($P = .007$), with higher disease severity score ($P = .025$) and with a higher prevalence of PAH ($P = .032$) and diabetes ($P = .028$); in addition they were more frequently on statins ($P = .014$), had lower predicted DLCO ($P = .044$), lower eGFR ($P = .005$), higher total and LDL cholesterol levels ($P = .022$ and 0.011, respectively), glycemia ($P = .037$), ESC scores ($P = .002$) and homocysteine ($P = .009$) (Table 3).

In multivariate analysis, predicted DLCO/AV ($P = .004$) was found lower in patients with plaques, while ESC score was higher ($P = .005$) (Table 4).

Patients with distal LLs were older ($P = .049$) than patients with no plaques, with a higher mRSS ($P = .007$), with lower HDL cholesterol ($P = .018$), more often ACA positive ($P = .021$), with ILD ($P = .035$) and PAH ($P = .020$) (Table 3).

Multivariate analysis (Table 4) showed that only PAH was significantly more frequent in patients with plaques ($P = .027$ in model 1 and $P = .006$ in model 2), also after correcting for ESC and diabetes.

3.4 | UL artery involvement

Only seven patients showed UL plaques. All had ulnar involvement and 2 had also radial and humeral plaques. No patients had subclavian involvement. In univariate analysis, patients with plaques had worse predicted FVC, DLCO and DLCO/VA ($P = .021$, .023 and .02, respectively), more frequent ulcers ($P = .012$ for active and $P = .004$ for previous), higher disease severity score ($P = .043$) and were more often on anti-endothelin treatment ($P = .050$) (Table 2).

In multivariate analysis we performed 2 models as previously explained (Table 4). We preferred previous to active ulcers for the analysis given the bigger sample size in this group. Model 1 showed that only a trend for lower predicted FVC and DLCO/VA to be associated with UL plaques ($P = .066$ and $P = .078$, respectively). In model 2 predicted FVC was shown to be significantly associated with plaques ($P = .023$).

3.5 | Combined analysis

We then analyzed data according to the number of vascular sites involved, that is, carotid and/or proximal LLs and/or distal LLs. We did not consider UL involvement since no differences in its prevalence were found between patients with and without plaques in other sites. Scleroderma patients with carotid plaques more frequently had also plaques at the LLs: 37 out of 73 cases (50.7%) vs 12 out of 67 cases (17.9%), $P < .001$. Table 5 summarizes univariate analysis that showed both traditional cardiovascular risk factors and disease characteristics to be associated with an increased number of sites involved. When we performed the multivariate analysis only male gender was found to be a risk factor for multi-site involvement (data not shown).

TABLE 3 Differences between patients with plaques at lower limb arteries and those without

	Proximal lower limbs plaques			Distal lower limb plaques		
	No	Yes	*P*	No	Yes	*P*
Age[a]	53.8 (14.6)	64.5 (12.2)	**.007**	56.9 (21.0)	62.2 (11.0)	**.049**
Disease duration, y[b]	10 (10)	12 (10)	.074	10.0 (11.0)	15.5 (14)	ns
BMI, kg/m^{2}[a]	23.9 (4.2)	24.9 (3.6)	ns	23.7 (6.1)	24.4 (8.0)	ns
FVC, predicted (%)[a]	101 (23)	104 (22)	ns	102 (26)	101 (29)	ns
DLCO, predicted (%)[a]	70 (21)	61 (19)	**.044**	66 (27)	55 (45)	ns
DLCO/AV, predicted (%)[a]	77 (21)	68 (23)	.094	74 (21)	74 (31)	ns
mRSS[b]	7.5 (8.5)	7.0 (6.0)	ns	7 (7)	9 (5)	**.007**
Disease severity score, 15[b]	5 (3)	6 (3)	**.025**	5 (3)	7 (4.5)	ns
Creatinine, mg/dL[a]	0.7 (0.2)	0.8 (0.3)	.06	0.7 (0.2)	0.8 (0.2)	**.044**
eGFR using CKD-EPI formula, mL/min/1.73 m^{2}[a]	91 (19)	79 (18)	**.005**	91 (15)	78 (19)	**.07**
Total cholesterol, mg/dL[a]	183 (39)	197 (33)	**.022**	188 (34)	187 (37)	ns
HDL cholesterol, mg/dL[a]	58 (20)	60 (28)	ns	60 (20)	52 (13)	**.018**
LDL cholesterol, mg/dL[a]	103 (28)	118 (33)	**.011**	103 (40)	111 (46)	ns
Triglycerides, mg/dL[a]	90 (54)	95 (66)	ns	91 (57)	100 (57)	ns
Glucose, mg/dL[a]	83 (12)	89 (16)	**.037**	84 (16)	89 (19)	ns
Homocysteine, µmol/L[a]	12.0 (22.8)	16.6 (10.2)	**.009**	11.8 (6.7)	15.9 (11.5)	.056
ESR, mm/h[a]	17 (23)	23 (27)	**.081**	19 (22)	31 (42)	ns
CRP, mg/L[b]	3 (p)	3 (0)	ns	3 (1)	3 (2)	ns
ESC score[b]	1 (2)	2 (2)	**.002**	1 (2)	2 (2)	ns
Female[c]	91 (93.8)	33 (76.7)	**.007**	123 (90.4)	10 (71.4)	.056
Smoker[c]						
Never	65 (67.0)	22 (51.2)	ns	83 (61.0)	7 (50.0)	ns
Previous	24 (24.7)	15 (34.9)		39 (28.7)	6 (42.9)	
Active	8 (8.2)	6 (14.0)		14 (10.3)	1 (7.1)	
Endothelin receptor antagonists[c]	10 (10.3)	5 (11.6)	ns	16 (11.8)	4 (28.6)	.095
Iloprost	90 (92.8)	39 (92.9)	ns	125 (92.6)	14 (100.0)	ns
Current immunosuppressants[c]	33 (34.0)	13 (30.2)	ns	45 (33.1)	3 (21.4)	ns
Previous immunosuppressants[c]	39 (40.2)	15 (34.9)	ns	53 (39.0)	3 (21.4)	ns
HCQ[c]	24 (24.7)	6 (14.0)	ns	27 (19.9)	3 (21.4)	ns
Current steroid treatment	16 (16.5)	12 (27.9)	ns	28 (20.6)	1 (7.1)	ns
Autoantibodies[c]						
Scl70	40 (41.2)	14 (32.6)	ns	54 (39.7)	3 (21.4)	**.021**
ACA	39 (40.2)	22 (51.2)		56 (41.2)	11 (78.6)	
ANA	18 (18.6)	7 (16.3)		26 (19.1)	0 (0.0)	
Cutaneous pattern[c]						
Limited	58 (59.8)	28 (65.1)	ns	84 (61.8)	11 (78.6)	ns
Diffuse	39 (40.2)	15 (34.9)		52 (38.2)	3 (21.4)	
ILD[c]	29 (29.9)	16 (37.2)	ns	49 (36.0)	1 (7.1)	**.035**
PAH[c]	1 (1.1)	4 (9.5)	**.032**	4 (3.0)	3 (21.4)	**.02**
Active ischemic digital ulcers[c]	9 (9.3)	3 (7.0)	ns	10 (7.4)	2 (14.3)	ns
Previous ischemic digital ulcers[c]	29 (29.9)	12 (27.9)	ns	42 (30.9)	5 (35.7)	ns
Scleroderma renal crisis[c]	1 (1.1)	0. (0.0)	ns	1 (0.8)	0 (0.0)	ns
Statin[c]	8 (8.2)	10 (23.3)	**.014**	18 (13.2)	4 (28.6)	ns

(Continues)

TABLE 3 (Continued)

	Proximal lower limbs plaques			Distal lower limb plaques		
	No	Yes	*P*	No	Yes	*P*
Hypercholesterolemia[c]	35 (36.1)	15 (34.9)	ns	49 (36.0)	7 (50.0)	ns
Blood hypertension[c]	13 (13.4)	9 (20.9)	ns	20 (14.7)	2 (14.3)	ns
Diabetes mellitus[c]	2 (2.1)	5 (11.6)	**.028**	6 (4.4)	1 (7.1)	ns

Abbreviations: ACA, anticentromere antibodies; ANA, antinuclear antibodies; BMI, body mass index; CDK-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRP, C-reactive protein; DLCO, diffusion lung for carbon monoxide; eGFR, estimated glomerular filtration rate; ESC, European Society of Cardiology; ESR, erythrocyte sedimentation rate; FVC, forced vital capacity; HCQ, hydroxychloroquine; HDL, high density lipoproteins; ILD, interstitial lung disease; IMT, intima-media thickness; LDL, low density lipoprotein; mRSS, modified Rodnan skin score; PAH, pulmonary artery hypertension; RCS, Raynaud's condition score; VA, alveolar volume; VAS, visual analog scale.

[a]Values expressed as mean (SD).

[b]Values expressed as median (interquartile range).

[c]Values expressed as absolute number (%).

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

In addition, cIMT was higher in patients with proximal and/or distal LL involvement (0.10 ± 0.04 vs 0.09 ± 0.02, $P < .001$) and patients with a cIMT > 0.09 had 2.6-fold increased risk of having a multi-district vasculopathy (95% CI 1.3-5.3, $P < .009$). No differences in cIMT were found for UL involvement.

3.6 | ESC

We studied ESC score to predict cIMT as surrogate of developing atherosclerotic events. A significant correlation between cIMT and ESC was found (Spearman's correlation .300, $P < .001$) and patients with a cIMT > 0.09 cm had higher ESC scores (0 ± 2 vs 1 ± 2, $P = .002$). Indeed, ESC score was found to perform fairly in predicting a cIMT > 0.09 cm (area under the curve [AUC] 0.646, $P = .003$) and showed a very low sensitivity but a high specificity in identifying patients at high risk, that is with an ESC score ≥5% (49.2% and 97.4%, respectively). When considering an ESC score ≥1% as a marker of increased cIMT, it showed a specificity of 53.8% and a sensitivity of 70.5%.

3.7 | Early disease

Patients were divided according to the disease duration, that is ≤5 years or >5 years. Those with an early disease (39, 25.2%) were significantly younger than those with a longstanding disease (46.2 ± 14.0 vs 61.9 ± 12.5, $P < .001$). Carotid and distal LL plaques were more common in the latter (54.4% vs 33.3%, $P = .023$, and 12.6% vs 0.0%, $P = .020$, respectively). In contrast, UL and proximal limb plaques were as common in early disease patients as in longstanding ones (5%-2% vs 2.6%, $P = .677$ and 32.4% vs. 25.7%, $P = .459$).

4 | DISCUSSION

In this study we have evaluated the macrovascular involvement in patients with SSc by performing a DUS of carotid, UL and LL and by collecting information on disease and cardiovascular risk factors (CRF). We have found that macrovascular involvement is quite common and that traditional CRF and some disease characteristics are associated with the development of plaques, not only in the univariate analysis that may be affected by age and disease duration, but also in multivariate models. In addition, we have confirmed that cIMT may be a useful red flag for macrovasculopathy also at LLs. Finally, ESC was found to perform fairly also in identifying SSc patients with subclinical atherosclerosis.

Table 6 summarizes the most important and recent studies on macrovascular involvement in SSc. Prevalence of carotid plaques was significantly variable, ranging from 11.8% to 65.5% with our study showing results in line with Nordin et al[9] and Schiopu et al.[18] Comparing the prevalence of LL and UL involvement is not easy given the use of different methods to define these manifestations. It is worth noticing that, although the prevalence of plaques is not uncommon, only a small proportion of patients had hemodynamically significant stenosis.

In our cohort we found that carotid and LL involvement is quite common and that, in multivariate analysis, traditional CRF are important determinants of proximal LL atherosclerosis with a trend for carotid and distal LL involvement. Similar results, although with a less complete evaluation of traditional CRF, have been reported in other studies.[11,19,20] Traditional CRF may play a role in macrovasculopathy, especially at proximal LLs, also in SSc, so the rheumatologist should always assess cardiovascular risk in these patients in order to prevent the development of its possible complications. When treating cardiovascular risk in SSc the rheumatologist should keep in mind there is some evidence that statins, apart from lowering blood cholesterol, may have favorable effects also on the fibrotic and vascular mechanisms involved in SSc pathogenesis.[21]

In addition, ESR was found to be higher in patients with carotid plaques. There is strong evidence that inflammation increases the risk of atherosclerosis not only in the general population,[22] but also in SSc patients.[18] In particular, Ozen et al[19] and Sedky et al[20] have recently found an increase in ESR in SSc patients with subclinical atherosclerosis. Although ESR is more variable than CRP in assessing

TABLE 4 Multivariate analysis of possible determinants of plaques

Carotid		*B*	Wald	Significance	Exp(*B*)	95% CI for Exp(*B*)	
						Inferior	Superior
Model 1	Age	0.148	10.604	**.001**	1.16	1.061	1.268
	Disease severity score (15)	0.284	4.488	**.034**	1.328	1.021	1.726
	eGFR	0.023	1.517	.218	1.024	0.986	1.063
	Glucose (mg/dL)	0.019	0.571	.450	1.019	0.971	1.069
	ESR (mm/h)	0.043	3.564	.059	1.044	0.998	1.091
	Homocysteine (μmol/L)	−0.036	0.768	.381	0.965	0.89	1.046
	Female gender	−1.246	2.201	.138	0.288	0.055	1.492
	Cutaneous pattern (limited)	2.514	11.654	**.001**	12.35	2.917	52.294
	Blood hypertension	0.328	0.166	.684	1.388	0.286	6.734
	ESC	0.302	3.194	.074	1.353	0.971	1.884
	Diabetes mellitus	0.476	0.24	.624	1.609	0.24	10.766
	ESR (mm/h)	0.038	6.642	**.010**	1.039	1.009	1.069
	Cutaneous pattern (limited)	1.456	10.587	**.001**	4.290	1.784	10.314
	Disease severity score (15)	0.124	2.298	.130	1.132	0.964	1.328
Model 1	PAH	−1.401	1.502	.351	0.246	0.013	4.683
	Statin	−0.595	0.737	.420	0.552	0.130	2.338
	Diabetes mellitus	−1.413	1.109	.203	0.243	0.028	2.139
	Female gender	−1.942	0.989	**.050**	0.143	0.021	0.997
	Age	0.064	0.037	.083	1.066	0.992	1.146
	DLCO/AV (predicted, %)	−0.039	0.019	**.040**	0.961	0.926	0.998
	Creatinine (mg/dL)	0.219	1.524	.886	1.245	0.063	24.697
	LDL cholesterol	0.011	0.009	.243	1.011	0.993	1.030
	Glucose (mg/dL)	−0.001	0.028	.977	0.999	0.946	1.056
	Homocysteine (μmol/L)	0.040	0.044	.359	1.041	0.955	1.135
	ESR (mm/h)	0.023	0.019	.224	1.024	0.986	1.063
	Disease severity score (15)	−0.070	0.145	.629	0.932	0.702	1.239
	ESC	0.474	7.900	**.005**	1.606	1.154	2.234
	Diabetes mellitus	−1.626	3.196	.074	0.197	0.033	1.170
	DLCO/AV (predicted, %)	−0.028	8.080	**.004**	0.972	0.954	0.991
Model 1	Age	−0.033	0.367	.544	0.968	0.870	1.076
	Disease duration	−0.099	1.161	.281	0.906	0.756	1.085
	mRSS	0.121	0.715	.398	1.129	0.852	1.495
	eGFR	−0.039	1.491	.222	0.961	0.902	1.024
	HDL cholesterol (mg/dL)	−0.055	1.777	.182	0.947	0.873	1.026
	ESR (mm/h)	−0.003	0.006	.939	0.997	0.927	1.072
	Homocysteine (μmol/L)	0.077	2.479	.115	1.080	0.981	1.189
	Female gender	−0.566	0.272	.602	0.568	0.068	4.762
	Endothelin receptor antagonists	0.012	0.000	.995	1.012	0.032	31.708
	Autoantibodies	−0.789	0.534	.465	0.454	0.055	3.770
	ILD	20.279	0.000	.998	6.413 × 108	0.000	–
	PAH	4.237	4.900	**.027**	71.429	1.623	–
Model 2	ESC	0.370	2.926	.087	1.448	0.947	2.213
	Diabetes mellitus	−0.248	0.045	.833	0.780	0.078	7.800
	PAH	2.422	7.486	**.006**	11.264	1.988	63.832

(Continues)

TABLE 4 (Continued)

Carotid		*B*	Wald	Significance	Exp(*B*)	95% CI for Exp(*B*)	
						Inferior	Superior
Model 1	FVC (predicted, %)	−0.101	3.371	**.066**	0.904	0.811	1.007
	Previous ischemic digital ulcers	18.813	0.000	.995	1.4 × 108	0.000	–
	DLCO/AV (predicted, %)	−0.092	3.105	**.078**	0.912	0.823	1.010
	Endothelin receptor antagonists	4.142	1.541	.215	62.903	0.091	4.353 × 104
	Disease severity score (15)	0.125	0.272	.602	1.134	0.708	1.815
Model 2	ESC	−0.592	0.940	.332	0.553	0.167	1.830
	Diabetes mellitus	17.152	0.000	.999	2.8 × 1010	0.000	–
	FVC (predicted, %)	−0.049	5.181	**.023**	0.952	0.912	0.993
	DLCO/AV (predicted, %)	−0.039	3.261	.071	0.962	0.922	1.003

Abbreviations: DLCO, diffusion lung for carbon monoxide; eGFR, estimated glomerular filtration rate; ESC, European Society of Cardiology; ESR, erythrocyte sedimentation rate; FVC, forced vital capacity; HDL, high density lipoproteins; ILD, interstitial lung disease; LDL, low density lipoproteins; mRSS, modified Rodnan skin score; PAH, pulmonary artery hypertension; VA, alveolar volume; VAS, visual analog scale.
Statistically significant values ($P<.05$) are indicated in bold.

inflammation and may be affected by many factors, such as age and gender, our result was confirmed also after correcting for them, so its increase in vasculopathy patients may be actually related to the role of inflammation in atherosclerosis.

Some disease characteristics have been found to increase the risk of macrovascular involvement independently from traditional CRF. Subjects with limited pattern were shown to have an increased risk of carotid plaques. Nordin et al[9] has previously reported a similar result for anti-centromere antibodies after correction for gender, age and disease duration. These antibodies have been found to be also associated with a lower ABPI.[23] Although in our cohort ACAs were showed to be more frequent in patients with carotid plaques only in univariate analysis, our results on limited pattern seems to support that those patients with a more pronounced microvascular than fibrotic process have an increased risk of carotid atherosclerosis, supporting a possible link between micro- and macrovascular involvement. On the other hand, one may argue that patients with a diffuse pattern or anti-Scl70 antibody positivity are more often on immunosuppressive drugs; it is worth noticing that, in our cohort, this treatment was found to be protective against carotid plaques in univariate analysis. Although the role of inflammation in atherosclerosis is well known, there is still a lack of data on the possible role of immunotherapy to prevent its progression.[24] We speculate that the higher prevalence of carotid plaques in limited SSc may be related to different factors, such as a possible role of microvascular damage or a less frequent use of immunosuppressive treatment, although other factors, such as other treatments or the degree of inflammation, cannot be ruled out and further studies are needed. Also, for proximal and distal LLs a possible link with microvasculopathy could be hypothesized. Indeed, low DLCO/AV and PAH, both markers of microvascular disease, were more frequent in patients with proximal or distal LL involvement. No data in the literature are available for a proper comparison since different methodologies were used to assess LL arteriopathy. Nevertheless, SSc patients were reported to have an increased risk of vasculopathy in 2 out 3 studies present in the literature. Again, Nordin et al[9] have found that ACA + patients suffered more often from ischemic peripheral vascular disease defined as intermittent claudication + ankle-brachial index (ABI) <0.9 or peripheral arterial thrombosis/embolus (confirmed by angiogram or Doppler flow studies). ACAs are a well-known risk factor for PAH but no analysis on this were reported probably because of the small number of patients with both or either of these characteristics. In addition, Ozen et al[19] have recently found that PAH was more common in patients with increased cIMT, further supporting a possible link between micro- and macrovasculopathy via a common pathologic pathway such as endothelial dysfunction.[25,26] Although these data support an intriguing link between micro- and macrovasculopathy, there is contrasting evidence on videocapillaroscopy. It was found to be related to ulnar involvement by Lescoat et al[27] while Schioppo et al[28] found no correlation. It is worth noticing that these vessels are not a usual target of atherosclerosis and do not clearly reflect large vessel involvement, so further studies are needed to clarify this issue. Indeed, also in our study, traditional risk factors were not found to be independently associated with UL involvement. This could be expected since ulnar involvement is an unusual finding in atherosclerosis, whereas it may be a possible peculiar manifestation of SSc given its high prevalence,[10,29] as partially supported by significantly higher disease severity scores in patients with plaques in univariate analysis. In our experience the involvement of UL arteries was observed in a very little subgroup of patients, different from the results previously reported in other studies.[10,29] Although our data showed that ulnar vasculopathy is a risk factor for digital ulcers only in univariate analysis, that may be affected by disease duration, this may further support other previous studies since the lack of significance in multivariate analysis may be caused by both the small number of patients with UL vasculopathy and the correlation between disease severity score and ulcers (actually when we re-run the analysis without Medsger score, ulcers showed statistical

TABLE 5 Differences between patients with 0 or 1 site with plaques and those with 2 or 3 sites involved

		Sites with plaques		
		0 or 1	2 or 3	*P*
Age[a]		53.9 (14.3)	66.1 (11.9)	**<.001**
Disease duration, y[b]		10 (10)	12 (10)	.094
BMI, kg/m^{2}[a]		23.9 (4.1)	25.1 (3.9)	ns
FVC, predicted (%)[a]		102 (21)	102 (26)	ns
DLCO, predicted (%)[a]		69 (20)	61 (22)	**.046**
DLCO/AV, predicted (%)[a]		76 (20)	69 (26)	ns
mRSS[b]		8 (7)	8 (7)	ns
Disease severity score, 15[b]		5 (3)	6 (3)	**.023**
Creatinine, mg/dL[a]		0.7 (0.2)	0.8 (0.3)	**.031**
eGFR using CKD-EPI formula, mL/min/1.73 m^{2}[a]		91 (19)	79 (18)	**.007**
Total cholesterol, mg/dL[a]		185 (34)	191 (34)	ns
HDL cholesterol, mg/dL[a]		60 (19)	61 (15)	ns
LDL cholesterol, mg/dL[a]		106 (28)	113 (34)	ns
Triglycerides, mg/dL[a]		99 (47)	110 (47)	ns
Glucose, mg/dL[a]		83 (12)	91 (16)	**.001**
Homocysteine, µmol/L[a]		13.7 (6.6)	18.4 (10.3)	**.007**
ESR, mm/h[a]		17 (23)	24 (30)	**.038**
CRP, mg/L[b]		3 (0)	3 (0)	ns
ESC score[b]		0 (2)	2 (1)	**<.001**
Female[c]	F	97 (94.2)	27 (73.0)	**<.001**
Smoker[c]	Never	68 (66.0)	19 (51.4)	ns
	Previous	26 (25.2)	13 (35.1)	ns
	Active	9 (8.7)	5 (13.5)	ns
Endothelin receptor antagonists[c]		9 (8.7)	6 (16.2)	ns
Iloprost		94 (92.2)	35 (94.6)	ns
Current immunosuppressants[c]		36 (35.0)	10 (27.0)	ns
Previous immunosuppressants[c]		43 (41.7)	11 (29.7)	ns
HCQ[c]		25 (24.3)	5 (13.5)	ns
Current steroid treatment		19 (18.4)	9 (24.3)	ns
Autoantibodies[c]	Scl70	45 (43.7)	9 (24.3)	.060
	ACA	39 (37.9)	22 (59.5)	
	ANA	19 (18.4)	6 (16.2)	
Cutaneous pattern[c]	Limited	60 (58.3)	26 (70.3)	ns
	Diffuse	43 (41.7)	11 (29.7)	ns
ILD[c]	0	33 (32.0)	12 (32.4)	ns
PAH[c]	0	0 (0.0)	5 (13.9)	**<.001**
Active ischemic digital ulcers[c]	0	8 (7.8)	4 (10.8)	ns
Previous ischemic digital ulcers[c]	0	32 (31.1)	9 (24.3)	ns
Scleroderma renal crisis[c]	0	1 (1.0)	0 (0.0)	ns
Statin[c]	0	9 (8.7)	9 (24.3)	**.015**
Hypercholesterolemia[c]	0	39 (37.9)	11 (29.7)	ns
Blood hypertension[c]	0	14. (13.6)	8 (21.6)	ns
Diabetes mellitus[c]	0	3 (2.9)	4 (10.8)	.059

Abbreviations: ACA, anticentromere antibodies; ANA, antinuclear antibodies; BMI, body mass index; CDK-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRP, C-reactive protein; DLCO, diffusion lung for carbon monoxide; eGFR, estimated glomerular filtration rate; ESC, European Society of Cardiology; ESR, erythrocyte sedimentation rate; FVC, forced vital capacity; HCQ, hydroxychloroquine; HDL, high density lipoproteins; ILD, interstitial lung disease; IMT, intima-media thickness; LDL, low density lipoprotein; mRSS, modified Rodnan skin score; PAH, pulmonary artery hypertension; RCS, Raynaud's condition score; VA, alveolar volume; VAS, visual analog scale.

[a]Values expressed as mean (SD).

[b]Values expressed as median (interquartile range).

[c]Values expressed as absolute number (%).

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

significance). Indeed, both our group and other authors previously reported a positive association between necrosis at LL extremities and concomitant peripheral artery disease[30,31]; together these data suggest that a concomitant micro- and macrovascular involvement may favor the development of ischemic complications in SSc. To the best of our knowledge, the link between reduced FVC and ulnar plaques is a finding not previously reported. A possible explanation is that the fibrotic process may be also involved in ulnar artery involvement.

Interesting is the result that patients with early disease do not seem to have a lower risk of UL and proximal LL plaques than patients with a longer disease, although they are at a significantly younger age. We think more studies are needed to confirm this data that may further shed a light upon a possible role of SSc itself in causing macrovascular involvement.

We have confirmed that carotid atherosclerosis increases the risk of LL involvement. In particular, a cIMT > 0.09 gives a more than 2.6-fold increased risk of having plaques at the LLs. Given the high prevalence of macrovasculopathy in SSc patients, these data further stress the importance of performing a wide evaluation involving not only a carotid DUS but also of other sites.

Finally, ESC was found to fairly predict subclinical atherosclerosis with AUC of 0.646. This result differs from that reported by Ozen et al[19] who showed a poor performance of different cardiovascular

TABLE 6 Previous studies on macrovascular involvement in SSc

	No. patients	Age, y	Disease duration, y	No. controls	Results
Nordin et al, 2013[9]	111	62 ± 12	9.4 (5.6-17.4)	105 (healthy)	Carotid plaques: 48% vs 41% (*P* n.s.) Comparable IMT and ABPI Ischemic cardiopathy: 12% vs 4% (*P* = .03) Peripheral arteriopathy: 8% vs 1% (*P* = .02)
Frerix et al, 2012[11]	90	57.7 ± 14.4	7.1 ± 8	100 (SLE)	65.5% vs 49%: carotid or lower limbs plaques Comparable IMT
Schiopu et al, 2014[18]	46	48.6 ± 13.3	6.5 ± 5.2	46 (Healthy)	Carotid plaques: 45.6% vs 19.5% (*P* = .01) Comparable IMT
Ho et al, 2000[7]	54	57 (range: 31-82)	4 (range: 0.5-26)	43 (healthy)	Carotid plaques: 64% vs 35% (*P* = .007) Peripheral arteriopathy: 17% vs 0% (*P* = .003)
Bartoli et al, 2007[8]	53		8.8	53 (healthy)	IMT: 0.85 mm vs 0.68 mm (*P* < .03) ABI: 1.018 vs 1.091 (*P* > .3)
Ozen et al, 2016[19]	110	50.5 ± 11.9	6.8 ± 6.3	110 (RA) 51 (healthy)	Carotid plaques: 11.8% (SSc), 14.5% (RA), 2% (healthy) IMT: 0.68 ± 0.15 mm (SSc), 0.66 ± 0.14 mm (RA), 0.61 ± 0.10 mm (healthy)

Abbreviations: ABI, ankle-brachial index; ABPI, ankle-brachial pressure index; IMT, intima-media thickness; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSC, systemic sclerosis.

risk scores in SSc patients. This difference may be explained by the significantly lower cIMT and prevalence of carotid plaques in their cohort as compared to our population.

Given that atherosclerosis is quite common also in SSc patients and that carotid DUS is a good screening tool, since cIMT correlates well with both ESC score and macrovasculopathy in other sites, our study further stresses that a thorough cardiovascular screening of SSc patients may be effective and very important in particular if we consider that peripheral atherosclerosis is a well-known risk factor for cardiovascular events in the general population and statins and aspirin are recommended in its treatment.[32] In addition, cardiovascular events accounted for about 29% of non-SSc-related deaths in the large EUSTAR cohort,[33] so its prevention is an issue also in SSc.

In conclusion, this study shows that macrovascular involvement is quite common in SSc patients and that, apart from a possible role of traditional risk factors, some disease characteristics are significantly associated with atherosclerotic plaques. In addition, we further underline the importance of screening for macrovascular involvement at LLs in those SSc patients with an increased cIMT. Finally, ESC score was found to have a fair performance in predicting subclinical atherosclerosis also in SSc patients. Our study suggests that a complete evaluation of patients is mandatory for rheumatologists for a comprehensive approach to this disease, that is still without a specific treatment, and a multidisciplinary and tailored therapy may allow longer survival.

AUTHORS' CONTRIBUTION

The authors meet all 4 International Committee of Medical Journal Editors criteria for authorship. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by C. Caimmi, S. De Marchi, SL Bosello, A. Di Giorgio, A. Spinella, G. Astorino, G. Canestrari, E. Cocchiara. The first draft of the manuscript was written by C. Caimmi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Solomon JJ, Olson AL, Fischer A, et al. Scleroderma lung disease. *Eur Respir Rev*. 2013;22:6-19.
2. Condliffe R, Howard LS. Connective tissue disease – associated pulmonary arterial hypertension. *F1000Prime Rep*. 2015;7:06.
3. Au K, Singh MK, Bodukam V, et al. Atherosclerosis in systemic sclerosis: a systematic review and meta-analysis. *Arthritis Rheum*. 2011;63:2078-2090.
4. van Sijl AM, Peters MJ, Knol DK, et al. Carotid intima media thickness in rheumatoid arthritis as compared to control subjects: a meta-analysis. *Semin Arthritis Rheum*. 2011;40:389-397.
5. Brohall G, Odén A, Fagerberg B. Carotid artery intima-media thickness in patients with Type 2 diabetes mellitus and impaired glucose tolerance: a systematic review. *Diabet Med*. 2006;23:609-616.
6. Masoura C, Pitsavos C, Aznaouridis K, Skoumas I, Vlachopoulos C, Stefanadis C. Arterial endothelial function and wall thickness in familial hypercholesterolemia and familial combined hyperlipidemia and the effect of statins. A systematic review and meta-analysis. *Atherosclerosis*. 2011;214:129-138.
7. Ho M, Veale D, Eastmond C, Nuki G, Belch J. Macrovascular disease and systemic sclerosis. *Ann Rheum Dis*. 2000;59:39-43.
8. Bartoli F, Angotti C, Fatini C, et al. Angiotensin – converting enzyme I/D polymorphism and macrovascular disease in systemic sclerosis. *Rheumatology*. 2007;46:772-775.

9. Nordin A, Jensen-Urstad K, Björnådal L, Pettersson S, Larsson A, Svenungsson E. Ischemical arterial events and atherosclerosis in patients with systemic sclerosis: a population based case control study. *Arthritis Res Ther*. 2013;15:R87.
10. Stafford L, Englert H, Gover J, Bertouch J. Distribution of macrovascular disease in scleroderma. *Ann Rheum Dis*. 1998;57:476-479.
11. Frerix M, Stegbauer J, Dragun D, Kreuter A, Weiner SM. Ulnar artery occlusion is predictive of digital ulcers in SSc: a duplex sonography study. *Rheumatology*. 2012;51:735-742.
12. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. *Ann Rheum Dis*. 2013;72:1747-1755.
13. LeRoy EC, Black CM, Fleischmajer R, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol*. 1988;15:202-205.
14. Akesson A, Fiori G, Krieg T, van den Hoogen FH, Seibold JR. Assessment of skin, joint, tendon and muscle involvement. *Clin Exp Rheumatol*. 2003;21:s5-s8.
15. Medsger TA, Bombardieri S, Czirjak L, Scorza R, Della Rossa A, Bencivelli W. Assessment of disease severity and prognosis. *Clin Exp Rheumatol*. 2003;21:s42-s46.
16. Piepoli MF, Hoes AW, Agewall S, et al. European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts): Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur J Prev Cardiol*. 2016;23:NP1-NP96.
17. Grant EG, Benson CB, Moneta GL, et al. Carotid artery stenosis: gray-scale and Doppler US diagnosis – society of radiologists in ultrasound consensus conference. *Radiology*. 2003;229:340-346.
18. Schiopu E, Au KM, McMahon MA, et al. Prevalence of subclinical atherosclerosis is increased in systemic sclerosis and is associated with serum proteins: a cross-sectional controlled study of carotid ultrasound. *Rheumatology*. 2014;53:704-713.
19. Ozen G, Inanc N, Unal AU, et al. Subclinical atherosclerosis in systemic sclerosis: not less frequent than rheumatoid arthritis and not detected with cardiovascular risk indices. *Arthritis Care Res*. 2016;68:1538-1546.
20. Sedky MM, Fawzy SM, El Baki NA, El Eishi NA, El Bohy AM. Systemic sclerosis: an ultrasonographic study of skin and subcutaneous tissue in relation to clinical findings. *Skin Res Technol*. 2013;19:e78-e84.
21. Ladak K, Pope JE. A review of the effects of statins in systemic sclerosis. *Semin Arthritis Rheum*. 2016;45:698-705.
22. Koenig W. Inflammation and coronary heart disease: an overview. *Cardiol Rev*. 2001;9:31-35.
23. Wan MC, Moore T, Hollis S, Herrick AL. Ankle brachial pressure index in systemic sclerosis: influence of disease subtype and anticentromere antibody. *Rheumatology*. 2001;40:1102-1105.
24. Khambhati J, Engels M, Allard-Ratick M, Sandesara PB, Quyyumi AA, Sperling L. Immunotherapy for the prevention of atherosclerotic cardiovascular disease: promise and possibilities. *Atherosclerosis*. 2018;276:1-9.
25. Hofstee HM, Voskuyl AE, Vonk noordegraaf A, et al. Pulmonary arterial hypertension in systemic sclerosis is associated with profound impairment of microvascular endothelium-dependent vasodilatation. *J Rheumatol*. 2012;39:100-105.
26. Peled N, Shitrit D, Fox BD, et al. Peripheral arterial stiffness and endothelial dysfunction in idiopathic and scleroderma associated pulmo- nary arterial hypertension. *J Rheumatol*. 2009;36:970-975.
27. Lescoat A, Coiffier G, de Carlan M, et al. Combination of capillaroscopic and ultrasonographic evaluations in systemic sclerosis: results of a cross-sectional study. *Arthritis Care Res*. 2018;70:938-943.
28. Schioppo T, Orenti A, Boracchi P, De Lucia O, Murgo A, Ingegnoli F. Evidence of macro- and micro-angiopathy in scleroderma: an integrated approach combining 22-MHz power Doppler ultrasonography and video-capillaroscopy. *Microvasc Res*. 2019;122:125-130.
29. Park JH, Sung YK, Bae SC, Song SY, Seo HS, Jun JB. Ulnar artery vasculopathy in systemic sclerosis. *Rheumatol Int*. 2009;29:1081-1086.
30. Caramaschi P, Biasi D, Caimmi C, et al. Digital amputation in systemic sclerosis: prevalence and clinical associations. A retrospective longitudinal study. *J Rheumatol*. 2012;39:1648-1653.
31. Nihtyanova SI, Brough GM, Black CM, Denton CP. Clinical burden of digital vasculopathy in limited and diffuse cutaneous systemic sclerosis. *Ann Rheum Dis*. 2008;67:120-123.
32. Stone NJ, Robinson JG, Lichtenstein AH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2014;63:2889-2934.
33. Tyndall AJ, Bannert B, Vonk M, et al. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. *Ann Rheum Dis*. 2010;69:1809-1815.

How to cite this article: Caimmi C, De Marchi S, Bosello SL, et al. Ultrasonography involvement of carotid, upper and lower limb arteries in a large cohort of systemic sclerosis patients. *Int J Rheum Dis*. 2020;23:681–692. https://doi.org/10.1111/1756-185X.13824

Received: 26 March 2020 | Revised: 3 April 2020 | Accepted: 3 April 2020

DOI: 10.1111/1756-185X.13845

EXPERT COMMENTS

International Journal of Rheumatic Diseases | WILEY

Recent advances in pediatric rheumatology: October to December 2019

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1 | ADENOSINE DEAMINASE 2 AS A BIOMARKER OF MACROPHAGE ACTIVATION SYNDROME IN SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS

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Ann Rheum Dis 2019 Nov 9. pii: annrheumdis-2019-216030.

Macrophage activation syndrome (MAS) is a serious complication which is seen in several rheumatological illnesses in children and especially in children with systemic juvenile idiopathic arthritis (sJIA). Early diagnosis and prompt initiation of therapy remain the cornerstones of management. Lee et al. conducted a multicentric prospective study to evaluate adenosine deaminase 2 (ADA2) as a novel biomarker for MAS. The authors established normal range for blood ADA2 levels in 324 individuals (174 children and 150 adults) and compared with ADA2 levels measured in various pediatric inflammatory conditions: Kawasaki disease, n = 25; juvenile idiopathic arthritis, n = 63; oligoarthritis/polyarthritis, n = 19; enthesitis-related arthritis/psoriatic arthritis, n = 14; sJIA, n = 20; sJIA with MAS, n = 10; pediatric systemic lupus erythematosus, n = 14; juvenile dermatomyositis, n = 13. This study revealed that children with sJIA complicated by MAS had increased levels of ADA2. Further it was found that sensitivity and specificity of discriminating MAS and disease activity in sJIA was 86% and 94% respectively. ADA2 levels correlated with levels of ferritin, serum interleukin-18, chemokine (CXCL9) and lactate dehydrogenase but not with C-reactive protein and erythrocyte sedimentation rates. However, the precise role of ADA2 in MAS remains unclear. This study has implications for clinical management of patients with sJIA.

2 | SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS–ASSOCIATED LUNG DISEASE: CHARACTERIZATION AND RISK FACTORS

Grant S. Schulert, Shima Yasin, Brenna Carey, Claudia Chalk, Thuy Do, Andrew H. Schapiro et al.

Arthritis Rheum 71(11); November 2019: 1943–1954. https://doi.org/10.1002/art.41073

sJIA is a common inflammatory disease of childhood. However, there is paucity of literature on sJIA-associated lung disease. Schulert et al. have conducted a prospective study in 18 patients with sJIA and chronic lung disease and found that this subset of patients showed increased levels of IL-18 in serum. The authors report that risk factors for development of lung disease include age <2 years at disease onset (odds ratio [OR] 6.5), recurrent MAS (OR 14.5) and adverse reactions to tocilizumab (OR 13.6). Striking findings on computed tomography scan of the chest include septal and pleural thickening, tree-in-bud appearance and ground glass opacities with peripheral consolidation. Seventeen of the 18 patients studied had received tocilizumab for treatment of sJIA. It is conjectural whether exposure to tocilizumab has a role in development of lung disease in this condition. This is an important study in as much as it suggests that lung involvement in children with sJIA may be a silent cause of morbidity and may be more common than hitherto believed.

3 | INTRAVENOUS DOSING OF TOCILIZUMAB IN PATIENTS YOUNGER THAN TWO YEARS OF AGE WITH SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS:

RESULTS FROM AN OPEN-LABEL PHASE 1 CLINICAL TRIAL

Navita L. Mallalieu, Sunethra Wimalasundera, Joy C. Hsu, Wendy Douglass, Chris Wells, Inmaculada Calvo Penades et al.

Pediatr Rheumatol Online J 2019 Aug 22; 17(1): 57. https://doi.org/10.1186/s12969-019-0364-z.

Tocilizumab (TCZ) is a humanized monoclonal antibody that is directed against IL-6, one of the key cytokines involved in etiopathogenesis of sJIA. It is approved for use in children with sJIA older than 2 years. Mallalieu et al. report this multicenter single-arm study in 11 children with sJIA aged below 2 years. The authors evaluated outcome measures after completion of 12 weeks of therapy with TCZ at a dose of 12 mg/kg/dose every 2 weeks. The pharmacokinetics, efficacy and safety of TCZ were compared with the previously published TENDER study that had included children between 2-17 years (control group). The authors report that pharmacokinetics between study and control groups (blood level before the next dose: 39.8 [±14.3] vs. 57.5 [±23.3] μg/mL and maximum concentration post-dose: 288 [±40.4] vs. 245 [±57.2] μg/mL) were comparable. However, the incidence of serious hypersensitivity reactions was higher in study subjects than in controls (27.3% vs. 2.6%). This study has evaluated the efficacy and safety of TCZ in children with sJIA below the age of 2 for the first time.

4 | ADALIMUMAB IN JUVENILE IDIOPATHIC ARTHRITIS–ASSOCIATED UVEITIS: 5-YEAR FOLLOW-UP OF THE BRISTOL PARTICIPANTS OF THE SYCAMORE TRIAL

Sarah Horton, Ashley P. Jones, Catherine M. Guly, Ben Hardwick, Michael W. Beresford, Richard W. Lee et al.

Am J Ophthalmol 2019 Nov; 207: 170-174. https://doi.org/10.1016/j.ajo.2019.06.007.

Adalimumab (ADA) has been found to be useful in refractory non-infectious uveitis, especially when associated with juvenile idiopathic arthritis (JIA-U). However, there is paucity of information on long-term outcomes in children with JIA-U on adalimumab. In this single-center retrospective study, Horton et al. followed up 28 children with JIA-U who had participated in the SYCAMORE multicenter randomized controlled study for 5 years from the day of randomization. Out of 28 children who had participated in the trial, 12 were in the treatment arm and had received ADA for a period of 18 months. The authors report that 11 of these 12 children (92%) in the treatment arm had to be restarted on ADA due to flare (median duration for flare was 188 days; range 42-413 days). This study shows that the required duration of therapy with ADA in JIA-U is still not clear.

5 | EFFECTIVENESS OF LONG-TERM INFLIXIMAB USE AND IMPACT OF TREATMENT ADHERENCE ON DISEASE CONTROL IN REFRACTORY, NON-INFECTIOUS PEDIATRIC UVEITIS

Virginia Miraldi Utz, Sabrina Bulas, Sarah Lopper, Matthew Fenchel, Ting Sa, Mitul Mehta et al.

Pediatr Rheumatol Online J 2019 Nov 29; 17(1): 79. https://doi.org/10.1186/s12969-019-0383-9.

Non-infectious uveitis (NIU) is a significant reason for visual morbidity in children. Timely diagnosis and effective management are important determinants of visual outcome. Several tumor necrosis factor-α inhibitors have been used with promising results. In this single-center retrospective study, Utz et al. followed 27 children with refractive NIU. Eleven among these had rheumatoid factor negative polyarthritis, 3 had extended oligoarthritis and 1 each had enthesitis-related arthritis, psoriatic arthritis and undifferentiated arthritis. These patients had been on long-term infliximab (IFX) median duration 35 months (range 9–128). Authors report that odds of having disease control with IFX is 4.1-fold higher than children who had not received this agent. Further it was found that children with incomplete treatment adherence had 10.3-fold greater odds of having disease activity when compared to children with full adherence. It appears that long-term IFX is effective in controlling refractory NIU. However, as the numbers are small, the results need to be interpreted with caution.

6 | HIGH-DOSE INTRAVENOUS METHYLPREDNISOLONE IN JUVENILE NON-INFECTIOUS UVEITIS: A RETROSPECTIVE ANALYSIS

Anja Schnabel, Elisabeth Unger, Normi Brück, Reinhard Berner, Ursula Range, Annette Holl-Wieden et al.

Clin Immunol 2019 Dec 18; 211: 108327. https://doi.org/10.1016/j.clim.2019.108327

At present there is lack of consensus on management of NIU in children. Systemic corticosteroids are commonly used as bridge therapy to induce remission when conventional topical therapy fails to achieve remission. In this single-center retrospective study, Schnabel et al. evaluated the effectiveness of systemic corticosteroids in 56 children (93 eyes with uveitis) with active NIU who failed conventional topical steroid therapy. Children were administered intravenous methylprednisolone (IVMP) (10-30 mg/kg/d for 1-5 days) and therapy was repeated at monthly intervals. It was found that IVMP is effective in improving visual outcomes at 3 months ($P < .005$) and at 6 months ($P < .005$). The authors compared patients who had received 1 course of IVMP with children who had received 3-5 such courses. It was found that children who received 3-5 courses had fewer relapses, and required subsequent treatment

with biologic disease-modifying anti-rheumatic drugs less frequently (39% vs. 19%; *P* = .174). Larger, and multicenter randomized, studies are required to confirm these findings.

7 | PATTERNS OF B CELL REPLETION FOLLOWING RITUXIMAB THERAPY IN A PEDIATRIC RHEUMATOLOGY COHORT

Chace Mitchell, Courtney B. Crayne, Randy Q. Cron

ACR Open Rheumatol 2019 Aug 27; 1(8): 527-532. https://doi.org/10.1002/acr2.11074.

Rituximab (RTX) is a chimeric mouse-human monoclonal antibody for B cell CD20 receptors and has been used in several refractory autoimmune illnesses in children as well as adults. Depletion of B cells results in clinical improvement but the duration of depletion shows inter-individual variability. There is paucity of literature on B cell repletion following RTX therapy in children. Mitchell et al. conducted this single-center retrospective study in 112 children to evaluate the association between disease type (connective tissue disease, n = 74; vasculitis, n = 15; dermatomyositis, n = 11), number of doses of RTX and concurrent immunosuppression with B cell repopulation (CD19 levels) at 6 and 12 months post-therapy. The authors report that B cell depletion was not associated with disease type (*P* values at 6 and 12-months post-therapy were .49 and .596 respectively) or number of doses of RTX. Further, it also was found that B cell depletion was not associated with concurrent administration of other immunosuppressive therapy (*P* values at 6 and 12-months post-therapy were .091 and .087 with cyclophosphamide; .374 and .227 with mycophenolate mofetil; and .438 and .16 with methotrexate). This study shows that RTX was well tolerated in children. Given the unpredictable nature of B cell repopulation following RTX therapy as shown in this study, the physician should closely monitor B cell subsets for treatment response.

8 | THE ROLE OF AGE-SPECIFIC N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE CUT-OFF VALUES IN PREDICTING INTRAVENOUS IMMUNOGLOBULIN RESISTANCE IN KAWASAKI DISEASE: A PROSPECTIVE COHORT STUDY

Shuran Shao, Chunyan Luo, Kaiyu Zhou, Yimin Hua, Mei Wu, Lei Liu et al.

Pediatr Rheumatol Online J 2019 Sep 18; 17(1): 65. https://doi.org/10.1186/s12969-019-0368-8.

Kawasaki disease (KD) is the most common pediatric vasculitis and is characterized by development of coronary artery abnormalities in 15%-25% of children who do not receive intravenous immunoglobulin (IVIg). Approximately 5%-10% of patients show IVIg resistance and may not respond to the first dose of IVIg. Shao et al. reported a single-center prospective cohort study in 393 patients with KD and evaluated age-specific N-terminal pro-brain natriuretic peptide (Pro-BNP) cut-off values in predicting IVIg resistance. Pro-BNP is significantly elevated in the IVIg resistant group and the cut-off values for predicting IVIg resistance in all age groups was 3755 pg/mL (sensitivity 44.4%; specificity 84.1%). The cut-off value for children below 1 was 2480 pg/mL (sensitivity 75%; specificity 71.8%) while that for children aged 1-2 years was 2800 pg/mL (sensitivity 50%; specificity 77.9%). For children aged 2-6 years the cut-off was 3710 pg/mL (sensitivity 52.2 %; specificity 86.3%). This is a landmark study as it is for the first time that age-specific cut-off levels of Pro-BNP have been used for predicting IVIg resistance. The findings have important clinical implications.

9 | PREDICTIVE VALUE OF SERUM PROCALCITONIN FOR BOTH INITIAL AND REPEATED IMMUNOGLOBULIN RESISTANCE IN KAWASAKI DISEASE: A PROSPECTIVE COHORT STUDY

Shuran Shao, Chunyan Luo, Kaiyu Zhou, Yimin Hua, Mei Wu, Lei Liu et al.

Pediatr Rheumatol Online J 2019 Nov 27; 17(1): 78. https://doi.org/10.1186/s12969-019-0379-5

IVIg is the standard of care in managing children with KD. However, 10%-20% of individuals with KD may not respond to the initial dose of IVIg, thereby increasing the odds of developing coronary artery abnormalities. At present, there is no validated clinical or laboratory measure for predicting IVIg resistance. Shao et al. conducted this single-center prospective cohort study in 530 children with KD, to evaluate serum procalcitonin (PCT) in predicting initial and repeated IVIg resistance. Levels of serum PCT were significantly raised in individuals who had initial and repeated IVIg resistance when compared with IVIg responders. Serum PCT cut-off level of 1.48 ng/mL for initial IVIg resistance showed sensitivity and specificity of 53.9% and 71.8% respectively; a cut-off value of 2.88 ng/mL for repeated IVIg resistance showed sensitivity and specificity of 51.4% and 73.2% respectively. However, on multiple logistic regression analysis, levels of serum PCT were not found to be a reliable independent predictive factor of initial (*P* = .986) or repeated IVIg resistance (*P* = .751).

10 | SYSTEMIC ARTERY ANEURYSMS AND KAWASAKI DISEASE

Qu-ming Zhao, Chen Chu, Lin Wu, Xue-cun Liang, Shu-na Sun, Lan He et al.

Pediatrics 144(6); December 2019: e20192254

There is paucity of literature on development of systemic artery aneurysms (SAAs) in KD. In this prospective study, Zhao et al. screened for SAAs among 162 of 1148 children with KD who had the following risk factors: giant coronary artery aneurysms (CAAs);

coronary artery lesions that continued to progress but fell short of defining criteria for giant CAAs; KD that was refractory to 2 doses of intravenous immunoglobulin. Screening was carried out using peripheral imaging. The authors report that 23 of 162 children (14.2%) with CAAs in the cohort also had SAAs. Further, all individuals with SAAs had concurrent CAAs >8 *z* score. Risk factors for development of SAAs included younger age and longer duration of illness. In this study Zhao et al. have shown that SAAs may not be uncommon in children with KD. This is a new finding.

11 | SHORT-TERM FOLLOW-UP RESULTS OF CHILDREN WITH FAMILIAL MEDITERRANEAN FEVER AFTER CESSATION OF COLCHICINE: IS IT POSSIBLE TO QUIT?

Ayse Tanatar, Serife Gul Karadag, Hafize Emine Sonmez, Mustafa Cakan, Nuray Aktay Ayaz

Rheumatology (Oxford) 2019 Oct 1; 58(10): 1818-1821. https://doi.org/10.1093/rheumatology/kez156.

Colchicine is the mainstay of most treatment protocols for familial Mediterranean fever (FMF). However, there is limited published literature on the required duration of colchicine therapy in FMF. Tanatar et al. conducted this single-center retrospective study on 64 patients with FMF in whom colchicine was discontinued (median duration of intake was 18.2 months, range 6-148), either by physician or patient/parent. On median follow-up of 37.4 months (6.4-154.7), colchicine had to be restarted in 23/64 individuals. The authors report that younger age and shorter duration of therapy with colchicine are risk factors for restarting colchicine in children in whom it has been discontinued. This study has therapeutic implications for the pediatric rheumatologist.

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How to cite this article: Sudhakar M, Sathish Kumar L, Singh S. Recent advances in pediatric rheumatology: October to December 2019. *Int J Rheum Dis*. 2020;23:693–696. https://doi.org/10.1111/1756-185X.13845

Received: 7 January 2020 | Revised: 9 March 2020 | Accepted: 16 March 2020

DOI: 10.1111/1756-185X.13836

APLAR GRAND ROUND CASE

International Journal of Rheumatic Diseases | WILEY

Multiple jeopardy: Diagnostic and therapeutic challenges in vasculitic flare

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Abstract

A 57-year old gentleman had presented a year back with inflammatory oligoarthritis and vasculitic neuropathy, diagnosed as unclassifiable vasculitis, initiated on oral corticosteroids and intravenous cyclophosphamide (monthly X 6). His disease stabilized and he had been maintained on azathioprine, which had to be stopped due to acute pancreatitis with subsequent pseudocyst formation, requiring percutaneous drainage suspecting infection. Within a week of pseudocyst drainage, he developed sudden onset pain in left upper limb, with absent left upper limb pulses, loss of motor function of left hand, myocardial ischemia, and extensive thrombosis of the left upper limb arteries. Neuropathy in the left upper limb was either vasculitic, or ischemic due to arterial thrombosis. However, multifocal thrombosis suggested an ongoing vasculitic flare. In view of possible infected pancreatic pseudocyst, intravenous methylprednisolone pulse was contra-indicated. Hence, he was offered intravenous immunoglobulin (IVIG) therapy, despite the risk of potentially worsening the prevalent prothrombotic state. On the second day of IVIG, he developed transiently tingling and weakness of right hand with vasculitic rashes, which subsequently resolved, reaffirming the suspicion of vasculitic flare. After completing IVIG therapy, the weakness in his left hand had markedly improved. His myocardial ischemia had also recovered, with a repeat echocardiography showing normalization of prior left ventricular hypokinesia. In the intervening period, the pseudocysts were drained, following which he was initiated on rituximab. This case highlights numerous challenges in the initial diagnosis, distinguishing vasculitic from ischemic neuropathy, and the management of vasculitic flare during infection.

KEYWORDS

classification, classification criteria, diagnostic criteria, intravenous immunoglobulins, mononeuritis multiplex, vasculitis

1 | INTRODUCTION

Vasculitis can sometimes be difficult to diagnose and classify. Also, the treatment of active vasculitis co-existent with systemic infection is challenging. We present a gentleman with vasculitic flare, with mononeuritis multiplex and extensive arterial thrombosis, coexistent with an infected pseudocyst of the pancreas, with a stormy clinical course, ultimately responding to intravenous immunoglobulin therapy.

2 | CASE REPORT

A 57-year old gentleman presented to us in August 2016 with inflammatory oligoarthritis of both knee joints for the past two years, mononeuritis multiplex with bilateral foot drop, and inability to make a fist in the right hand, associated with palpable purpura, over the past 15 days. There was no history of fever, weight loss or other constitutional symptoms, skin nodules, cough, shortness of breath, hemoptysis, headache, nasal discharge or crusting, facial puffiness, pedal edema, oral or genital ulcers. His past history was relevant for chronic alcohol usage and smoking. Examination revealed a blood pressure of 120/80 mm Hg, palpable purpura over legs, bilateral common peroneal nerve involvement with right median and ulnar neuropathy; other systems were unremarkable. Erythrocyte sedimentation rate (42 mm/hr) and serum C-reactive protein (138 mg/L) were elevated, blood counts revealed leucocytosis (13 600/mm^3, 80% neutrophils), normal platelet count (305 000/mm^3), urinalysis revealed no proteinuria or active sediments, antinuclear antibodies and anti-neutrophil cytoplasmic antibodies (ANCA) by immunofluorescence, anti-proteinase 3 ANCA (PR3-ANCA) and anti-myeloperoxidase ANCA (MPO-ANCA) were negative. Rheumatoid factor (>728 iu) and anti-citrullinated peptide antibodies (ACPA) (>300 units), complement components C3 (201 mg/dL) and C4 (43.1 mg/dL) were elevated. While he could have been classified as rheumatoid arthritis[1] with rheumatoid vasculitis,[2] the pattern of joint involvement with knee oligoarthritis as the lone articular manifestation did not favour a clinical diagnosis of rheumatoid arthritis. He did not fulfil classification criteria or case definitions for polyarteritis nodosa,[3] granulomatosis with polyangiitis,[4,5] microscopic polyangiitis[5] or eosinophilic granulomatosis with polyangiitis,[5] or leprosy.[6] Cryoglobulins and cryofibrinogens were not detected. Serology for hepatitis B and C viruses were negative. Nerve conduction study confirmed axonal neuropathy,a sural nerve biopsy revealed features consistent with vasculitic neuropathy. Since systemic small and medium vessel vasculitis was not in doubt, although he did not fulfill criteria for any particular form of systemic vasculitis (thereby, unclassifiable vasculitis),[7] he was started on monthly cyclophosphamide 900 mg along with corticosteroids in tapering doses. With this, he had significant improvement, with arrest of the neuropathy and disappearance of purpuric rash. After completing six doses of monthly cyclophosphamide, remission was maintained with daily azathioprine. In July 2017, he developed acute pancreatitis (probably related to either alcohol or azathioprine), with formation of two pseudopancreatic cysts (Figure 1). Azathioprine was stopped, and he was continued on low dose corticosteroid alone. One of the pseudocysts had fluid collection with internal echoes, therefore, he underwent a percutaneous drainage procedure in mid-October 2017, with a further drainage procedure planned. At the time of drainage of the pseudocyst, the gentleman was independently functional for his daily activities and mobile, therefore, no prophylactic anticoagulation was warranted during the procedure.

A week after undergoing the aspiration of the pancreatic pseudocyst, he presented with acute onset pain and numbness in the entire left upper limb and in the chest for one day. Examination revealed absent pulses in the left upper limb. He did not have any movement in his left hand, with complete loss of function in the

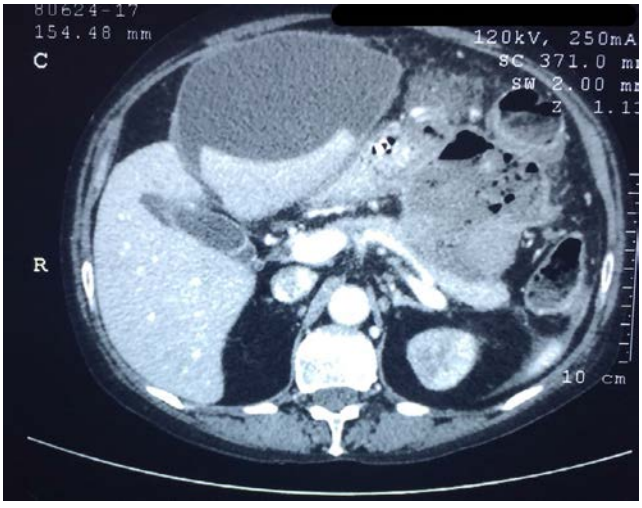

FIGURE 1 Well circumscribed, hypodense collection indenting the left lobe of the liver. Another irregular collection arising from the body of pancreas and lesser lac with some foci of air within, representing necrosis within the pseudocyst

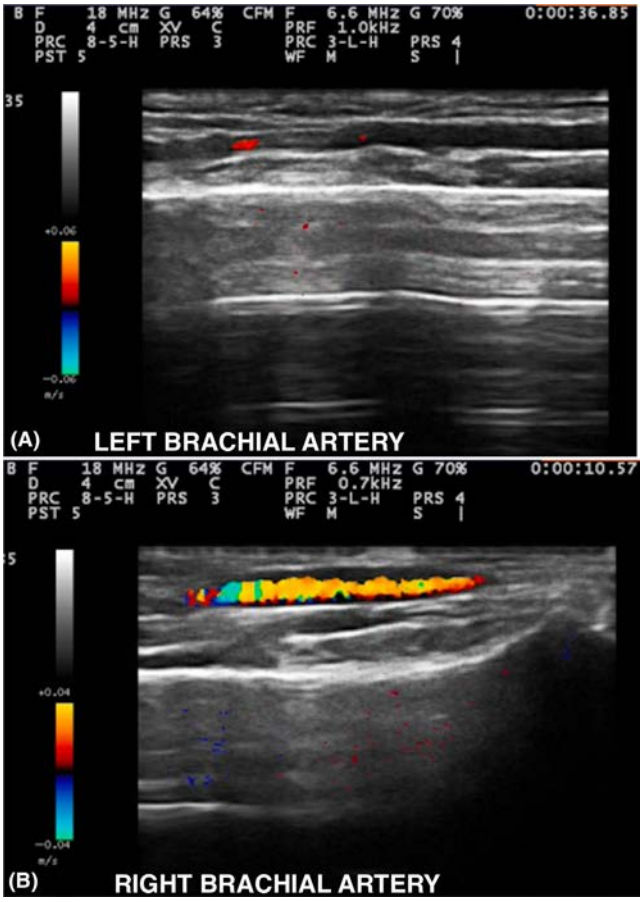

FIGURE 2 Colour Doppler Ultrasound of upper limb vessels showing almost complete absence of flow in left brachial artery (A), with normal flow in the right brachial artery (B)

motor distribution of median and ulnar nerves. Left wrist dorsiflexion was weak, suggesting left radial nerve involvement also. Blood counts revealed leucocytosis (26 500/mm^3, 85% neutrophils) and thrombocytosis (577 000/mm^3). Doppler ultrasound of the left upper limb revealed absent flow in the left axillary, brachial, and radial arteries with extensive thrombosis (Figure 2). An electrocardiogram revealed left anterior hemiblock,echocardiogram showed akinesia of the distal interventricular septum and the anterior wall of the left ventricle, with diminished ejection fraction of 40%, consistent with myocardial ischemia. Antiphospholipid antibodies were not tested as the clinical picture, despite the vascular thromboses, with acute-onset neuropathy, leucocytosis and thrombocytosis, did not favour antiphospholipid antibody syndrome. He had no fever or other constitutional symptoms or skin rash at that time. The loss of function in the left hand could have been due to either ischemia due to arterial occlusion, or relapse of vasculitis neuropathy. However, since multifocal arterial thrombosis can occur in variable vessel vasculitis like Behcet's disease,[8] and small-vessel vasculitis also predisposes to vascular thromboses,[9,10] we were of the opinion that this might have been due to endothelial injury from vasculitic flare. He required escalation of immunosuppression, and, in view of active vasculitis, pulsed intravenous methylprednisolone was considered, but was not feasible due to possible infected pseudocyst of the pancreas. Therefore, intravenous immunoglobulin (IVIG) 2 g/kg over 5 days was instituted. Low molecular weight heparin in therapeutic dose was initiated considering possible myocardial infarction. On the second day of hospitalization, while continuing IVIG, he transiently developed purpuric rashes on the right hand with tingling and numbness of the right hand, which disappeared over a few hours. While transient cutaneous rash could possibly have been due to IVIG therapy, the concomitant neuropathic features reaffirmed our suspicion of a vasculitic flare, and IVIG was continued. His further hospital course was complicated by lower gastrointestinal bleed requiring multiple transfusions of packed red blood cells, the cause of which was identified to be diverticulitis; this, however, necessitated stopping heparin. He was discharged two weeks after admission, by which time the motor function in his left hand had recovered to the extent that he was able to make a fist with 50% handgrip power. A repeat echocardiogram showed normalization of the previously documented regional wall motion abnormality, in spite of having stopped anticoagulation, thereby suggesting that the prior myocardial ischemia was part of the vasculitic flare. A definitive drainage procedure was performed for the pancreatic pseudocyst, and four weeks after the initial admission with vasculitic flare, he was initiated on rituximab (1 gram intravenous at 01 and 15 days). Three months after the flare of vasculitis, in January

TABLE 1 Differential diagnosis at initial presentation

Diagnosis	In favour	Against
Rheumatoid vasculitis[2]	• Mononeuritis multiplex • Arthritis • Sural nerve biopsy suggested vasculitic neuropathy • Positive for RF and ACPA	• He could have been classified as rheumatoid arthritis.[1] However, since making a clinical diagnosis of rheumatoid arthritis was debatable based on oligoarthritis of knees being the only joints involved, this diagnosis was not considered tenable
Granulomatosis with polyangiitis[4,5]	• Mononeuritis multiplex	• Absence of airway involvement, lung nodules, purpura[a], renal involvement • ANCA negative
Microscopic polyangiitis[5]	• Mononeuritis multiplex	• Absence of lung lesions, purpura[a], renal involvement • ANCA negative
Eosinophilic granulomatosis with polyangiitis[5]	• Mononeuritis multiplex	• Absence of asthma, eosinophilia, lung lesions
Polyarteritis nodosa[3]	• Mononeuritis multiplex • Sural nerve biopsy suggested vasculitic neuropathy	• Absence of constitutional features, hypertension, deranged renal function. • Negative serology for HBV and HCV
Leprosy[6]	• Mononeuritis multiplex • Oligoarthritis	• Absence of cutaneous features of leprosy • Neither slit skin smear, not the sural nerve biopsy demonstrated *Mycobacterium leprae*
Unclassifiable vasculitis[7]	• Mononeuritis multiplex • Arthritis • Sural nerve biopsy suggested vasculitic neuropathy • Not able to classify the presentation into any of the classification categories for vasculitis	None

Abbreviations: ACPA, Anti-citrullinated peptide antibody; ANCA, Anti-neutrophil cytoplasmic antibody; HBV, Hepatitis B virus; HCV, Hepatitis C virus; RF, Rheumatoid factor.

[a]Palpable purpura and vascular thromboses occurred at the time of vasculitic relapse 1 year 3 months later, and were not present at the initial admission.

2018, he continued to be well. Motor examination of the left hand revealed complete recovery of wrist dorsiflexion with more than 50% recovery of muscle power in the left median and ulnar nerve distribution. He was continued on intravenous rituximab 500 mg every 6 months. At the last follow-up visit in December 2019 (2 years 2 months after the last vasculitic flare, he continued to be well, with no recurrence or progression of neuropathy, other vasculitic symptoms, or arthritis.

3 | DISCUSSION

The management of this gentleman presented numerous challenges, viz. the primary diagnosis, management of vasculitic flare in the presence of infected pseudocyst and the use of IVIG in the presence of vascular thrombosis. We shall discuss each of these points separately.

Our patient had presented with inflammatory oligoarthritis, palpable purpura and vasculitic neuropathy. Although he had a small and medium vessel vasculitis, as discussed in Table 1, he did not fulfill classification criteria for any of the described forms of vasculitis (unclassifiable vasculitis).[7] A debatable point was whether the patient, who fulfilled classification criteria for rheumatoid arthritis,[1] could be labelled as having rheumatoid vasculitis. Elevated acute phase reactants at the first presentation could very well have been due to vasculitis, rather than due to the inflamed joints. Also, the classification criteria for rheumatoid arthritis particularly state the need to consider and exclude relevant differential diagnoses before choosing to utilize the said criteria to classify a patient.[1] Although rheumatoid vasculitis is generally a late manifestation associated with rheumatoid arthritis, occasional cases have been described early in the disease course.[11] Herein we would like to reiterate that classification criteria and nomenclature, such as those proposed by the Chapel Hill Consensus Conference for vasculitis and the American College of Rheumatology with the European League Against Rheumatism (EULAR) for rheumatoid arthritis, are meant for inclusion of a uniform population of patients in research studies.[12] These criteria are not meant for clinical diagnosis in day-to-day practice, since many such patients would not necessarily fulfill these criteria. Moreover, such classification criteria are meant to be applied once a clinical diagnosis has been made and inclusion of a patient in a study is being considered, rather than being inappropriately used to make clinical diagnosis.[12] In fact, misclassification of patients is recognized when classification criteria are used indiscriminately.[13] It is particularly important for the young Rheumatologist not to get overwhelmed by whether a patient fulfils criteria for a disease or not, and rather focus on the predominant manifestations that require treatment, in this instance, the vasculitic neuropathy and arterial thrombosis. This also brings in another debate that we had to consider, ie, whether the loss of function in the left hand was due to vasculitic neuropathy, or a result of the ischemia of the upper limb due to arterial occlusion. While there is no definitive way to quickly prove or disprove either hypothesis, considering the urgency of ongoing, potentially irreversible nerve damage, we decided to treat him with IVIG for the vasculitic flare, as well as with heparin. The subsequent appearance of skin purpura, and the recovery of neuropathy and cardiac ischemia, despite stopping heparin due to lower gastrointestinal bleed, retrospectively confirmed the vasculitic flare.

The gentleman presented with left upper limb arterial thrombosis. Active vasculitis can predispose to development of arterial and well as venous thromboses, classically in Behcet's disease, but also in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and large vessel vasculitides.[14] A recent meta-analysis showed patients with AAV to have an increased risk of myocardial infarction and stroke.[15] Another retrospective analysis of observational data from European vasculitis trials revealed that nearly a seventh of patients with granulomatosis with polyangiitis (GPA) or microscopic polyangiitis (MPA) developed a cardiovascular event within the first five years of disease.[16] Another report of 504 patients from a Chinese cohort of AAV revealed that, both traditional cardiovascular risk factors as well as higher disease activity at diagnosis predicted cardiovascular events in AAV.[17] The vasculitic nature of the arterial thromboses in the upper limb and, probably, the coronary circulation was likely in hindsight due to the response to IVIG (inspite of stopping heparin).

Another conundrum faced by us was the management of vasculitic flare in the presence of a potentially infected pseudocyst. This precluded the use of intravenous methylprednisolone pulses as first-line therapy, due to fear of exacerbation of infection.[18] In such instances, intravenous immunoglobulin (IVIG) was an option, since it shares a number of immunomodulatory functions with corticosteroids, without the associated increased infection risk.[19] A matter of concern was the presence of extensive arterial thrombosis, since IVIG can, per se, increase the risk of thrombosis.[20] However, since we felt that the thrombosis was due to endothelial injury as a consequence of active vasculitis, we decided to embark on administering IVIG after discussing the risks and potential benefits with the patient and family. Although evidence for the use of IVIG in active vasculitis is scant, a recent systematic review concluded evidence for benefit with IVIG in active AAV, with reductions in disease activity evident by 2 weeks of treatment,[21] as was also seen in our patient. IVIG may also be used as an adjunctive therapy in AAV when remission of disease cannot be attained despite standard therapeutic regimens.[22] Plasma exchange was another option which could have been utilized in our patient. The EULAR recommendations for the management of AAV recommend plasma exchange in the presence of diffuse alveolar haemorrhage or rapidly progressive renal failure,[22] however this modality also remains an option when high-dose corticosteroid therapy may not be feasible due to underlying infection. However, haemodynamic instability and myocardial ischemia were significant risk factors for complications associated with this therapeutic modality, therefore, it was not considered for our patient.

4 | CONCLUSION

The present case revealed diagnostic and therapeutic challenges. It is important for clinicians to understand that not all patients with disease present with a textbook phenotype. At times, presentations of rheumatic disease may be "unclassifiable" into definite disease subtypes. In such instances, it is important to treat the predominant manifestation. Misuse of classification criteria should be avoided for diagnostic purposes. Finally, in situations where there is potentially a life-threatening flare of rheumatic disease along with concomitant infection, IVIG remains an alternative option to high-dose corticosteroid therapy.

CONFLICTS OF INTEREST

Durga Prasanna Misra: None. Neeraj Jain: None. Gangadharan Harikrishnan: None. Vikas Agarwal: None.

AUTHOR CONTRIBUTIONS

All the authors contributed towards conception of the paper, acquisition, analysis and interpretation of data and approve of the final version of the paper. DPM, NJ and HG drafted the article. VA critically revised it for important intellectual content. All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ETHICAL CONSIDERATIONS

Written consent to publish was obtained from the patient.

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REFERENCES

1. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum*. 2010;62:2569-2581. https://doi.org/10.1002/art.27584.
2. Scott DG, Bacon PA. Intravenous cyclophosphamide plus methylprednisolone in treatment of systemic rheumatoid vasculitis. *Am J Med*. 1984;76:377-384.
3. Lightfoot RW Jr, Michel BA, Bloch DA, et al. The American. College of Rheumatology 1990 criteria for the classification of polyarteritis nodosa. *Arthritis Rheum*. 1990; 33:1088-1093.
4. Leavitt RY, Fauci AS, Bloch DA, et al. The American College of Rheumatology 1990 criteria for the classification of Wegener's granulomatosis. *Arthritis Rheum*. 1990;33:1101-1107.
5. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum*. 2013;65:1-11.
6. Haroon N, Agarwal V, Aggarwal A, Kumari N, Krishnani N, Misra R. Arthritis as presenting manifestation of pure neuritic leprosy - A rheumatologist's dilemma. *Rheumatology*. 2007;46:653-656. https://doi.org/10.1093/rheumatology/kel367.
7. Lamprecht P, Pipitone N, Gross WL. Unclassified vasculitis. *Clin Exp Rheumatol*. 2011;29:S81-85.
8. Wu X, Li G, Huang X, et al. Behçet's disease complicated with thrombosis: a report of 93 Chinese cases. *Medicine*. 2014;93:e263-e263. https://doi.org/10.1097/MD.0000000000000263.
9. Kang A, Antonelou M, Wong NL, et al. High incidence of arterial and venous thrombosis in antineutrophil cytoplasmic antibody–associated vasculitis. *The Journal of Rheumatology*. 2019;46:285-293.
10. Springer J, Villa-Forte A. Thrombosis in vasculitis. *Curr Opin Rheumatol*. 2013;25:19-25.
11. Parker B, Chattopadhyay C. A case of rheumatoid vasculitis involving the gastrointestinal tract in early disease. *Rheumatology (Oxford)*. 2007;46:1737-1738.
12. Aggarwal R, Ringold S, Khanna D, et al. Distinctions Between Diagnostic and Classification Criteria? *Arthritis Care Res*. 2015;67:891-897.
13. June RR, Aggarwal R. The use and abuse of diagnostic/classification criteria. *Best Pract Res Clin Rheumatol*. 2014;28:921-934.
14. Emmi G, Silvestri E, Squatrito D, et al. Thrombosis in vasculitis: from pathogenesis to treatment. *Thromb J*. 2015;13:15.
15. Houben E, Penne EL, Voskuyl AE, et al. Cardiovascular events in anti-neutrophil cytoplasmic antibody-associated vasculitis: a meta-analysis of observational studies. *Rheumatology (Oxford)*. 2017; 57(3):555-562.
16. Suppiah R, Judge A, Batra R, et al. A model to predict cardiovascular events in patients with newly diagnosed Wegener's granulomatosis and microscopic polyangiitis. *Arthritis Care Res*. 2011;63:588-596.
17. Bai YH, Li ZY, Chang DY, Chen M, Kallenberg CG, Zhao MH. The BVAS is an independent predictor of cardiovascular events and cardiovascular disease-related mortality in patients with ANCA-associated vasculitis: A study of 504 cases in a single Chinese center. *Semin Arthritis Rheum*. 2018;47:524-529.
18. Youssef J, Novosad SA, Winthrop KL. Infection Risk and Safety of Corticosteroid Use. *Rheum Dis Clin North Am*. 2016;42:157-x.
19. Gelfand EW. Intravenous immune globulin in autoimmune and inflammatory diseases. *N Engl J Med*. 2012;367:2015-2025.
20. Marie I, Maurey G, Herve F, Hellot MF, Levesque H. Intravenous immunoglobulin-associated arterial and venous thrombosis; report of a series and review of the literature. *Br J Dermatol*. 2006;155:714-721.
21. Shimizu T, Morita T, Kumanogoh A. The therapeutic efficacy of intravenous immunoglobulin in anti-neutrophilic cytoplasmic antibody-associated vasculitis: a meta-analysis. *Rheumatology (Oxford)*. 2019. https://doi.org/10.1093/rheumatology/kez311.
22. Yates M, Watts RA, Bajema IM, et al. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. *Ann Rheum Dis*. 2016;75:1583-1594.

How to cite this article: Misra DP, Jain N, Harikrishnan G, Agarwal V. Multiple jeopardy: Diagnostic and therapeutic challenges in vasculitic flare. *Int J Rheum Dis*. 2020;23:697–701. https://doi.org/10.1111/1756-185X.13836

Received: 7 March 2020 | Accepted: 7 March 2020

DOI: 10.1111/1756-185X.13831

COCHRANE CORNER

International Journal of Rheumatic Diseases

WILEY

Cochrane Rehabilitation

http://rehabilitation.cochrane.org

Are exercises beneficial for patients with rheumatoid arthritis of the hand?- A Cochrane review summary with commentary

1 | BACKGROUND

Rheumatoid arthritis (RA) is a chronic immune-mediated inflammatory rheumatic disease resulting from the complex interaction between genetic, constitutional and environmental triggers. It is polyarticular, but typically involves small joints of hands and feet. The disease requires lifelong monitoring and treatment and in the majority of patients limits daily functioning, quality of life and the ability to maintain work. The therapeutic approach to RA patients involves both pharmacological and nonpharmacological treatment aiming at preventing further structural damage, improving signs and symptoms, quality of life and increasing levels of functional independence.[2,3] People with RA are often referred to physical and occupational therapists to achieve these goals. The 3 most common components of the therapies they provide for hands with RA are exercise, joint protection advice, and provision of functional splinting and assistive devices.[4-6] Exercises are always individually planned and they are aimed at improving both the mobility and strength of the hand and variety of exercise types may be included (eg increasing and/or maintaining range of motion and strengthening exercises that use resistance). In addition to that, exercise programs may also incorporate the wrist due to the essential involvement of the wrist in functional activities of the hand.

2 | EXERCISE FOR RHEUMATOID ARTHRITIS OF THE HAND

2.1 | What is the aim of this Cochrane review?

The aim of this Cochrane review is to determine the benefits and harms of hand exercise in adult patients with RA (Williams et al 2018).[1]

2.2 | What was studied in the Cochrane review?

The population addressed in this review were adults (male and female), aged 18 years and older, diagnosed with RA lasting 5-14 years. In total, 7 studies involving in total 841 people (aged 20-94 years) were included in the review. Most studies used validated diagnostic criteria and involved home programs. Trials in which exercise for RA of the hand was compared with no treatment, usual care, placebo, medication, surgery, therapeutic modalities, or other non-exercise therapies were taken into account. All forms of exercise such as range of motion, stretching, and strength exercises and functional skills training were considered. Outcomes, assessing benefits and harms, were extracted and defined at 3 time point categories: short term (<3 months), medium term (3-11 months), and long term (12 months or beyond), and at the end of the trial for adverse events. For trials that reported outcomes at multiple time points, the longest follow-up was selected.[7] The major outcomes measures included: hand function, pain, hand impairment measures: power grip strength and pinch grip strength (tip-to-tip/tripod pinch grip), American College of Rheumatology 50 (ACR50) response criteria, patient adherence and adverse events due to exercise (eg exercise-induced injuries, increase in pain or in number of swollen or tender joints). The minor outcome measures included: hand impairment measures of range of motion, dexterity, deformity and hand stiffness, function assessed by Health Assessment Questionnaire, Disease Activity Score of 28 joints (DAS28), patient satisfaction, costs and change in splint or assistive device usage.

2.3 | Search methodology and up-to-dateness of the Cochrane review?

Cochrane Central Register of Controlled Trials (CENTRAL) (the Cochrane Library), MEDLINE, Embase, CINAHL, AMED, Physiotherapy

The aim of this commentary is to discuss the recently published Cochrane review "Exercise for rheumatoid arthritis of the hand"[1] by Williams MA et al (*This summary is based on a Cochrane review previously published in the Cochrane Database of Systematic Reviews 2018, Issue 7. Art. No.: CD003832.* https://doi.org/10.1002/14651858.CD003832.pub3 (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)* under the direct supervision of the Cochrane Musculoskeletal Group. This Cochrane Corner is produced in agreement with *International Journal of Rheumatic Diseases* by Cochrane Rehabilitation.

Evidence Database (PEDro), OTseeker, Web of Science, ClinicalTrials.gov and the World Health Organization International Clinical Trials Registry Platform (WHO ICTRP) were searched up to July 2017.

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

2.4.1 | Hand function

Very low-quality of evidence (due to risk of bias and imprecision) from 1 study indicated uncertainty about whether exercise improves hand function in the short term (up to 3 months), while moderate-quality evidence (due to risk of bias) from another study indicated that exercise compared to usual care probably slightly improves hand function in the medium term (3-11 months) and in the long term (12 months or beyond). People who did not exercise rated their function at 52.1 points. People who exercised rated their hand function 5 points higher in the medium term (3 to 11 months) and 4 points higher in the long term (12 months or beyond).

2.4.2 | Pain

Very low-quality evidence (due to risk of bias and imprecision) from 2 studies indicated uncertainty about whether exercise compared to no treatment improved pain in the short term. People who did not exercise rated their pain at 51.4 points. On a 0-100-mm pain scale (lower scores mean less pain), people who exercised rated their pain 28 mm lower in the short term, while those who did not exercise rated their pain at 68 mm. On a 0-100-point scale (lower scores mean less pain), people who exercised rated their pain 3 points lower in the medium and 4 points lower in the long term. Moderate-quality evidence (due to risk of bias) from 1 study indicated there is probably little or no difference between exercise and usual care on pain in the medium and long term. On a 0-100 scale, the absolute changes were −3% (95% CI −7% to 2%) and −4% (95% CI −8% to 1%), respectively.

2.4.3 | Grip strength

Very low-quality evidence (due to risk of bias and imprecision) from 3 studies (n = 141) indicated uncertainty about whether exercise compared to no treatment improved grip strength in the short term. People who exercised had 3% and 4% improvement in the left- and right-hand grip strength in the short term. People who did not exercise measured 14.3 kg and 15.6 kg, respectively. People who exercised had 1% improvement in the average grip strength of both hands in both medium and long term. People who did not exercise measured 13.2 kg.

High-quality evidence from one study showed that exercise compared to usual care has little or no benefit on mean grip strength (in kg) of both hands in the medium term, relative change 11% (95% CI −2% to 13%) and in the long term, relative change 9% (95% CI −5% to 23%).

2.4.4 | Pinch strength

Very low-quality evidence (due to risk of bias and imprecision) from 2 studies (n = 120) indicated uncertainty about whether exercise compared to no treatment improved pinch strength (in kg) in the short term. People who exercised had 4% and 6% improvement in the left- and right-hand pinch strength in the short term. People who did not exercise measured 1.2 kg and 1.2 kg, respectively. People who exercised had 2% and 3% improvement in the average pinch strength of both hands in the medium and long term. People who did not exercise measured 4 kg. High-quality evidence from 1 study showed that exercise compared to usual care has little or no benefit on mean pinch strength of both hands in the medium and long term. The relative changes were 8% (95% CI −4% to 19%) and 10% (95% CI −2% to 22%).

Moderate-quality evidence (due to risk of bias) from 1 study indicated that people who also received exercise with strategies for adherence were probably more adherent than those who received routine care alone in the medium term (risk ratio 1.31, 95% CI 1.15 to 1.48; n = 438) and number needed to treat to benefit 6 (95% CI 4 to 10). In the long term, the risk ratio was 1.09 (95% CI 0.93 to 1.28; n = 422).

Based on the available data, it is not possible to estimate potential risks or adverse events.

2.5 | How did the authors conclude on the evidence?

Based on the result, it is uncertain whether exercise improves hand function or pain in short, medium and long term. Furthermore, based on the evidence it is also uncertain if exercise improves grip and pinch strength in the short term and probably has little or no difference in the medium and long term. The ACR50 response is unknown. People who received exercise with adherence strategies were probably more adherent in the medium term than those who did not receive exercise, but with little or no difference in the long term. Based on the evidence, the risk of any adverse effects is uncertain. The quality of the evidence was very low to high across different outcomes. The quality of the evidence was lowered due to several problems: lack of blinding of participants to their allocated treatment and measurements, methods of allocation and small study sizes. Future research should consider hand and wrist function as their primary outcome, describe exercise following the template for intervention description and replication (TIDieR) guidelines and evaluate behavioral strategies.

2.6 | What are the implications of the cochrane evidence for practice in rehabilitation?

In order to prevent disability in RA patients, it is important to prevent structural damage (eg joint, cartilage, muscle, synovium) by early diagnosis and introduction of both pharmacological and non-pharmacological treatment modalities. Functional disability is mainly associated with disease activity in early RA or with radiographic joint

damage in patients with established disease and is often used as an outcome measure to assess the impact of disease over time. Due to the variable quality of current evidence, future studies should face higher quality standards in terms of conducting and reporting in order to evaluate the effectiveness of exercise therapy for the RA hand. Development of a core set of outcomes for conservative treatment for RA would improve the ability to synthesize evidence in this and similar areas. In addition to that, research to ascertain the clinically important change in hand function is also required. Another important issue in improving upon existing reporting of trials of exercise therapy is how authors should attempt to define, control, and report dosage of exercise and related adherence in accordance with the TIDieR guidelines.[8] With the majority of the studies included in this review evaluating short term effectiveness, there is a need for incorporating evaluation of long term effectiveness, especially for a chronic health condition such as RA. Future research to evaluate the efficacy of different modes of exercise intensity, frequency, and duration would therefore be a welcome addition to the evidence base.

ACKNOWLEDGEMENTS

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

CONFLICT OF INTEREST

The author declares no conflicts of interest.

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REFERENCES

1. Williams MA, Srikesavan C, Heine PJ, et al. Exercise for rheumatoid arthritis of the hand. *Cochrane Database Syst Rev*. 2018;(7):CD003832.
2. Smolen JS, Breedveld FC, Burmester GR, et al. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. *Ann Rheum Dis*. 2016;75(1):3-15.
3. Smolen JS, Landewé R, Bijlsma J, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis*. 2017;76:960-977.
4. Hammond A. Rehabilitation in rheumatoid arthritis: a critical review. *Musculoskeletal Care*. 2004;2(3):135-151.
5. Steultjens EE, Dekker JJ, Bouter LM, et al. Occupational therapy for rheumatoid arthritis. *Cochrane Database Syst Rev*. 2004;(1):CD003114.
6. Tuntland H, Kjeken I, Nordheim LV, Falzon L, Jamtvedt G, Hagen KB. Assistive technology for rheumatoid arthritis. *Cochrane Database Syst Rev*. 2009;(4):CD006729.
7. Deeks JJ, Higgins JPT, Altman DG. Chapter 9: Analysing data and undertaking meta-analyses. In: Higgins JPT, Green S, eds. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011)*. The Cochrane Collaboration; 2011.
8. Hoffman TC, Glasziou PP, Boutron I, et al. Better reporting of interventions: template for intervention description and replication (TIDieR) checklist and guide. *BMJ*. 2014;348:1687.

Received: 23 March 2020 | Accepted: 25 March 2020

DOI: 10.1111/1756-185X.13838

International Journal of Rheumatic Diseases

WILEY

CORRESPONDENCE

Shared susceptibilities between knee osteoarthritis and hip osteoarthritis

Dear Editor,

Recently, we have read the publication "Association of IL-17A-197G/A and IL-17F-7488T/C polymorphisms and osteoarthritis (OA) susceptibility: A meta-analysis"[1] with great interest. However, the meta-analyses involved in this article only addressed the risk of knee osteoarthritis (KOA). As is known, the prevalence of hip and KOA varies by ethnicity. Asian populations show a higher prevalence for KOA and a lower prevalence for hip OA (HOA), notably in Chinese and Japanese compared with populations of European descent.[2] These differences in the prevalence of KOA and HOA may also indicate genetic heterogeneity in ethnicity and predilection sites (knee or hip). Given that the interaction between genetic and environmental factors in OA is still not well understood and only a small fraction of the genetic determinants has been confirmed with biological explanations, it is necessary to elucidate the potential shared susceptibilities between KOA and HOA.

So far, there have been only four research reports on the interleukin (IL)17F gene and OA susceptibility in Han Chinese populations,[3-6] except the report from Gao et al[1] However, these four articles are all about KOA, not HOA. Moreover, two articles reported a significant association between rs763780 and KOA risk,[3,5] while the other two articles did not identify the association of rs763780 with KOA risk.[4,6] Different studies on KOA susceptibility in the same population have yielded conflicting results, which makes it even more necessary to conduct a deeper systematic genetic association analyses in Han Chinese populations and even other populations. Moreover, these four articles simply examined the association of the single nucleotide polymorphism (SNP) rs763780 with KOA risk, not including the common SNPs of the entire IL17F gene. It is unknown whether the SNP of rs763780 may coexist in linkage disequilibrium with other ungenotyped SNPs or variants to contribute to the risk of OA. What is more, there has been no study of the association of the IL17F gene with HOA risk in Han Chinese populations to date, so additional studies are particularly necessary to systemically evaluate the relationship between IL17F gene and HOA in different populations worldwide including Han Chinese populations.

CONFLICTS OF INTEREST

None.

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REFERENCES

1. Gao S, Mao C, Cheng J, Deng Q, Sheng W. Association of IL-17A-197G/A and IL-17F-7488T/C polymorphisms and osteoarthritis susceptibility: a meta-analysis. *Int J Rheum Dis*. 2020;23(1):37-46.
2. Xu L, Nevitt MC, Zhang Y, Yu W, Alibadi P, Felson DT. High prevalence of knee, but not hip or hand osteoarthritis in Beijing elders: comparison with data of Caucasian in United States. [Article in Chinese] *Zhonghua Yi Xue Za Zhi*. 2003;83:1206-1209.
3. Bai Y, Gao S, Liu Y, Jin S, Zhang H, Su K. Correlation between interleukin-17 gene polymorphism and osteoarthritis susceptibility in Han Chinese population. *BMC Med Genet*. 2019;20(1):20.
4. Jiang L, Zhou X, Xiong Y, Bao J, Xu K, Wu L. Association between interleukin-17A/F single nucleotide polymorphisms and susceptibility to osteoarthritis in a Chinese Population. *Medicine (Baltimore)*. 2019;98(12):e14944.
5. Lu F, Liu P, Zhang Q, Wang W, Guo W. Association between the polymorphism of IL-17A and IL-17F gene with knee osteoarthritis risk: a meta-analysis based on case-control studies. *J Orthop Surg Res*. 2019;14(1):445.
6. Zhang PL, Yang FM, Qiao ZZ, et al. Association between interleukin-17A and 17F single nucleotide polymorphisms and knee osteoarthritis]. [Article in Chinese]. *Zhonghua Yi Xue Za Zhi*. 2019;99(24):1870-1874.

Received: 30 March 2020 | Accepted: 2 April 2020

DOI: 10.1111/1756-185X.13844

CORRESPONDENCE

International Journal of Rheumatic Diseases

WILEY

Interleukin-17 in urine and serum of patients with nephritis

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Recent evidence suggests that interleukin-17 (IL-17) is involved in the pathogenesis of systemic lupus erythematosus (SLE) and it is a promising marker of disease activity in lupus nephritis (LN).[1] IL-17 induces the expression of various pro-inflammatory cytokines and chemokines, which increase the influx of various leukocyte subpopulations and thus, causes severe inflammation. These inflammatory mediators enhance the involvement of various leukocyte subpopulations, which ultimately leads to damage.[2,3]

Nordin et al published interesting data about IL-17 levels in 120 patients with SLE.[4] The authors showed an increase in the levels of IL-17 in the serum and urine of patients and their correlations with British Isles Lupus Assessment Group and SLE Disease Activity Index scores, which is consistent with other studies.[5,6] But IL-17 was not a sensitive or specific marker of SLE activity. In addition, there was no correlations of IL-17 levels in urine or serum with the histological classes of LN.

We also studied the levels of IL-17 in the urine and serum of patients with chronic glomerulonephritis (CGN). The levels of IL-17 in serum did not significantly differ from the levels in healthy individuals and did not depend on the activity of nephritis. On the contrary, the levels of IL-17A in the urine of patients with CGN were significantly higher at 3.05 (2.98-3.1) pg/mg creatinine (Cr) than that in healthy individuals 2.93 (2.92-2.94) pg/mg Cr The urinary IL-17A levels were not correlated with proteinuria (R^2 = 0.21, P = .29) and serum albumin (R^2 = 0.24, P = .21). These results indicate a connection between IL-17 levels in urine and inflammation of renal tissue in CGN patients.

Also, IL-17 levels in the urine were significantly higher in the CGN patients with advanced interstitial fibrosis and a renal dysfunction with glomerular filtration rate less than 60 mL/min/1.73 m^2 (2.97 [2.96-2.99] pg/mg Cr vs. 2.99 [2.98-3.00] pg/mg Cr in normal renal function, P < .05) regardless of histological forms. Currently, the role of IL-17 in fibrotic accumulation has been discussed. Severe fibrosis in murine radiation-induced lung disease was more pronounced with regard to reduction in Th1 cells in the presence of increased number of Th17 cells and higher levels IL-17.[7] IL-6 can induce glomerulosclerosis and interstitial fibrosis in the kidney directly or through activation of IL-17.[8] Changes in the Th17/Treg ratio may contribute to the development of advanced tissue fibrosis by transforming growth factor-β signaling through the activation of mitogen-activated protein kinases.[9]

Therefore, future research is needed to compare IL-17 levels in the urine with activity and sclerosis scores in the renal tissue and renal outcomes in LN.

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REFERENCES

1. Metoni A, Balanescu P, Dima A, et al. Interleukin-17 - association to silent lupus nephritis and disease activity. *J Transl Med Res*. 2015;20(2):71-75.
2. Crispín JC, Tsokos GC. IL-17 in systemic lupus erythematosus. *J Biomed Biotechnol*. 2010;2010:943254.
3. von Vietinghoff S, Ley K. Homeostatic regulation of blood neutrophil counts. *J Immunol*. 2008;181(8):5183-5188.
4. Nordin F, Shaharir SS, Wahab AA, et al. Serum and urine interleukin-17A levels as biomarkers of disease activity in systemic lupus erythematosus. *Int J Rheum Dis*. 2019;00:1-8.
5. Abdel Galil SM, Ezzeldin N, El-Boshy ME. The role of serum IL-17 and IL-6 as biomarkers of disease activity and predictors of remission in patients with lupus nephritis. *Cytokine*. 2015;76(2):280-287.
6. Mohamed AH, Badawy A, Elmasry E, et al. Serum level of Interleukin -17 in Systemic lupus erythematosus: clinical associations with disease activity and lupus nephritis. *Indian J Appl Res*. 2014;4(10):75-79.
7. Paun A, Bergeron M-E, Haston CK. The Th1/Th17 balance dictates the fibrosis response in murine radiation-induced lung disease. *Sci Rep*. 2017;7:11586.
8. Lei L, Zhao C, Qin F, et al. Th17 cells and IL-17 promote the skin and lung inflammation and fibrosis process in a bleomycin-induced murine model of systemic sclerosis. *Clin Exp Rheumatol*. 2016;100(5):14-22.
9. Shoukry NH, Fabre T, Molina MF, et al. Th17 cytokines drive liver fibrosis progression by regulating TGF-β signaling through activation of MAPKs. *J Immunol*. 2017;198(1 suppl):197.12.

The 2020 congress will be held on **31 August – 3 September** in Kyoto International Conference Center, Japan. Please do look out for updates by visiting the website.

APLAR aims to improve standards of clinical practice, teaching, and research in rheumatology across Asia Pacific. We are recognising the long-term efforts and dedication of centers in the region with a similar goal for excellence in the field. The certification programme we have initiated will award leading centers in Asia Pacific as Centers of Excellence based on three pillars (research, clinical practice, academia), pre-defined by a list of criteria set by APLAR.

We hope the centers in the region with an excellent track record in any of these pillars will participate in this programme as our goal is to establish reference centers that are best in class models for practice, teaching, and research in rheumatology. We believe this will enhance and enrich the 'best in class' experience for our trainees involved in the APLAR Fellowship programme. Further, this will also help us build a strong network of reference centers for collaborations and consultation within and among countries in the region.

APLAR awarded Centers of Excellence have been updated and information about these centers can be found on the website. Center of Excellence 2020 application will begin in March next year. Application information will be made available through the Member National Organisations of APLAR.

APLAR FELLOWSHIP GRANT

The Asia Pacific League of Associations for Rheumatology (APLAR) had awarded 2 applicants for the Fellowship Grant of 2019. They are embarking on their fellowship programme in the coming months. Successful candidates must have a long-term commitment to continue research or clinical work in his/her own country at the conclusion of the Fellowship. The grant is awarded to cover accommodation and subsistence costs. We congratulate the awardees and wish them a fruitful journey in their career paths.

APLAR RESEARCH GRANT

The Asia Pacific League of Associations for Rheumatology (APLAR) had awarded 2 applicants for the Research Grant of 2019. The grants are to assist the undertaking of research in either adult or paediatric rheumatology. The aims of the grant are to give the researcher an opportunity to start and do research within their own country of residence. In addition, we hope to promote and support basic and clinical research directed to the causes, prevention, and treatment of rheumatic diseases in the APLAR member society countries. This grant is to be used for consumables required for the research and not for salaries or fixed costs. It is expected that the research will be completed within one (1) year of the onset. The awarded candidates are encouraged to publish their work in the APLAR official journal – International Journal of Rheumatic Disease (IJRD) as part of their contribution.

APLAR-COPCORD GRANT

The Asia Pacific League of Associations for Rheumatology (APLAR) did not have any applicant for COPCORD grant 2019. We encourage interested candidates to send in their application during the application period for COPCORD grant 2020. The aims of the grant are to give the researcher an opportunity to study rheumatic disease in the community of their own country of residence. This grant is to be used for consumables required for the research and not for salaries or fixed costs. It is expected that the research will be completed within one (1) year of the onset.

All APLAR Grants are currently open for application. The closing date will be on 21 February 2020. APLAR Grants information on eligibility, criteria and application requirement can be found on the *website*.