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EDITORIAL

Costs of disease: The perspective matters

For many centuries, care for the ill was provided within families. For the poor and those without family, charitable organizations took over this task. At the turn of the 18th century, healthcare became slowly a public responsibility. In parallel, debates emerged how to finance healthcare. The Bismarckian and Beveridge systems served as the best-known examples which were introduced in the 19th century. Independent of the type of financing, the costs of diagnosing and treating illness became increasingly a key factor in organizing healthcare.[1] Since then, studies on the costs of disease have become increasingly common. "Costs" usually refers to the financial consequences of disease, although in some studies (typically costs-of-illness [COI] studies) the loss of health-related quality of life is also considered a cost ("intangible costs"), yet rarely expressed in monetary terms. Although the usefulness of studies investigating the costs of disease has been debated frequently,[2-7] knowledge of costs can inform the decision-making process on healthcare and social policies, and is useful to prioritize areas for future economic evaluation. Moreover, data on costs serve as a main component of cost-effectiveness studies.[4,8-10] However, in this setting of healthcare policies, the part of the costs of disease borne by patients and their families are not explicit and receive relatively little attention. Notwithstanding, financial impact of diseases on patients and families can be large and influential on social status and consequently on health behavior and health.

For correct interpretation of cost studies, it is essential to understand their concept and limitations. Cost studies are no economic evaluations: the costs are not weighted against health or other benefits, and there is no estimation of how costs might change with the introduction of a new intervention or change of policy. These studies also do not provide an indication of health: some diseases result in high individual suffering but only a limited utilization of resources. The impact on quality and quantity of life should therefore always be taken into account by policy-makers to put costs in a correct perspective.[11] Finally, although cost studies can be used to prioritize disease for economic evaluation or identify the most important cost components of a disease, they do not provide an indication of efficiency or waste of resources. Diseases associated with high costs might be interesting for policy-makers when prioritizing healthcare, but might not be preventable or treatable, or disease management might come at an inevitable high cost.

Cost studies, and health economic studies in general, can be conducted from several perspectives.[12] The adopted perspective guides which cost categories are included in the analysis, with the societal perspective being the most comprehensive as it includes all cost components, inside and outside of healthcare.[13] For this reason, health economists often prefer this perspective. However, costs can also be assessed from the specific perspective of the patient (Table 1). Patients and their families often incur significant disease-related costs. These can be related to healthcare use (out-of-pocket direct costs), non-healthcare uses (travels to care providers, over-the-counter drugs, informal care) but also to indirect costs caused by impaired working ability (loss of income and employment, early retirement, reduced career opportunities). The amount of costs borne by patients are relevant, as high costs might restrict patients' access to successful treatment or even lead to health inequalities and poverty in the long-term, which in itself has an effect on poor health.[14]

1 | A RECENT EXAMPLE - COSTS OF GOUT FROM THE PATIENT PERSPECTIVE

In this issue of *International Journal of Rheumatic Diseases*, Nathan et al. aimed to estimate the direct and indirect out-of-pocket costs of gout to Australian patients.[15] In their population study, 79 patients with gout completed an open, web-based questionnaire, which contained questions on their resource use and work productivity in the last 12 months. The median (mean; range) total direct out-of-pocket costs were estimated at AU$200 (AU$666; AU$0-AU$7088) per year. Medical costs, especially those related to medication and consultations with care providers, accounted for the majority (>99%) of these direct costs. The median (mean; range) number of missed work days was 0.25 (3.2; 0-65) in the last 12 months, which was valued at AU$60. Some patients were unable to afford medical care for gout, in particular medication. Nathan et al. need to be commended for their efforts of investigating a relevant aspect of chronic illness. Formally not a COI study (as intangible costs were not included), their study estimated both direct and indirect costs from the patient perspective. As such, it instigates a discussion on the costs of disease from the perspective of patients and their families, and specifically on the role of the indirect costs associated with short- and long-term absence from work (sick leave, work disability, early retirement) and (more arguable) of presenteeism (ie, decreased productivity while working).

TABLE 1 Overview of the costs borne by society and patients for different cost categories

Category[a]	Societal perspective	Patient perspective
Healthcare, such as therapies, diagnostics, drug prescriptions	"True" cost (independent of who pays for them, this includes patients' co-payments)	Only the part of the costs paid by patient or their family (contributions, co-payments)
Non-healthcare, such as travel costs, membership to society, over-the-counter drugs, informal care	Market price	Market price
Other sectors, such as costs related to paid work	Cost resulting from reduction in production of goods and services	Income loss as a consequence of ill health (eg, in case of sick leave or work disability, or if career prospects are affected)

[a]Healthcare and non-healthcare costs are often called "direct costs" as they are directly related to health services. Healthcare refers to costs borne by the healthcare system, while non-healthcare refers to costs borne by parties outside the healthcare system. Costs in other sectors are not directly related to health services, and are called "indirect costs".

2 | ESTIMATING PRODUCTIVITY COSTS - CHALLENGING, BUT NECESSARY

Productivity changes related to paid work can be due to absenteeism (being absent from work) or presenteeism (decreased at-work productivity). For absenteeism, a distinction is made between temporary absenteeism, also referred to as sick leave, and permanent absenteeism (work disability, early retirement). Productivity changes are an important aspect of the costs of disease. This is especially true for chronic diseases that manifest themselves in patients of working age, for which the consequences of such diseases on work outcomes can span multiple decades. The relevance of productivity changes for society has been highlighted in cost studies of rheumatic diseases (including gout), where these costs of productivity changes accounted for substantial parts (often > 50%) of total costs.[16-18] The far majority of cost studies in gout adopted either a societal or payer perspective, while studies on the burden of productivity costs for patients with gout are mostly lacking.[16]

2.1 | Assessment of productivity changes

Measuring changes in "production" as a consequence of illness is a challenging but essential first step to estimate indirect costs for patients and society, and even recommended in guidelines on health economic studies.[19] To assess loss of "production of good and services", the *time (days, hours)* a person is not able to work is usually regarded as an appropriate surrogate of true loss of production. Next, a *person's wage* is commonly used as an appropriate monetary value of lost production and converts time into costs. In the study by Nathan et al., the authors have chosen to use survey data and respondents had to indicate "the number of (half days) days absent because of gout in the past 12 months".[15] As they correctly state, longer assessment periods to detect relatively rare events (sick leave) will improve precision when the sample size is small. Also, while longer periods of recall are prone to inaccuracy, in clinical studies a balance has to be made between frequency of measurement, and study design, feasibility and patient burden.[20,21] Absence of specification in the survey whether calendar or work days should be counted, might add to inaccuracy. For short spells, persons might count work days missed, while in case of long-term absence patients might find it easier to estimate the calendar days absent. Also, patients might find it difficult to decide whether sick leave is attributable to gout or other health problems, and this is especially relevant when the prevalence of comorbidities is high (as in gout and thus in this study). An employed gout patient admitted 2 weeks to hospital because of worsening heart failure, who experienced during his stay a gout attack lasting 4 days, how would this be reported in a survey asking to report days absent due to gout? Or what about the 2 days sick leave because of colchicine induced diarrhea? To avoid such discussions and to allow benchmarking with the general population, it is advised to collect data on absence related to any health problem. To complicate measurement of loss of production related to sickness absence, when a gout flare is short and a patient returns to work after 2 or 3 working days, it is likely colleagues took over the most urgent tasks during normal working hours and the patient catches up on unfinished tasks himself upon return. These so-called compensation mechanisms might reduce production loss of short-term sickness toward zero. Of note, they could also result in more stress (eg, due to increased workload at times), which can be considered intangible costs and are often not quantified in monetary terms.

Assessment of *presenteeism* comes with additional challenges, which are as of yet not fully resolved, and is a topic of continuing research. First of all, it is important to recognize differences in the underlying concepts among instruments assessing presenteeism. Some of these focus on the consequences for society (productivity/efficiency), while others mainly aim to measure the patient's experience (difficulty/inability) when performing work and have no (primary) economic objective.[22,23] Patients in this survey were asked to rate the extent to which gout problems affected their productivity, as a surrogate for the percentage of "productive" time loss.[24,25] Alternatively, researchers could have asked to rate the extra hours patients would have needed to catch up unfinished tasks.[26] To estimate the indirect costs, the % productive time lost (or the extra time to catch lost production) can be converted into monetary terms, again using wages. Clearly, these different approaches result in very different estimates of productive time lost and thus indirect costs.[22,27] Although a gold standard is lacking, the extra time

needed to catch up on lost work is a more conservative approach, avoiding overestimation and reducing the risk of stigmatization of persons with a chronic disease.[28] Having said this, the jury of health economists is not out whether presenteeism results in any loss of production (and indirect costs) at all, or whether the greater majority of a person's presenteeism is compensated. Whatsoever, in the current study, Nathan et al. recognized they did not ask patients to indicate the number of hours they would have normally worked (in a week), preventing them from calculating the working time lost due to presenteeism. Even if that information would have been available, recall of presenteeism should be accounted for when appraising the results. Presenteeism likely fluctuates strongly over time, and it is unlikely patients can estimate their averaged presenteeism over the past year.

2.2 | Productivity changes – who pays the price?

Apart from general measurement issues in relation to accuracy, the most important issue when estimating indirect costs from a patient perspective, is the question which costs matter in this perspective. While restrictions to participate in the work process will likely impact health-related quality of life, there is not necessarily a cost impact for the patients or their families. This would only be the case if (a) wage is affected in case of sick leave or work disability, (b) when recurrent sick leave would affect career prospects and thus future income, or (c) when free (unpaid) time is used to catch up the unfinished work. It would be interesting to know whether in this study the reported days of sick leave indeed resulted in reduced income, and whether the study might underestimate income loss of employment or early retirement because of health. To answer these questions, insights into social security and income compensation in Australia, and how income is affected in case of sick leave opposed to work disability, would be helpful. Knowledge on such elements of a study's context benefits the interpretation of productivity costs borne by patients, and it should be encouraged to report them. If income loss, in particular due to unemployment and work disability for medical reasons, was indeed underestimated in this study, it is easier to understand the economic hardships reported by a substantial proportion of patients (33%). This underlines the importance of careful assessment of *all* aspects of productivity: the productivity costs borne by patients and their family are real and do affect their health and welfare.

3 | CONCLUDING REMARKS

Chronic disease often has economic consequences for patients and their families. These consequences go beyond costs for medical care and medication. Patients are at risk of losing income and future earnings, which can put substantial strain on households. These costs can also affect access to care, putting patients and their families in a vicious circle. Appropriate assessment of productivity changes, valued in the context of the relevant social security regulations and income compensation measures, is necessary to correctly appreciate the extent of costs of disease borne by patients. Research on costs of gout and their consequences from the patient perspective, of which the study by Nathan et al. is a first step,[15] is necessary to identify where and how to support these patients and break the circle.

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EDITORIAL

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Slowly melting the urate snow in joints: Explaining gout attacks to patients

1 | INTRODUCTION

Generally, gout patients believe that a gout attack or flare occurs when serum urate levels (SU) are abnormally high. However, gout attacks often occur even with a normal SU. Furthermore, a gout attack does not always occur when the SU exceeds 7.0 mg/dL, eg, 10 mg/dl. This phenomenon makes it difficult not only for patients but general practitioners to understand the relationship between gout attacks and SU. It causes confusion about how to regulate SU in various situations of gout treatment. These misunderstandings can cause treatment to be interrupted or terminated before gout control is achieved. As a result, gout attacks will recur, risking complications such as urolithiasis formation, progressive renal damage, and the eventual formation of multiple tophi.

We designed a simple and effective method that we are implementing to improve patient compliance. In order to help patients easily understand the need for continuous long-term treatment, we have devised explanatory illustrations that utilize snowfall, snow cover (Figure 1), avalanches (Figures 2 and 3), and slowly melting snow (Figures 4 and 5) to depict various states of SU conditions and successful treatment.[1]

2 | URATE LEVELS ARE EQUAL IN SERUM AND JOINT FLUID

In body fluid with pH 7.4, 99% of uric acid is dissociated or exists as urate, and most urate exists in the form of monosodium urate (MSU).

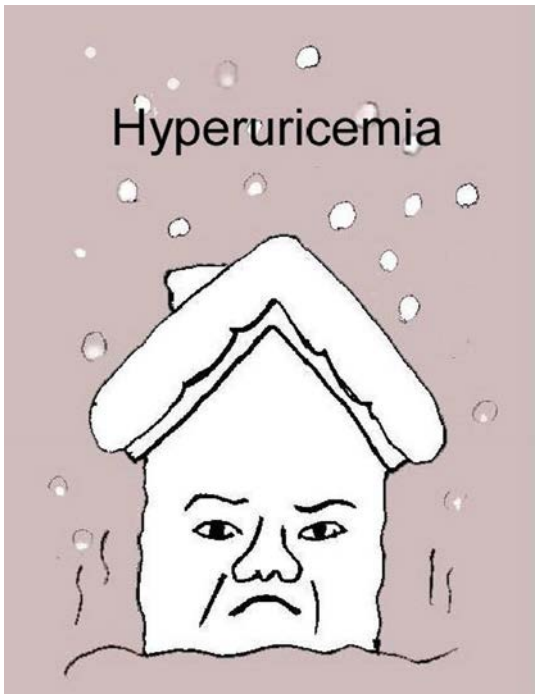

FIGURE 1 Hyperuricemia. We compared hyperuricemia to snowfall and the state of accumulating monosodium urate crystals to snow cover. When snowfall continues, snow cover gradually increases on the roof (joint lining). The snow cover eventually reaches a dangerous level on the roof.

FIGURE 2 Crystal shedding. When the monosodium urate burden exceeds the limit of stability, triggered by a variety of stimuli, a block of crystals sheds into the joint cavity. This crystal shedding (avalanche) evokes acute gouty arthritis, otherwise known as a gout attack or a flare.

FIGURE 3 Unexpected gout attacks. Notice that after an avalanche occurs, the remaining snow cover becomes very unstable and easily breaks off. Over-administration of urate-lowering drugs often evokes unexpected, repeated, and prolonged gout attacks. This instability might persist for 2-3 months.

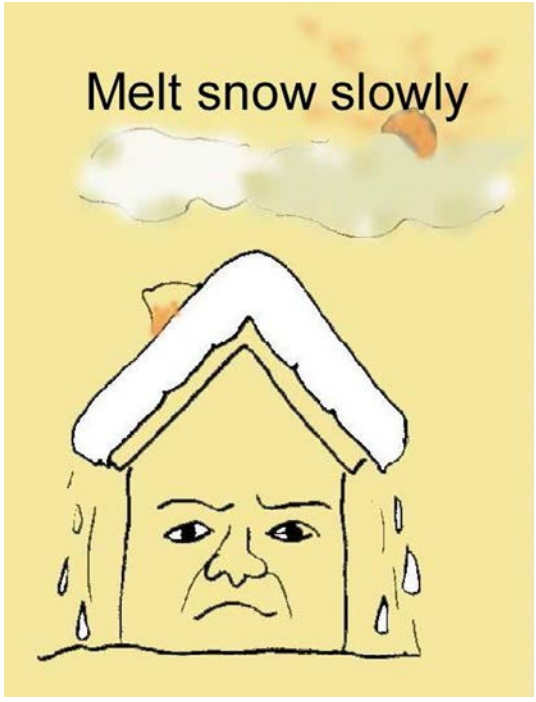

FIGURE 4 Slowly melting snow. It is extremely important that the snow cover on the roof should be melted slowly and gently, so that avalanches will gradually disappear. By steadily controlling serum urate, gout attacks become less severe and less frequent.

FIGURE 5 No more gout attacks. When the snow has melted away (accumulated monosodium urate crystals have disappeared), avalanches (gout attacks) no longer occur.

Urate has a small molecular weight (168 g/mol) and passes freely from serum into the synovium and synovial fluid. Therefore, measuring SU effectively measures the urate level in joint fluid.

The upper limit of MSU solubility in body fluids with pH 7.4 approximates at 7.0 mg/dL (0.42 mmol/L).[2] This value indicates a branch point concerning whether MSU is soluble or insoluble in the joint fluids. When SU and hence synovial fluid urate exceeds 7.0 mg/dL (hyperuricemia), MSU crystals gradually accumulate on the surface of joint synovium as microtophi (Figure 1).

3 | CRYSTAL SHEDDING

When surface MSU accumulation exceeds the limit of stability, triggered by a variety of stimuli, a block of crystals is shed from the microtophi into the joint cavity. This concept was proposed as "crystal shedding".[3,4] Macrophages and neutrophils recognize the needle-like MSU crystals dislodged from microtophi into joint fluid as harmful enemies and start reacting violently against the crystals. Here, an acute gouty arthritis or gout attack is evoked (Figure 2).

4 | SLOWLY MELTING URATE SNOW IN JOINTS

For about a month after a gout attack, the crystals accumulated on the joint lining are very unstable and easily break off. Abrupt

reduction of SU due to over-administration of urate-lowering drugs often evokes unexpected, repeated, and prolonged gout attacks (Figure 3). If the treatment procedure is not successful, such instability might persist for 2-3 months or more. At this stage, it is extremely important for accumulated crystals to be dissolved slowly and gently, as shown by the illustration of the sun slowly melting the snow on the roof (Figure 4). This is recommended in the 2020 American College of Rheumatology Guidelines for Management of Gout, which state that a "lower starting dose of urate-lowering therapy reduces the risk of flares associated with initiation."[5]

5 | FOR COMPLETE REMISSION OF GOUT ATTACKS

As the crystals accumulated on the inner surface of the joint gradually decrease, the occurrence of crystal shedding gradually diminishes, and eventually avalanches occur no longer (Figure 5). When this condition is attained, SU should be controlled using the minimum dose of urate-lowering drugs that achieves a target SU with 6.0 mg/dL. In the illustrations, this stage is depicted as cloudy or pale sun shining down on the roof and the absence of snow accumulation.

Lifestyle disorders such as regular overeating, drinking, and obesity resist normalization of SU. When management and guidance from the doctor or patient compliance is suboptimal, SU cannot be maintained within the target value. No matter how much time passes, the accumulated snow will not melt and gout attacks will continue to occur.

These explanatory illustrations utilizing snowfall, snow cover, and an avalanche to depict various states of SU conditions have proven very useful to understanding the relationship between gout attacks and SU, and has improved patient compliance for gout treatment.

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SUPPORTING INFORMATION

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REVIEW

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Effect of mannose-binding lectin gene polymorphisms on the risk of rheumatoid arthritis: Evidence from a meta-analysis

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Abstract

Background: The effect of mannose-binding lectin (MBL) gene polymorphisms on susceptibility of rheumatoid arthritis (RA) were evaluated in ethnically different populations, whereas the results were always inconsistent.

Materials and methods: Fourteen articles involving 36 datasets were recruited to evaluate the association between MBL gene polymorphisms and rheumatoid arthritis in a meta-analysis. The random or fixed effect models were used to evaluate the pooled odds ratios (ORs) and their corresponding 95% confidence intervals (CIs).

Results: Stratified analysis by ethnicities was conducted and the result revealed that rs1800450 (T vs C, OR = 1.32, 95% CI: 1.04-1.67, $P < .05$) and MBL-A/O (T vs C, OR = 1.20, 95% CI: 1.08-1.34, $P < .001$) were strongly associated with RA in Brazilian populations. In addition, the significant relationship between rs11003125 (T vs C, OR = 1.16, 95% CI: 1.06-1.26, $P < .05$) with RA were also observed in East Asian populations. Meanwhile, the inverse associations between rs5030737 with RA in East Asians and rs1800450 with RA in Indians were acquired. However, no association between any MBL polymorphism with RA susceptibility was confirmed in Caucasians.

Conclusions: The structural polymorphisms in exon 1 of MBL gene may significantly contribute to susceptibility and development of RA in Brazilian and Indian populations, whereas the functional polymorphisms in the promoter region were more likely to associate with RA in East Asians.

KEYWORDS

mannose-binding lectin, meta-analysis, polymorphism, rheumatoid arthritis

1 | INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterized by persistent synovitis, systemic inflammation and presence of autoantibodies.[1] RA affects 0.5% ~2% of the world population and the prevalence rate was 0.2%~0.4% in China.[2,3] At present, RA is one of the major diseases that seriously destructs life quality of patients and leads to the loss of labor capacity.[4] Although the pathogenesis of RA has not been fully clarified, it is generally believed that environmental, genetic and autoimmune factors may play a vital role in the onset of RA and genetic factors account for about 60% of RA susceptibility.[5]

Mannose-binding lectin (MBL) is a calcium-dependent collagen lectin secreted by hepatocytes and plays an important role in innate immunity by opsonizing mannose- and N-acetylglucosamine-rich microorganisms and activating macrophages and complements.[6] The mutation of MBL gene can decrease the level of plasma MBL which may relate to immune deficiency.[7] The MBL gene is mapped on the long arm of chromosome 10q11.2-10q21 and contains 4 exons.[8] Exon 1 has three functional single nucleotide polymorphisms (SNPs) at codons 54 (allele B, rs1800450), 57 (allele C, rs1800541), and 52 (allele D, rs5030737) in the structural part of the gene.[9] In addition, there are SNPs at positions −550 (allele L, rs11003125) and −221 (allele X, rs7096206) in the promoter region.[10] These variant alleles were associated with lowing serum MBL levels. Previous studies found that MBL gene variants can contribute to the susceptibility of acquired immune deficiency syndrome (AIDS), tuberculosis, systemic lupus erythematosus (SLE), Crohn's disease, RA and other infectious diseases.[11-15] However, the associations between MBL gene polymorphisms and RA were studied in various countries and ethnicities, but the results were inconsistent.[8,16,17] Tsutsumi et al.[18] found that codon 52 and 57 mutations of MBL gene were absent or extremely rare in Japanese; homozygous codon 54 mutation of the MBL gene was significantly increased in patients with autoimmune disorders. In addition, promotor regions of MBL gene suggested that individuals with absent or extremely low serum MBL were at risk of having autoimmune disorders.[19] Moreover, the B variant of the MBL2 gene may be associated with protection from RA in an Indian cohort and the promoter polymorphism rs1800450 seemed to have some roles in disease progression.[8] However, Stanworth et al.[20] declared that no evidence was found to support the association of MBL allele with protection from RA in Caucasian populations and genotype frequencies were similar in case and control groups. Similarly, the negative results were confirmed in a Japanese population.[18]

Although many studies have been conducted so far to investigate the relationship between MBL gene polymorphisms and susceptibility of RA, the results were inconsistent. This discrepancy may be attributed to small sample sizes, low statistical power, different genetic background, or clinical heterogeneity. Meanwhile, inclusion of data that did not satisfy the requirement of meta-analysis will produce a spurious association. Therefore, in order to reduce the limitations of a single study and to overcome the possible random errors, a large-scale meta-analysis involving multifarious ethnicities and multiple polymorphisms was performed in this study.

2 | MATERIALS AND METHODS

2.1 | Identification of eligible studies

To analyze the roles of MBL gene polymorphisms in susceptibility of RA, all published literature before June 2020 that researched the relationship between MBL gene polymorphisms and RA risk were included. The electronic databases were used including PubMed databases (National Center for Biotechnology, National Library of Medicine), CNKI (China National Knowledge Infrastructure), and Web of Science to retrieve articles by using the keywords "MBL gene", "codon 54 (allele B, rs1800450)", "codon 57 (allele C, rs1800541)", "codon 52 (allele D, rs5030737)", "-550 (allele L, rs11003125)", "-221 (allele X, rs7096206)", "polymorphism" connected to "RA", "rheumatoid arthritis" without language restrictions. Finally, we extracted data from the published articles, not including meetings or any conference abstracts. All of studies were conducted with case–control or nested case–control design. The diagnosis of RA was according to the American College of Rheumatology (ACR) criteria and proper genotyping methods in most of the studies.[21-23]

Three functional single nucleotide polymorphisms (SNP) in codons 54 (allele B), 57 (allele C), and 52 (allele D) were associated with changes in the structure and functional deficiency of protein. In codon 54, an A to G substitution alters an aspartic acid to a glycine at the protein level. In codon 57 there is a G to A substitution (glycine to glutamic acid), and in codon 52 a C to T substitution leads to a change from arginine to cysteine. Altogether, the presence of any variant alleles above has been collectively labeled O, while the simultaneous absence of variants at the 3 positions has been called allele A, the wild-type allele.[24]

2.2 | Selection criteria and data extraction

Such major criteria must be followed for included studies: (a) original papers containing complete data; (b) case–control or cohort studies that assessed the association of MBL gene polymorphisms with RA; (c) sufficient data to calculate the odds ratio (OR) or *P* value; (d) relevant RA outcomes were angiographically confirmed according to the ACR criteria;[25] (e) the genotype distribution in the control group for each individual study should follow Hardy–Weinberg equilibrium (HWE).[26,27] The primary reasons for excluded studies: (a) case report, review or meta-analysis articles; (b) deviation from the major selection criteria; (c) overlapping or that supplied inadequate data; (d) repeated publications or the same authors employed similar data in different papers, the data was only used once.

The study data were extracted based on standard protocols.[28] Disagreement was settled by a consensus between all authors. Where essential information was not presented in articles, every effort was made to contact the authors. All procedures conformed to the guidelines for meta-analysis of observational studies in epidemiology.[29] The following information were extracted independently by individuals in our study: first author, year of publication, ethnicity, study design, types of RA, HWE status among controls, sample size of cases and controls, number of genotypes and allele frequency.

2.3 | Statistical analysis

We calculated the allele frequency for each study in allele counting method; the HWE was tested by using the Chi-square test. We

TABLE 1 The basic information of included studies in this meta-analysis

Study	Y	Ethnicity	Polymorphisms	Sample size		Genotypes						Allele frequencies (%)			
						Control			Case			Control		Case	
				Control	Case	CC	CT	TT	CC	CT	TT	C	T	C	T
Bhawna et al.[8]	2005	Indian	rs5030737	119	120	104	15	0	100	20	0	93.7	6.3	91.7	8.3
Bhawna et al.[8]	2005	Indian	rs5030737	145	120	132	13	0	100	20	0	95.5	4.5	91.7	8.3
Hou et al.[47]	2013	Chinese	rs5030737	378	280	362	15	0	278	2	0	97.9	2.1	99.6	0.4
Stanworth et al.[20]	1999	Caucasian	rs1800450	114	182	84	28	2	142	35	5	86.0	14.0	87.6	12.4
Ip et al.[46]	2000	Chinese	rs1800450	196	211	153	42	1	143	64	4	88.8	11.2	82.9	17.1
Horiuchi et al.[18]	2000	Japanese	rs1800450	105	59	66	29	10	42	13	4	76.7	23.3	82.2	17.8
Tsutsumi et al.[53]	2001	Japanese	rs1800450	129	95	88	39	2	58	32	5	83.3	16.7	77.9	22.1
Bhawna et al.[8]	2005	Indian	rs1800450	119	120	72	47	0	106	14	0	80.3	19.7	94.2	5.8
Bhawna et al.[8]	2005	Indian	rs1800450	145	120	94	49	2	106	14	0	81.7	18.3	94.2	5.8
Min et al.[49]	2006	Chinese	rs1800450	48	93	36	12	0	66	24	3	87.5	12.5	83.9	16.1
Hou et al.[47]	2013	Chinese	rs1800450	378	280	231	138	10	195	76	9	79.2	20.8	83.2	16.8
Isabela et al.[16]	2014	Brazilian	rs1800450	200	156	148	45	7	96	55	5	85.3	14.7	79.2	20.8
Isabela et al.[16]	2014	Brazilian	rs1800450	120	156	79	41	0	96	55	5	82.9	17.1	79.2	20.8
Bhawna et al.[8]	2005	Indian	rs1800451	119	120	111	8	0	110	10	0	96.6	3.4	95.8	4.2
Bhawna et al.[8]	2005	Indian	rs1800451	145	120	129	16	0	110	10	0	94.5	5.5	95.8	4.2
Ip et al.[46]	2000	Chinese	rs7096206	174	115	119	50	5	68	41	6	82.8	17.2	76.5	23.5
Bhawna et al.[8]	2005	Indian	rs7096206	119	120	70	43	6	60	50	10	76.9	23.1	70.8	29.2
Bhawna et al.[8]	2005	Indian	rs7096206	90	120	46	32	12	60	50	10	68.9	31.1	70.8	29.2
Min et al.[49]	2006	Chinese	rs7096206	48	50	38	9	1	33	15	2	88.5	11.5	81.0	19.0
Isabela et al.[16]	2014	Brazilian	rs7096206	200	156	130	58	12	109	38	9	79.5	20.5	82.1	18.9
Isabela et al.[16]	2014	Brazilian	rs7096206	120	156	91	28	1	109	38	9	87.5	12.5	82.1	17.9
Hou et al.[17]	2020	Chinese	rs7096206	400	380	232	160	8	230	143	7	77.9	22.1	79.3	20.7
Ip et al.[46]	2000	Chinese	rs11003125	174	115	48	87	39	20	56	39	52.6	47.4	41.7	58.3
Bhawna et al[8]	2005	Indian	rs11003125	119	120	16	54	49	15	54	51	36.1	63.9	35.0	65.0
Bhawna et al.[8]	2005	Indian	rs11003125	100	120	17	42	41	15	54	51	38.0	62.0	35.0	65.0
Min et al.[49]	2006	Chinese	rs11003125	48	50	17	23	8	15	25	10	59.4	40.6	54.0	46.0
Hou et al.[17]	2020	Chinese	rs11003125	400	380	111	225	64	100	181	99	56.0	44.0	50.2	49.8
Jacobsen et al.[51]	2000	Caucasian	MBL-A/O	250	68	157	86	7	35	28	5	80.0	20.0	72.1	27.9
Koert et al.[45]	2008	Caucasian	MBL-A/O	194	218	120	65	9	128	81	9	78.6	21.4	77.3	22.7

(Continues)

TABLE 1 (Continued)

Study	Y	Ethnicity	Polymorphisms	Sample size		Genotypes						Allele frequencies (%)			
						Control			Case			Control		Case	
				Control	Case	CC	CT	TT	CC	CT	TT	C	T	C	T
Fernanda et al.[19]	2012	Brazilian	MBL-A/O	345	322	207	120	18	171	131	20	77.4	22.6	73.4	26.6
Fernanda et al.[19]	2012	Brazilian	MBL-A/O	244	300	148	83	13	160	123	17	77.7	22.3	73.8	26.2
Fernanda et al.[19]	2012	Brazilian	MBL-A/O	101	22	59	37	5	11	8	3	76.7	23.3	68.2	31.8
Isabela et al.[16]	2014	Brazilian	MBL-A/O	200	156	119	73	8	75	73	8	77.8	22.2	71.4	28.6
Isabela et al.[16]	2014	Brazilian	MBL-A/O	120	156	62	58	0	75	73	8	75.8	24.2	71.4	28.6
Malthe et al.[48]	2014	Caucasian	MBL-A/O	383	301	159	193	31	130	143	28	66.7	33.3	66.9	33.1
Malthe et al.[48]	2014	Caucasian	MBL-YA/O	374	315	150	193	31	144	143	28	65.9	34.1	68.4	31.6

Note: C, represent wild-type allele; T, represent minor allele; MBL-A/O, the presence of any of rs5030737, rs1800450, rs1800451 has been collectively labeled O, while the simultaneous absence of variants at the 3 positions has been called allele A, the wild-type allele; MBL-YA/O, the MBL-A/O and presence of rs7096206.

employed pooled ORs and 95% confidence intervals (CIs) to evaluate the strength of association between polymorphisms and RA for every eligible study.

The methodology of Cochran's Q-statistic was used to evaluate the heterogeneity, which is similar to the previous study in our lab.[22,23] If the *P* value in heterogeneity test was higher than 0.1, the fixed effect model was used. Moreover, the random effect model was used. We used the following formula to quantify the effect of heterogeneity: $I^2 = 100\% \times (Q - df)/Q$.[30] The proportion of between-study variability attributable to heterogeneity was indicated by I^2 value, and I^2 values of 25%, 50% and 75% were considered to be of low, moderate and high heterogeneity, respectively. If study groups revealed no heterogeneity, the similar results were produced in fixed and random effects models and, otherwise the random effects model usually produced wider CIs than the fixed effects model.[31] In this meta-analysis, *P* value of less than .05 was considered as statistically significant.

In order to get exacting search results, we evaluated possible publication bias by Egger's linear regression test. If *P* value <.05 the statistical publication bias was considered. Moreover, the Begg's test also used a funnel plot to evaluate the publication bias.[32] For sensitivity analysis, we removed 1 study orderly from the total and tested residual studies.[33] All standard methods in this meta-analysis were conducted in a previous study by us. Statistical analysis was carried out using the software program STATA15.0 (Stata Corporation).

3 | RESULTS

3.1 | Studies included in the meta-analysis

In this meta-analysis, totally 318 relevant articles were searched. After reading titles and abstracts, we excluded irrelevant studies, leaving 105 articles for further reading. Then, we excluded 54 articles, because of no data, insufficient data, repeated date, family-based studies and not referring to RA. Thus, 51 articles met the study inclusion criteria. Lastly, 15 articles that included insufficient data, 5 articles in which the control populations deviated from HWE and 18 reviews or meta-analysis researches about MBL gene polymorphisms were excluded.[25,32,34-44] After filtering, 13 eligible studies involving 36 data sets were finally included.[8,16-20,24,45-53] Eventually, 13 studies provided 5972 cases and 6663 controls: codon 54 (allele B, rs1800450), 1472 patients and 1554 controls; codon 57 (allele C, rs1800541), 240 patients and 264 controls; codon 52 (allele D, rs5030737), 520 patients and 642 controls; −550 (allele L, rs11003125), 785 patients and 841 controls; −221 (allele X, rs7096206), 1097 patients and 1151 controls; MBL-A/O, 1858 patients and 2211 controls were pooled to evaluate the relationship between SNPs of MBL and RA in the meta-analysis (Table 1). The flowchart of selecting articles is presented in Figure 1.

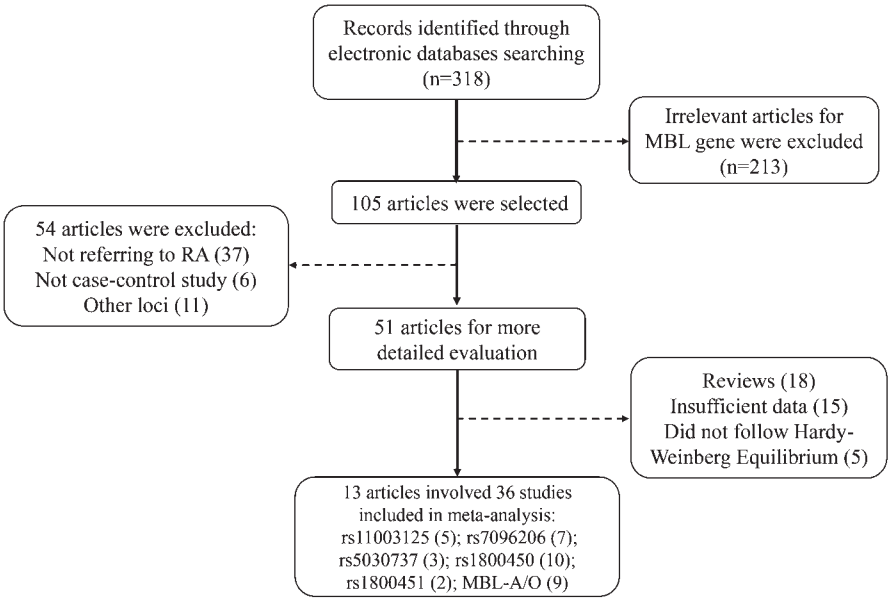

FIGURE 1 The process of the articles selected in this meta-analysis

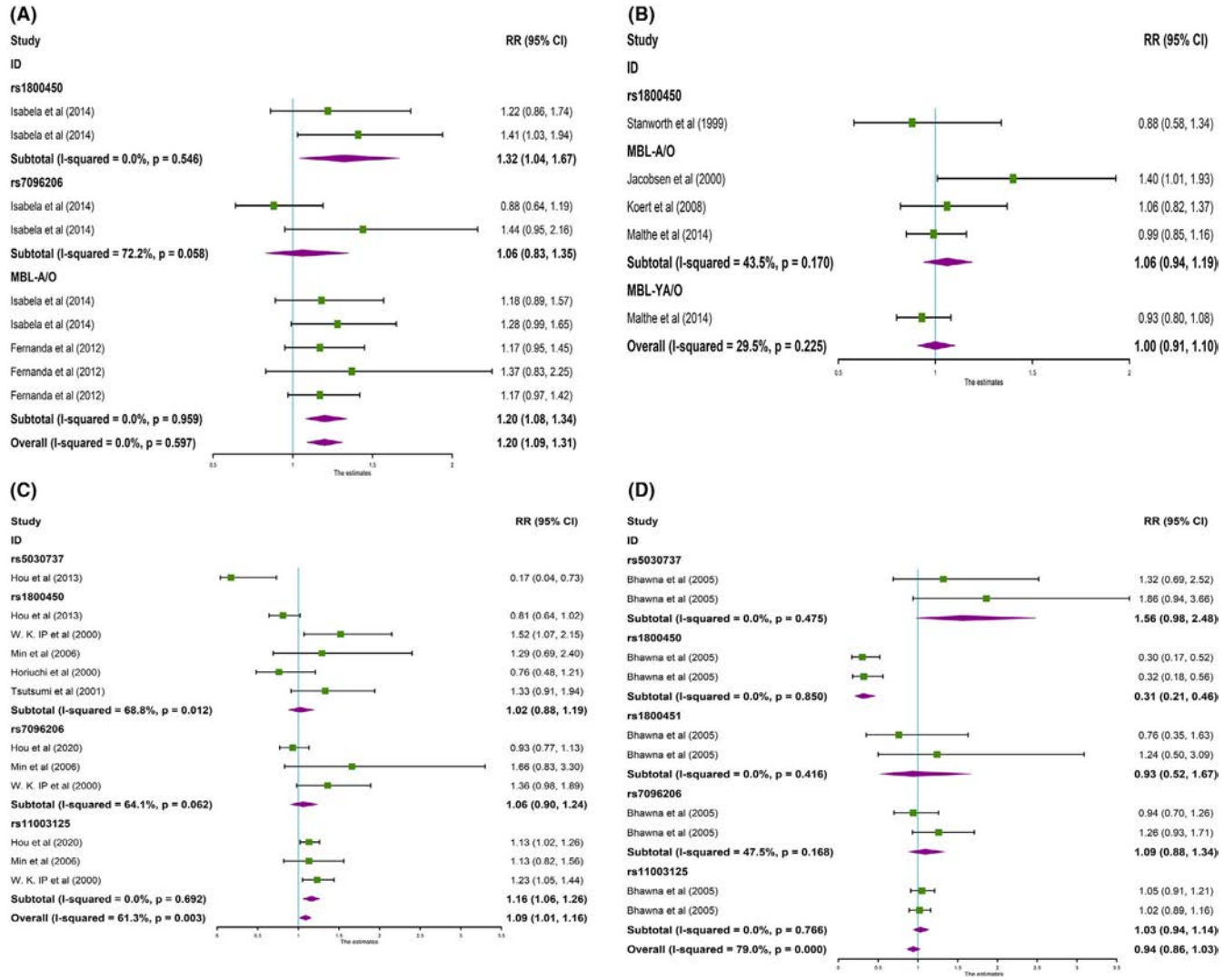

FIGURE 2 Forest plot for the meta-analysis of allele model (T vs C). A, MBL gene polymorphisms and RA in Brazilians. B, MBL gene polymorphisms and RA in Caucasians. C, MBL gene polymorphisms and RA in East Asians. D, MBL gene polymorphisms and RA in Indians

TABLE 2 The association between MBL polymorphisms and RA risk in meta-analysis

Sub-group analysis	No. of data sets	No. of cases/controls		Allele model (T vs C)			Dominant model (CC vs TT + CT)			Recessive model (TT vs CC + CT)		
		Cases	Controls	OR (95% CI)	P_{OR}	P_H	OR (95% CI)	P_{OR}	P_H	OR (95% CI)	P_{OR}	P_H
Brazilian												
rs1800450	2	754	740	1.32 (1.04-1.67)*	<.05	.546	1.30 (1.04-1.62)*	<.05	.225	1.55 (0.59-4.12)	.377	.141
rs7096206	2	736	752	1.06 (0.83-1.35)	.666	.058	1.00 (0.78-1.27)	.969	.146	1.54 (0.74-3.20)	.248	.070
MBL-A/O	5	2432	2479	1.20 (1.08-1.34)**	<.001	.959	1.18 (1.07-1.31)*	<.05	.890	1.39 (0.95-2.05)	.093	.379
Overall	9	3922	3971	1.20 (1.09-1.31)***	<.0001	.597	1.17 (1.07-1.27) ***	<.0001	.489	1.44 (1.04-1.99)*	<.05	.298
Caucasian												
rs1800450	1	409	260	0.88 (0.58-1.34)	.556	NA	0.84 (0.55-1.26)	.391	NA	1.57 (0.31-7.94)	.588	NA
MBL-A/O	3	1510	2092	1.06 (0.94-1.20)	.357	.170	1.04 (0.94-1.16)	.463	.176	1.20 (0.81-1.78)	.372	.310
MBL-YA/O	1	829	1003	0.93 (0.80-1.08)	.326	NA	0.91 (0.80-1.03)	.141	NA	1.07 (0.66-1.75)	.779	NA
Overall	5	2748	3355	1.00 (0.91-1.10)	.973	.225	0.98 (0.90-1.06)	.592	.180	1.16 (0.86-1.57)	.335	.623
East Asian												
rs5030737	1	562	772	0.17 (0.04-0.73) *	<.05	NA	0.18 (0.04-0.78) *	<.05	NA	NA		
rs1800450	5	1735	2017	1.02 (0.88-1.19)	.769	.012	0.99 (0.85-1.14)	.872	.011	1.44 (0.81-2.56)	.214	.426
rs7096206	3	1320	1492	1.06 (0.90-1.24)	.495	.062	1.04 (0.90-1.21)	.567	.082	1.28 (0.63-2.61)	.499	.647
rs11003125	3	1649	1800	1.16 (1.06-1.26) *	<.05	.692	1.05 (0.98-1.13)	.133	.338	1.56 (1.25-1.94)	<.0001	.783
Overall	12	5266	6081	1.09 (1.02-1.17)*	<.05	.003	1.02 (0.96-1.09)	.531	.005	1.52 (1.24-1.84)	<.0001	.851
Indian												
rs5030737	2	520	556	1.56 (0.98-2.48)	.062	.475	1.56 (1.00-2.44)	.053	.459	NA	NA	NA
rs1800450	2	508	628	0.31 (0.21-0.46)***	<.0001	.850	0.31 (0.21-0.46)***	<.0001	.766	0.24 (0.01-4.98)	.357	NA
rs1800451	2	500	552	0.93 (0.52-1.67)	.802	.416	0.93 (0.52-1.64)	.798	.406	NA	NA	NA
rs7096206	2	620	529	1.09 (0.88-1.34)	.434	.168	1.12 (0.92-1.36)	.268	.392	0.94 (0.52-1.71)	.836	.130
rs11003125	2	792	714	1.03 (0.94-1.14)	.524	.766	1.03 (0.96-1.11)	.415	.579	1.03 (0.83-1.28)	.760	.985
Overall	10	2940	2979	1.07 (0.98-1.18)***	.184	<.0001	1.09 (1.00-1.19)	.085	<.0001	1.00 (0.82-1.23)	.981	.510

Abbreviations: C, represent wild-type allele; 95%CI, 95% confidence interval; OR, odd ratio; P_{OR}, *P* value for the test of association; P_H, *P* value for heterogeneity analysis; T, represent minor allele; NA, none.

*$P_{OR} < .05$.

**$P_{OR} < .001$.

*** $P_{OR} < .0001$.

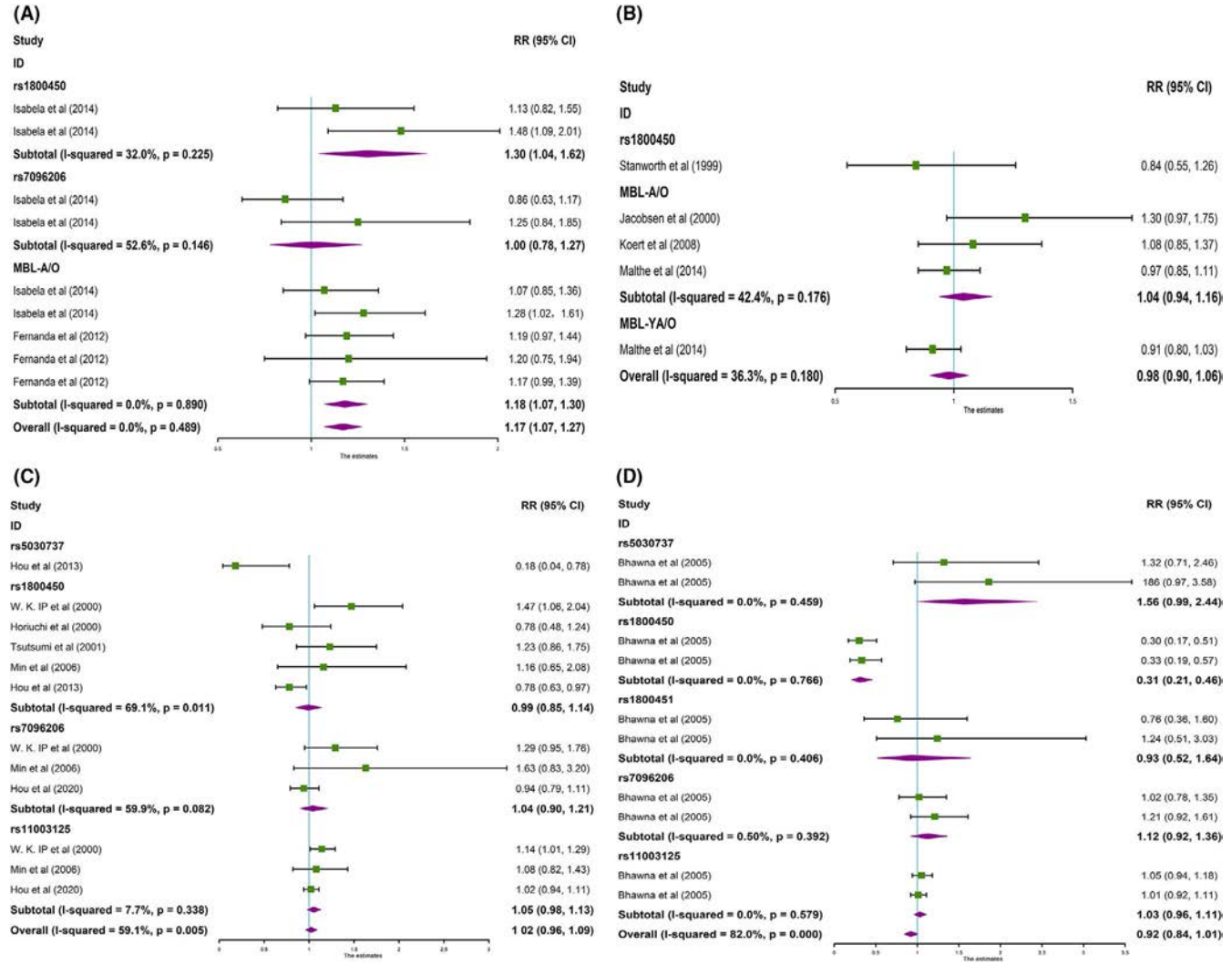

FIGURE 3 Forest plot for the meta-analysis of allele model dominant model (CC vs TT + CT). A, MBL gene polymorphisms and RA in Brazilians. B, MBL gene polymorphisms and RA in Caucasians. C,, MBL gene polymorphisms and RA in East Asians. D, MBL gene polymorphisms and RA in Indians

3.2 | Meta-analysis results

In this meta-analysis we recruited allele model, dominant gene model and recessive gene model to confirm the association between 5 MBL SNPs with RA in multiple ethnicities. The results of stratification by ethnicity revealed the heterogeneity had disappeared ($P > .01$, $I^2 < 30\%$; Figure 2A-D).

3.3 | Mannose-binding lectin SNPs and RA in Brazilians

The results found that rs1800450 (T vs C, OR = 1.32, 95% CI: 1.04-1.67, $P_{OR} < .05$) and MBL-A/O (T vs C, OR = 1.20, 95% CI: 1.08-1.34, $P_{OR} < .001$) were strongly associated with RA in a Brazilian population (Table 2, Figure 2A). Meanwhile, the overall study displayed the same significant association (T vs C, OR = 1.20, 95% CI: 1.09-1.31, $P_{OR} < .001$), and no heterogeneity ($P_H = .597$) (Table 2, Figure 2A). In addition, rs1800450 (TT + TC vs CC, OR = 1.30, 95% CI: 1.04-1.62, $P_{OR} < .05$) and MBL-A/O (TT + TC vs CC, OR = 1.18, 95% CI: 1.07-1.31, $P_{OR} < .05$) were strongly related to RA in the dominant model (Table 2, Figure 3A), whereas, the association was weak in the recessive gene model (Table 2, Figure 4A). However, pooled associations of rs1800450 and MBL-A/O not only in the dominant model (TT + TC vs CC, OR = 1.17, 95% CI: 1.07-1.27, $P_{OR} < .0001$) were strong (Table 2, Figure 3A), but also in the recessive model (TT vs TC + CC, OR = 1.44, 95%CI: 1.04-1.99, $P_{OR} < .05$) (Table 2, Figure 4A).

3.4 | MBL SNPs and RA in East Asians

The significant relationship between rs11003125 (T vs C, OR = 1.16, 95% CI: 1.06-1.26, $P_{OR} < .05$) with RA susceptibility was observed in East Asian populations (Table 2, Figure 2C). Meanwhile, significant association was found in the recessive gene model (TT vs TC + CC,

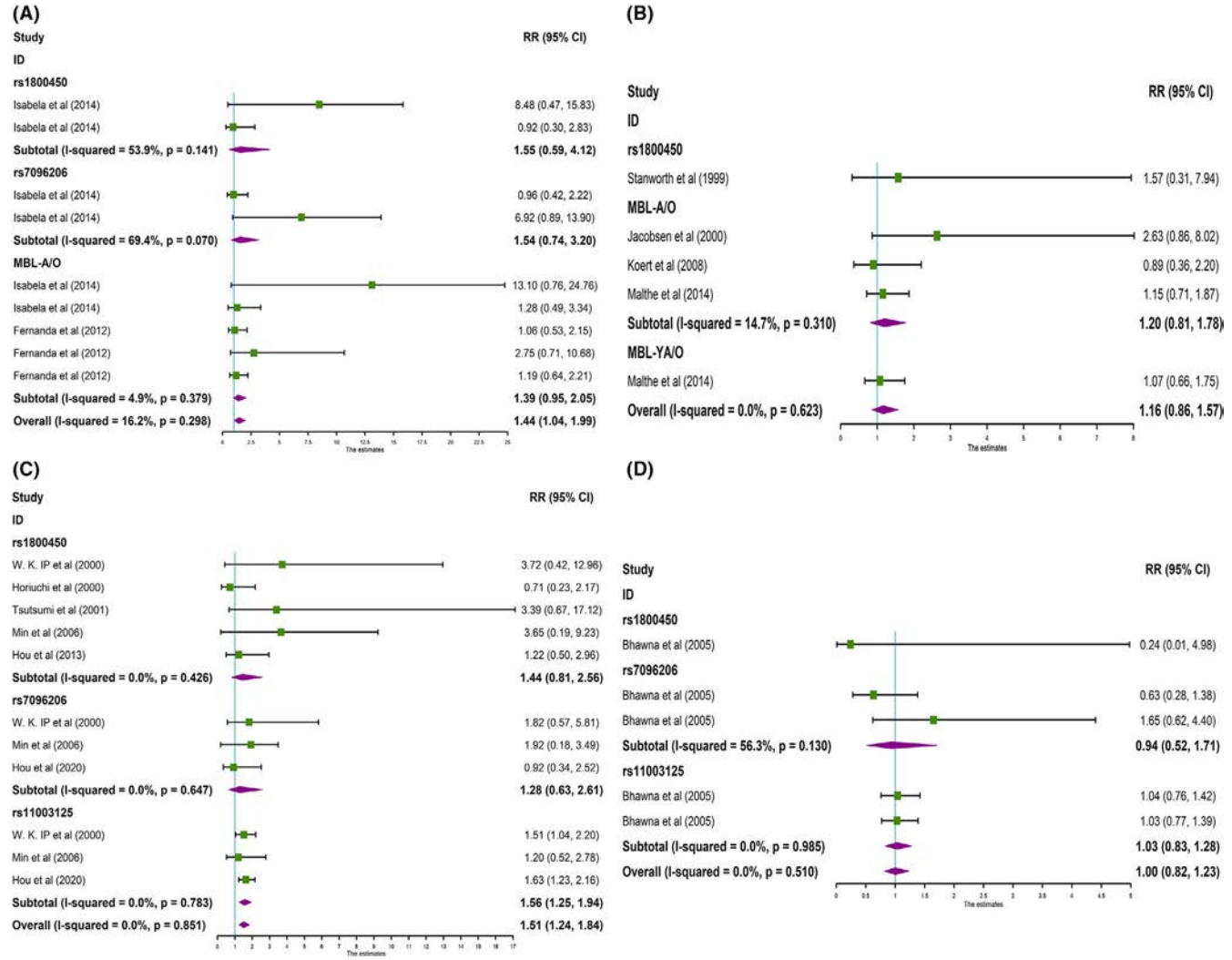

FIGURE 4 Forest plot for the meta-analysis of recessive model (TT vs CC + CT). A, MBL gene polymorphisms and RA in Brazilians. B, MBL gene polymorphisms and RA in Caucasians. C, MBL gene polymorphisms and RA in East Asians. D, MBL gene polymorphisms and RA in Indians

OR = 1.56, 95% CI: 1.25-1.94, $P_{OR} < .0001$) (Table 2, Figure 4C). The rs5030737 (T vs C, OR = 0.17, 95% CI: 0.04-0.73, $P_{OR} < .05$) was reversely associated with RA in East Asians (Table 2, Figure 2C), and the reverse association was maintained in the dominant model (TT + TC vs CC, OR = 0.18, 95% CI: 0.04-0.78, $P_{OR} < .05$) (Table 2, Figure 3C). However, pooled associations of rs11003125 and rs5030737 were observed, but the heterogeneity ($P_H < .01$, $I^2 > 30\%$) was also found.

3.5 | MBL SNPs and RA in Indians

In this stratification, the heterogeneity was resolved. The rs1800450 (T vs C, OR = 0.31, 95% CI: 0.21-0.46, $P_{OR} < .0001$) was reversely associated with RA in an Indian population (Table 2, Figure 2D). Meanwhile, the reverse association was maintained in the dominant model (TT + TC vs CC, OR = 0.31, 95% CI: 0.21-0.46, $P_{OR} < .0001$) (Table 2, Figure 3D).

3.6 | MBL SNPs and RA in Caucasians

In this meta-analysis, 5 studies involved rs1800450 and pooled MBL-A/O polymorphisms to research the association with RA in Caucasians. However, the results showed that no association between any MBL polymorphism with RA susceptibility was confirmed in Caucasian ($P_{OR} > .05$) (Table 2, Figures 2, 3 and 4B).

3.7 | Comparing allele frequency of MBL SNPs to the 1000 genome phase 3 population

We compared allele frequencies of different ethnicities in our meta-analysis to 1000 genome allele frequencies in Table 3. In view of the sample size and population, the allelic frequencies of MBL polymorphisms in this meta-analysis were consistent with the allelic frequencies in the 1000 Genome Project East Asian ancestry and Caucasians.

TABLE 3 The allele frequency comparison between the meta-analysis and 1000 Genomes Project

		Meta-analysis (alleles frequencies)				1000 genomes (alleles frequencies)	
		Case		Control			
Polymorphisms	Populations	C	T	C	T	C	T
rs5030737	Brazilian	NA	NA	NA	NA	1.00	0.00
	Caucasian	NA	NA	NA	NA	0.94	0.06
	East Asian	1.00	0.00	0.98	0.02	1.00	0.00
	Indian	0.92	0.08	0.95	0.05	0.97	0.03
	All	0.96	0.04	0.97	0.03	0.97	0.03
rs1800450	Brazilian	0.79	0.21	0.84	0.16	0.85	0.15
	Caucasian	0.88	0.12	0.86	0.14	0.86	0.14
	East Asian	0.82	0.18	0.82	0.18	0.85	0.15
	Indian	0.94	0.06	0.81	0.19	0.78	0.22
	All	0.84	0.16	0.83	0.17	0.88	0.12
rs1800451	Brazilian	NA	NA	NA	NA	0.97	0.03
	Caucasian	NA	NA	NA	NA	0.99	0.01
	East Asian	NA	NA	NA	NA	1.00	0.00
	Indian	0.96	0.04	0.95	0.05	0.98	0.02
	All	0.96	0.04	0.95	0.05	0.92	0.08
rs11003125	Brazilian	NA	NA	NA	NA	0.47	0.53
	Caucasian	NA	NA	NA	NA	0.61	0.39
	East Asian	0.49	0.51	0.55	0.45	0.55	0.45
	Indian	0.35	0.65	0.37	0.63	0.60	0.40
	All	0.45	0.55	0.51	0.49	0.69	0.31
rs7096206	Brazilian	0.82	0.18	0.83	0.18	0.13	0.87
	Caucasian	NA	NA	NA	NA	0.22	0.78
	East Asian	0.79	0.21	0.80	0.20	0.19	0.81
	Indian	0.71	0.29	0.73	0.27	0.13	0.87
	All	0.78	0.22	0.80	0.20	0.20	0.80
MBL-A/O	Brazilian	0.73	0.27	0.77	0.23	NA	NA
	Caucasian	0.70	0.30	0.71	0.29	NA	NA
	All	0.72	0.28	0.74	0.26	NA	NA

Abbreviations: C, represent wild-type allele; T, represent minor allele.

However, there was distinction between the allele frequencies in Indians and the 1000 Genomes Project. Meanwhile, allele frequency of rs7096206 was inconsistent in any ethnicity compared to the 1000 Genomes Project.

3.8 | Publication bias and sensitivity analysis

Begg's funnel plot and Egger's test were performed to estimate publication bias (Figure 5A-D). No evidence of publication bias for MBL gene polymorphisms under the allele genetic model was found in any ethnicity. In addition, no significant difference was found in the Egger's test, suggesting no obvious bias of publication in the present meta-analysis. We also conducted sensitivity analysis to assess the influence of individual studies on the pooled ORs. We found the pooled OR was not substantially altered, when a single study involved in the meta-analysis was deleted each time (Figure 6A-D).

4 | DISCUSSION

The comprehensive meta-analysis confirmed that the biological roles of 5 loci in different ethnicities were distinct. It was verified that the structural polymorphisms in exon 1 of MBL gene may significantly contribute to susceptibility and development of RA in Brazilian and Indian populations, whereas the functional polymorphisms in the promoter region were more likely to associate with RA in East Asians.

MBL was structurally and functionally similar to C1q, and shared the same phagocytic receptor on phagocytes, platelets, and endothelial

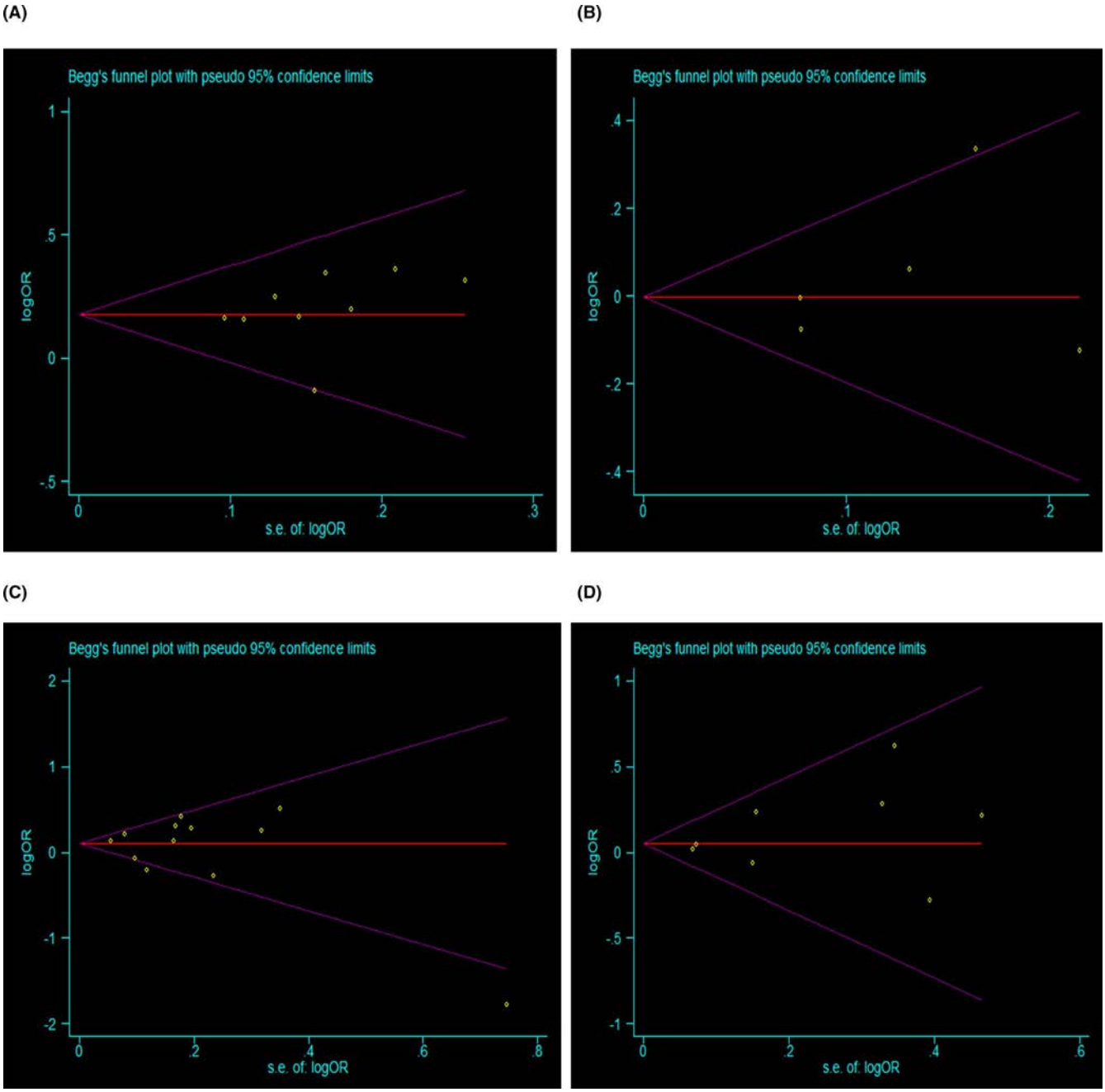

FIGURE 5 Begg's funnel plot of publication bias in the meta-analysis of the association of MBL gene polymorphisms with RA risk. A, MBL gene polymorphisms and RA in Brazilian. B, MBL gene polymorphisms and RA in Caucasian. C, MBL gene polymorphisms and RA in East Asian. D, MBL gene polymorphisms and RA in Indian

cells.[54] MBL plays a key role in the innate immune system by activating complements and macrophages, and by inducing opsonization. MBL mediates lectin-dependent activation of the complement pathway, and resembles C1q in terms of structure and function.[55] Low serum levels of MBL may result in impaired opsonization of complement-containing immune complexes.[56] The activation of MBL variants could contribute to damage tissue and consequently to disease severity. Inversely, deficiencies of complement proteins may enhance autoimmunity.[15] Considering that, the lectin pathway is involved in the clearance of pathogens and apoptotic bodies that may act as potential autoimmune initiators, deficiencies of components could enhance susceptibility and severity of some rheumatic disorders.[36,57,58] The functional MBL exon 1 codon 54 (allele B), codon 57 (allele C), and codon 52 (allele D) variants cause structural changes of the MBL basic unit, producing a lower molecular weight protein and reduced serum MBL levels.[16] Besides the exon 1 variant alleles, SNPs at promoter −550 (allele L) and −221 (allele X) have been associated with low serum MBL levels.[48]

A low MBL level caused by *MBL* variant alleles has been associated with human immunodeficiency virus and hepatitis C virus infections, and with SLE.[13,15,59] Since MBL2 variants are the major

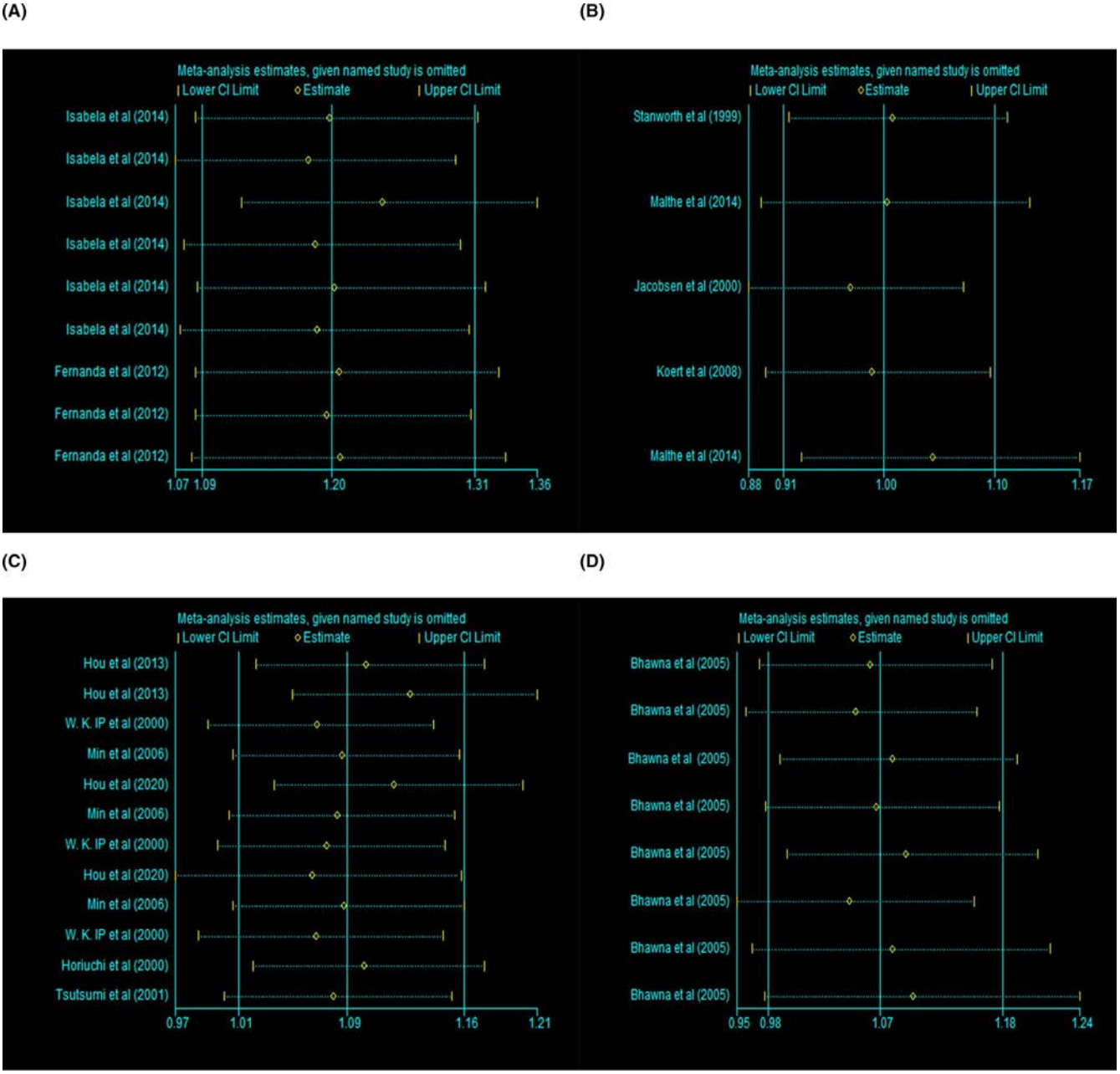

FIGURE 6 Sensitivity analysis to assess the stability of the meta-analysis. A, MBL gene polymorphisms and RA in Brazilian. B, MBL gene polymorphisms and RA in Caucasian. C, MBL gene polymorphisms and RA in East Asian. D, MBL gene polymorphisms and RA in Indian

determinants of MBL circulating levels, various studies demonstrated that variations on MBL serum levels seem to influence RA development and prognosis in different ways.[60] Although MBL2 low-producing polymorphisms were associated with increased susceptibility to RA, disease progression and clinical manifestations, the B variant was reported to confer protection against RA in an Indian population.[8] High producing genotype YA/YA conferred an increased risk of myocardial infarction and death in RA patients with ischemic heart disease.[61,62] Similarly, high producing MBL2 genotypes enhanced the risk of cardiovascular disease in patients with rheumatic fever.[54,63] Nevertheless, no association between RA and MBL2 polymorphisms was reported by others.[64]

In addition, a meta-analysis was conducted with 8 researches by others and this found \ a significant association between the *MBL* D allele and RA in the overall population (OR = 1.708, $P = .023$).[42] An association was also found between the *MBL* L allele and RA in the overall group (OR = 1.936, $P = .005$), as well as between the *MBL* X allele and RA in the overall group (OR = 1.582, $P = .001$). Their meta-analysis demonstrated an association between the MBL D, L, and X alleles and the risk of RA. However, the mixed ethnic population and limited sample size may make their results unreliable, or serious deviation from the real situation. Moreover, Stefanie et al.[35] also conducted a meta-analysis and the results showed that MBL2 low-producing OO and XX genotypes do not confer higher risk to

RA, even when data were analyzed according to the cohort's ethnicity. Due to the diversity of MBL2 alleles and divergent concepts about high and low-producing genotypes, they analyzed first only exon 1 polymorphisms and classified the data according to the presence of AA, AO and OO genotypes. Of course, so far, some research had reported that there was no association found between rs1800450 and RA, which was contradictory with our findings.[43] It is normal that such distinct consequences were obtained in separated studies. RA is considered to be a common multifactor autoimmune disease due to its complicated pathogenesis. It was validated that body mass index (BMI) and smoking will significantly contribute to susceptibility and development of RA. In addition, the gender difference was the key role in RA morbidity. However, lack of BMI level in participants might lead to inconsistent results. These phenomena and discrepancies need further investigation on the basis of large sample size. Moreover, the concentration of MBL may be regulated by other mechanisms than by variants on the MBL2 gene; additional studies including both polymorphisms and functional assays could give a better insight into the relationship between MBL and RA.

Although we revealed some new discoveries in this study, there were still several limitations which should be taken into consideration. In our study, the overall sample size is large, but the size of each study is relatively small; the smallest sample is 50 cases and 48 controls, and we need numerous data to validate the relationship between MBL SNPs and RA for further study in Caucasian populations. Second, in stratification analysis, the number of studies included in each ethnicity was unbalanced, some just for one study. Additionally, we are unable to analyze the actual impact of immanent factors on RA because of the incomplete data. Meanwhile, how the interaction of genes with environmental factors and genes with dietary models relate to the risk of RA is unclear. Further efforts should be put on investigating the association of the functional mutations in the MBL gene with RA, and the interactions of potential gene-gene and gene-environment factors should be comprehensively analyzed.

5 | CONCLUSIONS

We conducted a meta-analysis to evaluate the effects of MBL polymorphisms (rs1800450, rs1800541, rs5030737, rs11003125, rs7096206) on the risk of RA. The structural polymorphisms in exon 1 of MBL gene may significantly contribute to susceptibility and development of RA in Brazilian and Indian populations, whereas the functional polymorphisms in the promoter region were more likely to associate with RA in East Asians. Meanwhile, the reverse association between rs5030737 with RA in East Asians was displayed. However, the polymorphisms in exon 1 of MBL gene lacked the connection with RA.

CONFLICT OF INTERESTS

The authors declare they have no competing interests.

AUTHOR CONTRIBUTIONS

KQL and JJX made substantial contributions to the conception; JJX designed the work; GC, ZY and MCQ interpreted data; WTT for the main data analysis; XBZ created new software used in the work. LZ and YMZ drafted the work or substantively revised it. All authors reviewed and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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REVIEW

Health-related quality of life in rheumatoid arthritis: Systematic review and meta-analysis of EuroQoL (EQ-5D) utility scores from Asia

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Abstract

Introduction: Region-specific health-related quality of life (HRQoL) scores or utility values are representative and pivotal for economic evaluations as they are influenced by the value judgment of the local population. This study systematically reviewed and pooled EuroQoL-5 Dimension (EQ-5D) utility scores of rheumatoid arthritis (RA) across primary studies from Asia.

Methods: Studies reporting EQ-5D utility scores among adult RA patients from Asian countries were systematically searched in PubMed-Medline, Scopus and Embase since inception through February 2020. Selected studies were systematically reviewed and study quality assessment was performed. Meta-analysis was performed using a random-effect model with subgroup and meta-regression analysis to explore heterogeneity.

Results: Among 1391 searched articles, 37 studies with 31 983 participants were systematically reviewed and meta-analysis was conducted among 31 studies. The pooled EQ-5D scores and EQ-5D visual analog score were 0.66 (95% CI 0.63-0.69, $I^2 = 99.65\%$) and 61.21 (50.73-71.69, $I^2 = 99.56\%$) respectively with high heterogeneity. For RA patients with no, low, moderate and high disease activity based on Disease Activity Score (DAS)-28, the pooled EQ-5D scores were 0.78 (0.65-0.90), 0.73 (0.65-0.80), 0.53 (0.32- 0.74), and 0.47 (0.32-0.62), respectively. On meta-regression, age of patients ($P < .05$) was positively associated and use of glucocorticoids ($P < .05$) was inversely associated with utility values.

Conclusion: Lower EQ-5D scores were associated with severe disease activity, increasing age and female gender among RA patients. The study provides pooled EQ-5D scores for RA patients that are useful inputs for cost-utility studies in Asia.

KEYWORDS

EQ-5D-3L health utility, EQ-5D-5L, rheumatoid arthritis

Prospero registration: PROSPERO 2020 CRD42020165263

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease that is associated with pain and swelling in the wrist, elbows, knees, ankles and small joints of the hands and feet.[1,2] The condition is progressive with constant pain and often disabling which limits the individual's daily activities.[1] Thus, the quality of life (QoL) of RA patients is highly compromised in several domains, such as physical health, level of independence, environment and personal beliefs.[3]

In clinical trials, patient-reported outcomes and QoL measures obtained using health-related quality of life (HRQoL) instruments are often considered to study the impact of any intervention on RA patients.[4-8] QoL can be calculated using several generic (EuroQoL 's Five-Dimensional Questionnaire [EQ-5D], Short Form [SF]-6D, Health Utilities Index)[9] and disease-specific questionnaires (Rheumatoid Arthritis Quality of Life).[10] EQ-5D is one of the most commonly used HRQoL instruments and is also a preferred method for evaluating health state utilities involved in health technology assessment (HTA) as recommended by the National Institute for Health and Care Excellence (NICE).[11] The EQ-5D is a descriptive and visual analog scale (EQ-5D-VAS) framework that measures health status in 5 dimensions, such as mobility, self-care, usual activities, pain/discomfort and anxiety/depression.[12-14] Each dimension has 5 (EQ-5D-5L) levels (earlier 3 levels EQ-5D-3L) of responses the scores of which are converted to a single summary index number (utility) ranging from 0 (death) to 1 (perfect health), but values below 0 are possible and reflect health states considered worse than death. On a vertical VAS of 0 to 100, the EQ-5D-VAS reports the patient's self-assessed health.[15]

In recent decades, several Asian countries, including India, have moved toward evidence-based healthcare decision-making, witnessing a rapid rise in the number of cost-utility analyses (CUA) targeting cancer (24.6%), infectious diseases (13.7%), cardiovascular diseases (8.6%), and musculoskeletal and rheumatological diseases (5.7%).[16-19] Health state utility values are pivotal for CUA for which local utility data is preferred to obtain more robust results. Previous systematic reviews were primarily based on the EQ-5D studies from Central and Eastern European countries,[20] or from Australia.[21] Further, the existing systematic reviews[20,21] have not explored the utility scores of RA unique to different disease activities. Despite numerous primary studies on RA-EQ-5D in Asian countries,[22-24] a SRMA (Systematic Review and Meta-Analysis) specific to Asian countries is lacking. SRMA could provide more precise, representative regional estimates as well as improve the generalizability of individual study findings across Asian countries. Therefore, we systematically reviewed literature to identify EQ-5D utility scores of RA from Asia to provide a pooled estimate of EQ-5D utility and EQ-5D VAS scores. Additionally, we aimed to pool EQ-5D values separately based on disease severity to bridge the information gap in this respect.

2 | METHODS

We conducted a systematic review adhering to the guidelines of Preferred Reporting Items of Systematic reviews and Meta-Analysis[25] and the protocol was registered at PROSPERO (PROSPERO ID: CRD42020165263). Studies reporting EQ-5D utility scores among adult RA patients from Asian countries were systematically searched with no language restrictions using key terms from PubMed-Medline, Scopus and Embase databases since inception through February 2020. In order to develop the key search terms, we used PICO approach (ie population [adult RA patients], intervention [none], comparator [none] and outcome [EQ-5D utility]). Conventional sensitivity and precision maximizing strategy was adopted during the selection of studies. Detailed search terms and search strategies are reported in Appendix A. The last search was performed on 5 February 2020. In line with the objectives, studies from Asia that reported EQ-5D scores among adult patients with RA were included in the systematic review. Studies involving other arthritis, not having sufficient information, were excluded. Lastly, reviews, commentaries, letters to editors, editorials, conference abstracts and methodological papers were exempted from the review.

2.1 | Screening and reviewing of studies

The reviewers (MH, MK and BSB) independently performed the title and abstract screening of the studies obtained from electronic databases for their inclusion using the Rayyan-web app for systematic reviews.[26] At least 2 of 3 authors (MH, MK and BSB) independently reviewed all the full text of articles identified during screening. The final list of studies that met the inclusion criteria was prepared on the mutual agreement of authors after eliminating duplicates.

2.2 | Data collection, extraction, analysis and management

From included studies, relevant information required to achieve the proposed objectives including participant details and characteristics (study design, age and gender distributions, medication details, body mass index, rheumatoid factor [RF], C-reactive protein [CRP], Disease Activity Score of 28 joints [DAS-28], comorbidities, etc.) were extracted using a data extraction form prepared on Microsoft Excel (Version 2016). In addition, author names, study title, year of publication, study period, sample size and contact details of corresponding author and so on, were recorded. Data on central tendency (mean/median) and dispersion (SD) / (SE) / interquartile range (IQR) / 95% confidence interval (CI) for the primary outcome variable EQ-5D utility scores and other available parameters such as age, disease duration, DAS-28 score were independently extracted from included studies.

2.3 | Risk of bias assessment

Risk of bias was assessed using the Newcastle–Ottawa Scale (NOS) for cohort and case-control studies,[27] AXIS tool for cross-sectional

studies[28] and Version 2 of the Cochrane risk of bias tool for randomized trials (RoB-2) for randomized control trials (RCTs).[29] NOS assesses the risk of bias for subject selection, comparability and the assessment of outcome/exposure in case-control and cohort studies. A study with a score ≥ 7 is considered to be of high quality, although not a standard criterion.[27] AXIS tool consists of 20 components with 3 responses including Yes, No and Do not know.[28] RoB-2 assesses the risk of bias in RCTs in the 5 domains including randomization process, deviation from intended intervention, missing outcome data, measurement of outcome and selection of reported results.[29] Two authors (MH and MK) independently assessed the quality of included studies, and disagreements were resolved by consensus.

The effect size, the means of EQ-5D utility scores were pooled. Heterogeneity among included studies was assessed using visual inspection of forest plots, the Cochran-Q test and I^2 statistics. Pooled results with $I^2 > 25\%$ or Cochrane-$Q > 0.1$ were suggestive of heterogeneity. The random-effects model with the DerSimonian and Laird method was used if heterogeneity was present; otherwise, a fixed-effect model was employed.[30] Further, on observing heterogeneity, meta-regression involving the available confounders (age of patients, disease duration, the proportion of patients on glucocorticoid /methotrexate/biological therapy) was explored to identify the source of heterogeneity and bubble plots were generated. A particular variable included in the meta-regression model was regarded as a possible source of heterogeneity when the I^2 was reduced by ≥50%.

Publication bias was assessed using funnel plot (asymmetry) or Egger's test ($P < .05$) when more than 10 studies were available to measure the pooled effect[31,32] (Figure S2). Further, subgroup analysis was performed to investigate the influence of study design, disease activity, EQ-5D-5L or EQ-5D-3L and the country of the participants. However, the subgroup analysis was conducted only if sufficient (at least ≥ 2) studies were available for each subgroup. The changes in the heterogeneity (I^2) were considered to decide (interpret) the influence of particular subgroup analysis. Data was recorded using a Microsoft Excel sheet (version 16) and analysis was performed using Stata version 16 (2019).[33] All results were considered statistically significant at $P < .05$, except for the subgroup analysis and heterogeneity test, wherein $P < .10$ was regarded as significant. Lastly, a separate analysis was conducted by pooling of means of EQ-5D based on separate groups such as disease activity, gender, disease duration, rheumatoid factor (RF) positivity, glucocorticoid users, biologics users, as reported in the primary literature.

3 | RESULTS

The electronic search retrieved 1391 articles. After the removal of duplicates, screening of abstracts and titles, 146 studies were considered for full-text review. After full-text scrutiny with inclusion and exclusion criteria, 37 studies were selected for qualitative synthesis.[22-24,34-67] As 4 studies did not provide SDs for EQ-5D scores[43,44,64,67] and 2 studies did not provide the EQ-5D score,[46,66] the meta-analysis was performed with 31 studies. The selection of studies is described in the PRISMA flowchart (Figure 1) and characteristics of included studies is tabulated in Table 1. Studies included 31 983 participants with a mean age of 56.5 years. The study sample size ranged from 17 to 5317 participants (mean = 909, median = 221, IQR = 123, 817). Seventeen studies (46%) were conducted in Japan, 7 studies (19%) in Republic of Korea, 4 studies (11%) in China, 4 studies in Thailand (11%), 2 studies (5%) in India, 1 each (2.5%) from Russia, Taiwan, and Turkey. Fifteen out of 37 studies were cohort studies, 14 were cross-sectional, 7 were case-control and 1 was a RCT. Twenty-one out of 37 studies assessed the utility scores using the EQ-5D-3L and 5 studies used the EQ-5D-5L. Eleven studies did not mention the EQ-5D 3L/5L version.[22,35,38,42,44,45,51-54,59] In 15 out of 37 studies, RA patients were diagnosed using the American College of Rheumatology (ACR) 1987 criteria[68] and 22 studies did not mention the diagnosis criteria for RA. The median RA disease duration available from 26 studies was 9.4 (7.2-9.9) years. Among the included studies, comorbidities were reported only in 22% (8/37) of the studies, with diabetes mellitus (3%-10%) and hypertension (10%-45%) being the most predominant ones. The other comorbidities reported were osteoporosis, stroke, coronary artery disease dyslipidemia, anxiety and depression.

3.1 | Risk of bias assessment

NOS scores for observational studies included in our systematic review ranged from 3 to 9 with a mean of 6.6 and a median of 6 (5.25-9). A summary of risk of bias as assessed using NOS for case-control and cohort studies is shown in Figures S3 and S4. Among case-control studies, 57% of the studies had a low risk of bias (score ≥ 7) and 43% of the studies had a high risk of bias (score ≤ 7) where "selection and definition of controls" were the questions with the lowest count of stars contributing to high risk of bias. Among cohort studies, the lowest count of stars was for the question "selection of non-exposed cohort", with 40% of studies showing a low risk of bias (score > 7); 41% of studies earned 2 stars for comparability with regards to age, gender and additional adjustment.

We appraised the methodological quality of 15 cross-sectional studies included in the systematic review using the AXIS tool. For 11 out of 20 questions related to aim of the study, study design, definition of the target population, measurement of appropriate outcome, tools used for measurement of outcomes, statistical significance, reproducibility of methods, description of basic data, description of results in the methods, justification of discussion and conclusion, ethical approval and informed consent, the response was "Yes" in all the studies, signifying a low risk of bias for these questions. Limitations of the study were discussed in 80% (12/15) of the studies. Information related to funding sources and conflicts of interest were present in 87% (13/15) of studies. The sample frame was taken from an appropriate population in 53% of studies (8/15) and details regarding the same were not clear in 27% (4/15) of studies. The selection process was likely to select participants in 27% (4/15) of

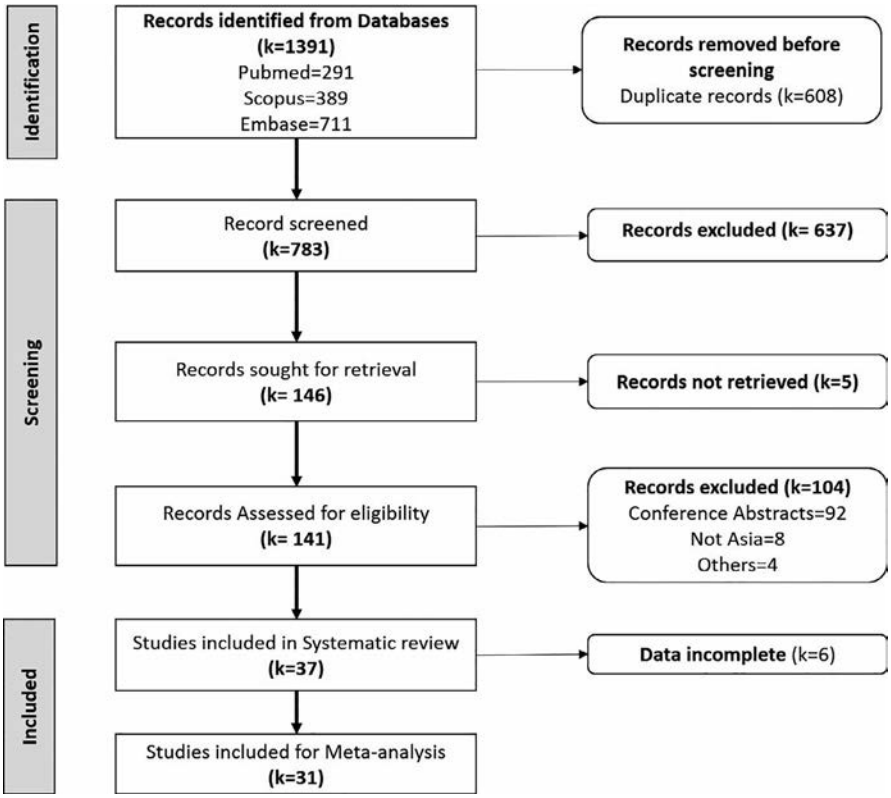


FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart of selection of studies. The flow chart illustrates the number of articles included and excluded at various steps

studies. Sample size justification, internal consistency of results and appropriate information about non-responders were given only in 20% (3/15) of studies, indicating a high risk of bias in these aspects. Only 2 studies (13%) had mentioned measures undertaken to address and categorize non-responders, whereas in one study (7%), the response rate raised concerns about non-response bias (Figure S3).

RoB-2 was used to assess the risk of bias in one RCT[60] included in the systematic review for which the risk of bias was low in all the 5 domains, including randomization process, deviation from intended intervention, missing outcome data, measurement of outcome and selection of reported results.

3.2 | Results of pooling

3.2.1 | Overall pooling of EQ-5D

Thirty-one studies reported utility scores of RA patients.[22-24,34-42,45,47-63,65] The pooled mean EQ-5D score was 0.66 (95% CI 0.63-0.69) with high heterogeneity $I^2 = 99.65\%$ (Figure 2A). The pooled EQ-5D VAS scores of RA patients were 61.21 (50.73-71.69) with high heterogeneity ($I^2 = 99.56\%$) which was reported in 9 among the 31 studies[11,22-24,36,40,52,54,57,60] (Figure 2B).

On subgroup analysis, the EQ-5D utility values significantly differed between different study designs, with EQ-5D values of case-control 0.65 (0.49-0.80), cohort studies 0.60 (0.54-0.66), cross-sectional studies 0.72 (0.68-0.75). On subgroup analysis the EQ-5D values significantly differed between RA patients with different severities with the observation of higher EQ-5D utility values with less severe disease activity such as high 0.52 (0.46-0.57), moderate 0.72 (0.67-0.77), low 0.74 (0.70-0.78), remission 0.82 (0.77-0.87) and among studies with no disease severity information 0.58 (0.50-0.65). On subgroup analysis the EQ-5D utility values significantly differed between the studies that used EQ-5D-3L 0.64 (0.60-0.67) and EQ-5D-5L 0.79 (0.70-0.87) among studies with no clear reporting of EQ-5D questionnaire 0.64 (0.57-0.70). On subgroup analysis the EQ-5D scores significantly differed between the countries in which studies were conducted such as India 0.40 (0.35-0.45), Japan 0.68 (0.65-0.71), Republic of Korea 0.67 (0.57-0.76), Russia 0.40 (0.35-0.45), Taiwan 0.67 (0.64-0.70), Thailand 0.81 (0.74-0.87) and China 0.58 (0.42-0.72) (Table 2a).

Meta-regression was performed with the utility as a dependent variable and age, disease duration and percentage of glucocorticoid, methotrexate and biologics users as independent variables. With age ($R^2\%$ <.01, coefficient = 0.127, SE = 0.002, $P < .05$) and glucocorticoids users ($R^2\% = 35.93$, coefficient = −0.004, SE = 0.001, $P < .05$) as independent variables, an inverse relation was observed with EQ-5D score. However, with an increasing percentage of methotrexate users ($R^2\% = 9.77$, coefficient = 0.002, SE = 0.001, $P = .05$), the EQ-5D scores improved but was not statistically significant. Disease duration and use of biologics showed no association with EQ-5D values (Figure S1).

Some of the studies reported EQ-5D values of specific subgroups of RA patients.[22,36,38] These were separately pooled if at least 3 studies reported such specific subgroup EQ-5D utility values. Three studies reported utility scores of male and female RA patients.

TABLE 1 Characteristics of included studies

Author_y	Country	Study design	Sample size (n)	Mean age, y	DAS-28 Mean ± SD	Disease duration Mean ± SD	RA treatment				
							Glucocorticoids users (%)	Methotrexate users (%)	Other csDMARDs users (%)	NSAIDs users (%)	Biologics users (%)
Anno_2018	Japan	Case-control	191	62.30 ± 12.6	2.80 ± 1.3	18.1 ± 11.7	20.4	80.6	17.2	NA	48.7
Asai_2019	Japan	Cross-sectional	49	62.00 ± 10.0	1.50 ± 0.4	11.0 ± 8.0	29	NA	16	NA	63
Bae_2018	Republic of Korea	Cross-sectional	2000	56.00 ± 11.8	NA	8.1 ± 6.9	69.1	NA	88.8 (including MTX)	74.9	11.3
Chen_2017	Taiwan	Cross-sectional	219	52.25 ± 12.9	NA	5.7 ± 3.3	NA	NA	NA	NA	NA
Cho_2013	Republic of Korea	Cross-sectional	225	53.20 ± 10.9	3.28 ± 1.2	13.0 ± 9.3	NA	NA	NA	NA	NA
Fukuda_2013	Japan	Cross-sectional	385	62.89 ± 12.5	3.53 ± 1.2	9.4 ± 9.2	NA	NA	NA	NA	NA
Ghosh_2012	India	Cohort	79	51.31 ± 2.1	NA	NA	NA	NA	NA	NA	NA
Hattori_2018	Japan	Cross-sectional	1005	63.20 ± 13.2	2.80 ± 1.1	13.6 ± 11.2	32.3	59.3	NA	NA	42.7
Hirata_2018	Japan	Cross-sectional	1455	65.00 ± 11.9	4.10 ± 0.9	7.7 ± 9.6	NA	NA	NA	NA	NA
Hoshi_2016	Japan	Cross-sectional	5043	59.67 ± 12.6	3.20 ± 1.2	11.0 ± 9.6	50.9	63.6	91.4	64.1	4.4
Hu_2017	China	cross-sectional	133	60.39 ± 12.0	NA	NA	NA	NA	NA	NA	NA
Ishikawa_2017	Japan	Cohort	119	62.00 ± 17.3	3.99	14.0 ± 44.8	60	68	NA	NA	19
Ishikawa_2019	Japan	Cohort	276	64.00 ± 17.5	3.12 ± 1.0	16.0 ± 59.8	63	64	NA	NA	25
Kang_2019	Republic of Korea	Cross-sectional	45	55.80 ± 12.8	NA	6.1 ± 6.3	NA	NA	NA	NA	NA
Katchamart_a_2019	Thailand	Cross-sectional	464	59.15 ± 11.4	3.50 ± 0.8	11.5 ± 8.3	NA	NA	NA	NA	NA
Katchamart_b_2019	Thailand	Cross-sectional	443	59.70 ± 11.7	3.15 ± 1.1	8.4 ± 11.5	NA	NA	NA	NA	NA
Kim_2016	Republic of Korea	Cross-sectional	3557	55.28 ± 11.8	2.86 ± 1.1	9.7 ± 7.5	NA	NA	NA	NA	NA
Kojima_a_2018	Japan	Cohort	126	65.40 ± 10.7	3.04 ± 1.0	17.4 ± 11.8	NA	57.9	NA	NA	29.6
Kojima_b_2018	Japan	Cross-sectional	435	64.20 ± 11.8	NA	17.1 ± 10.8	56.9	59.1	NA	NA	23
Li_2017	China	Cohort	49	59.90 ± 11.9	5.75 ± 1.2	NA	NA	NA	NA	NA	NA
Lipina_2019	Russia	Case-control	138	41.70 ± 14.3	NA	8.7 ± 6.6	NA	NA	NA	NA	NA
Munchey_2018	Thailand	Case-control	221	57.83 ± 9.3	NA	4.0 ± 4.0	32.6	82.81	17.20	32.6	NA
Nakajima_2015	japan	Cohort	5317	61.07 ± 12.5	3.00 ± 1.0	8.3 ± 9.6	43.1	70.4	80.9	57.9	11.7
Pal_S_2019	India	Cohort	211	43.95 ± 11.6	5.86 ± 1.0	4.8 ± 4.0	77.73	14.22	NA	NA	NA
Park_EH_2019	Republic of Korea	Case-control	120	58.30 ± 12.1	3.56 ± 1.0	7.6 ± 6.5	NA	NA	NA	NA	NA
Park_H_2017	Republic of Korea	Cohort	140	67.50 ± 9.9	NA	NA	NA	NA	NA	NA	NA
Paton_2018	China	Case-control	133	NA	NA	NA	NA	NA	NA	NA	NA
Seto_2013	Japan	Cohort	5038	59.40 ± 13.1	3.29 ± 1.14	13.2 ± 9.6	46.8	68.5	91	NA	8.7

(Continues)

TABLE 1 (Continued)

Author_y	Country	Study design	Sample size (n)	Mean age, y	DAS-28 Mean ± SD	Disease duration Mean ± SD	RA treatment				
							Glucocorticoids users (%)	Methotrexate users (%)	Other csDMARDs users (%)	NSAIDs users (%)	Biologics users (%)
Sung_2017	Republic of Korea	Cohort	1236	52.68 ± 12.7	5.39 ± 1.3	NA	87.14	86.08	NA	NA	NA
Taibanguay_2019	Thailand	RCT	119	56.50 ± 11.7	3.22 ± 1.0	8.0 ± 7.4	36.13	78.99	NA	NA	16.81
Takeuchi_2017	Japan	Cohort	1808	55.47 ± 13.4	4.79 ± 1.4	6.6 ± 7.9	NA	89.38	NA	NA	NA
Tanaka_a_2018	Japan	Cohort	629	58.87 ± 13.5	5.01 ± 1.2	6.0 ± 8.3	NA	81.72	NA	NA	NA
Tanaka_b_2020	Japan	Cohort	357	58.00 ± 13.8	5.35 ± 1.2	6.9 ± 9.6	37.3	70.9	NA	NA	NA
Uehara_a_2013	Japan	Cohort	25	62.20 ± 7.8	NA	15.7 ± 8.0	NA	NA	NA	NA	NA
Uehara_b_2013	Japan	Cohort	17	61.00 ± 9.0	NA	16.8	NA	NA	NA	NA	NA
Ulutatar_2018	Turkey	Case-control	106	50.97 ± 11.1	NA	NA	NA	NA	NA	NA	NA
Zhang_2017	China	Case-control	70	40.13 ± 15.4	NA	NA	NA	NA	NA	NA	NA

Abbreviations: csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; DAS-28, Disease Activity Score of 28 joints; NA, not available; NSAIDs, nonsteroidal anti-inflammatory drugs; RCT, randomized controlled trial.

On analysis of pooled EQ-5D utility scores, male RA patients (0.74, 0.66-0.82) had better EQ-5D scores than female RA patients (0.65, 0.53-0.77).[22,36,38] Three studies reported the EQ-5D scores in RA with varying grades of disease activity which was determined based on the DAS-28 score.[22,24,36,38] The pooled EQ-5D scores of RA patients with no, low, moderate and high disease activity were 0.78 (0.65-0.90), 0.73 (0.65-0.80), 0.53 (0.32-0.74) and 0.47 (0.32-0.62) respectively (Figure 3). Three studies reported the EQ-5D scores of RA patients who were RF positive (0.65 [0.51-0.80]) and RF negative (0.67 [0.56-0.78]) which showed high heterogeneity and did not differ significantly.[22,36,38] The pooled EQ-5D scores of RA disease duration < 5 years (0.66, 0.58-0.75), 5-10 years (0.67, 0.55-0.80) and >10 years were similar.[22,24,38] Three studies had reported the EQ-5D values of RA patients under the treatment of corticosteroids and biologics.[22,36,58] The pooled EQ-5D scores were 0.68 (0.63-0.73) and 0.67 (0.61-0.74) for corticosteroids and biologics respectively (Table 2b).

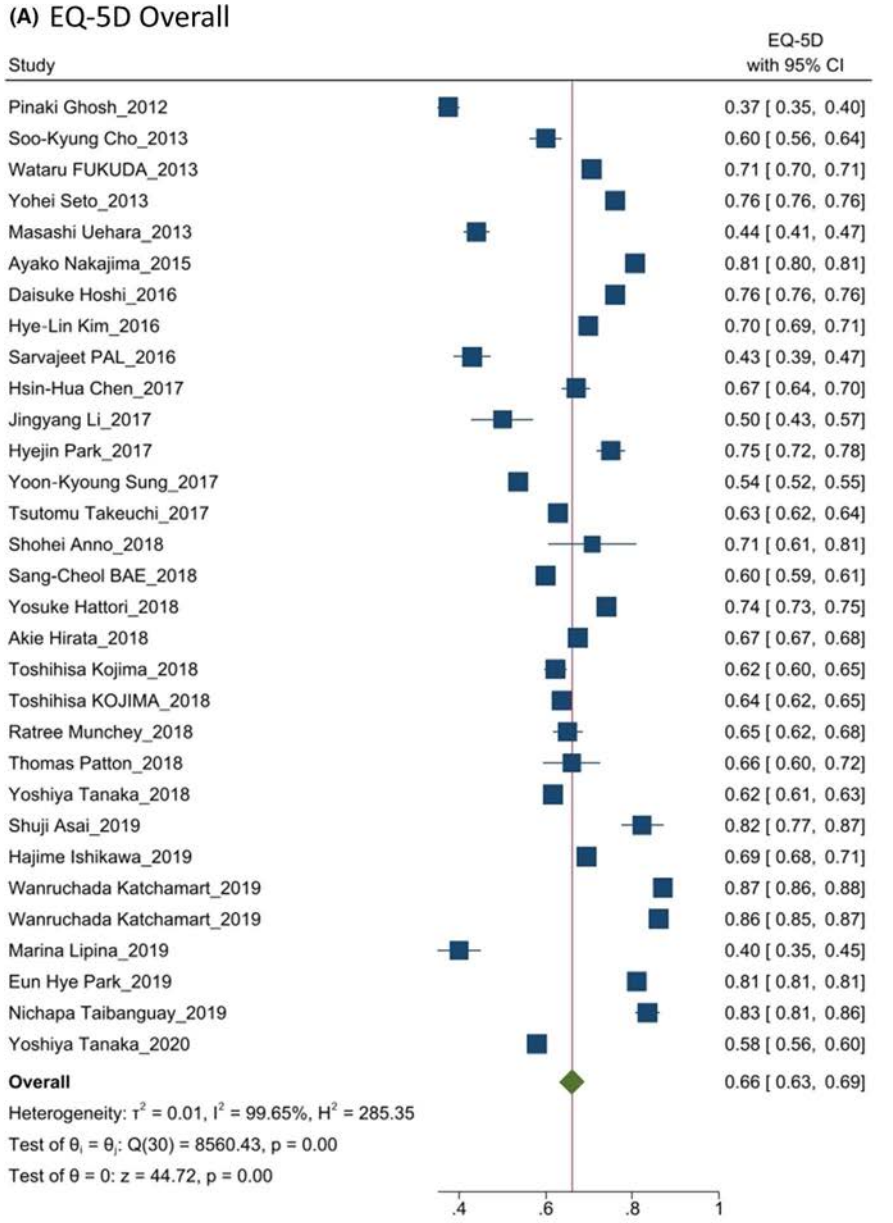
4 | DISCUSSION

The present systematic review and meta-analysis was conducted to synthesize the evidence on EQ-5D utility scores of RA from Asia and provide the pooled estimate of EQ-5D utility value as well as the EQ-5D VAS. We have also synthesized EQ-5D values for various subgroups among RA patients such as based on varying grades of disease, gender, duration of illness, RF positivity status, to bridge the knowledge gap in this respect. We observed that the utility of RA patients with high disease activity (DAS-28 ≥ 5) was very low compared to the overall utility.

In general, health utilities may be influenced by various factors, including age, gender, sample source, comorbidities, urban/rural, and so on.[69] The utility values tend to decrease with increasing age as the health status gradually declines as people grow older. To explore the influence of age on utility, we conducted meta-regression analysis, which revealed an inverse relation of age with EQ-5D score as observed in other studies.[70] In most of the studies that reported on gender-specific health utility values, it was noted that men had a better HRQoL compared to women.[70-72] Our synthesized evidence also supports this observation where male RA patients had better EQ-5D scores than female RA patients.

Apart from the demographic factors, comorbidity also has an important role in the variation of health utility values. It has been evidenced by previous findings that the value of utility in people suffering from a disease further drops with other comorbid conditions.[69] However, in our systematic review, we were not able to examine the impact of comorbidities on the utility value of RA as most of the study participants had multimorbidity. Thus, it was not possible to identify RA only patients to compare the utilities between RA only and RA with comorbidities.[73]

Disease activity is the most significant factor that could affect the utility of RA patients. For RA, a single utility value may not be sufficient, as RA patients suffer from multiple grades of disease



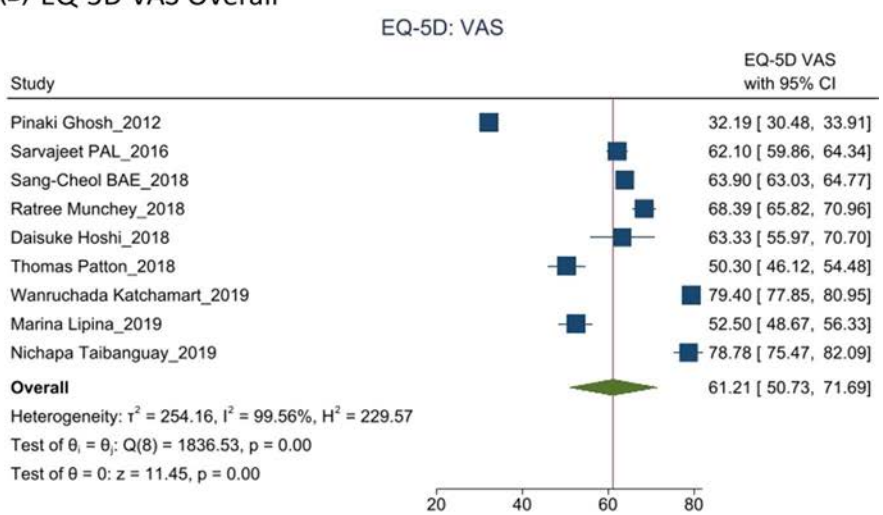


FIGURE 2 Forest plot of EuroQoL 's Five-Dimensional Questionnaire (EQ-5D) utility and EQ-5D visual analog score (VAS) scores for rheumatoid arthritis (RA). A, Pooled overall EQ-5D scores for RA. B, Pooled EQ-5D VAS scores for RA

TABLE 2 Results of EuroQoL 's Five-Dimensional Questionnaire (EQ-5D) among rheumatoid arthritis, subgroup analysis and meta-analysis based on patients' characteristics

Subgroups	Number of studies	EQ-5D mean (95% CI)	I^2 (%)	*P* value
a. Subgroup analysis				
Study design				
Case-control	5	0.65 (0.49, 0.80)	98.91	<.01
Cohort	13	0.60 (0.54, 0.66)	99.70	<.01
Cross-sectional	12	0.72 (0.68, 0.75)	99.48	<.01
RCT	1	0.83 (0.81, 0.86)		
Country				
India	2	0.40 (0.35, 0.45)	79.56	.03
Japan	15	0.68 (0.65, 0.71)	99.56	<.01
Republic of Korea	6	0.67 (0.57, 0.76)	99.78	<.01
Russia	1	0.40 (0.35, 0.45)		
Taiwan	1	0.67 (0.64, 0.70)		
Thailand	4	0.81 (0.74, 0.87)		
China	2	0.58 (0.42, 0.74)	90.73	<.01
Disease activity				
Remission	1	0.82 (0.77, 0.87)		
Low	8	0.74 (0.70, 0.78)	99.11	<.01
Moderate	9	0.72 (0.67, 0.77)	99.81	<.01
High	4	0.52 (0.46, 0.57)	94.02	<.01
No data on disease activity	9	0.58 (0.50, 0.65)	98.78	<.01
EQ-5D version				
EQ-5D-3L	19	0.64 (0.60, 0.67)	99.69	<.01
EQ-5D-5L	5	0.79 (0.70, 0.87)	99.15	<.01
Not available	7	0.64 (0.57, 0.70)	99.65	<.01
Overall	31	0.66 (0.63, 0.69)	99.65	<.01
b. Meta-analysis based on patient characteristics				
Gender				
Male	3	0.74 (0.66, 0.82)	95.66	<.01
Female	3	0.65 (0.53, 0.77)	99.57	<.01
Disease duration				
Less than 5 y	3	0.66 (0.58, 0.75)	95.86	<.01
5-10 y	3	0.67 (0.55, 0.80)	97.79	<.01
More than 10 y	3	0.64 (0.52, 0.76)	96.46	<.01
Rheumatoid factor (RF)				
RF positive	3	0.65 (0.51, 0.80)	99.61	<.01
RF negative	3	0.67 (0.56, 0.78)	98.23	<.01
Rheumatoid arthritis treatment				
Biologics	3	0.67 (0.61, 0.74)	95.73	<.01
Corticosteroids	3	0.68 (0.63, 0.73)	99.06	<.01

activity that have an immense effect on their QoL. Thus, the EQ-5D values should be unique for no (remission), low, moderate, or high RA disease. DAS-28 is the most frequently used tool for measuring disease activity in RA, while Simple Disease Activity Index and Crohn Disease Activity Index are other indicators of disease activity reported in included studies. We categorized as remission, low, moderate and high disease activity based on the mean DAS-28 scores and did a subgroup analysis to identify the EQ-5D values specific for disease activity. Since the subgroup analysis is carried out with mean DAS-28 values reported in individual studies, the pooled

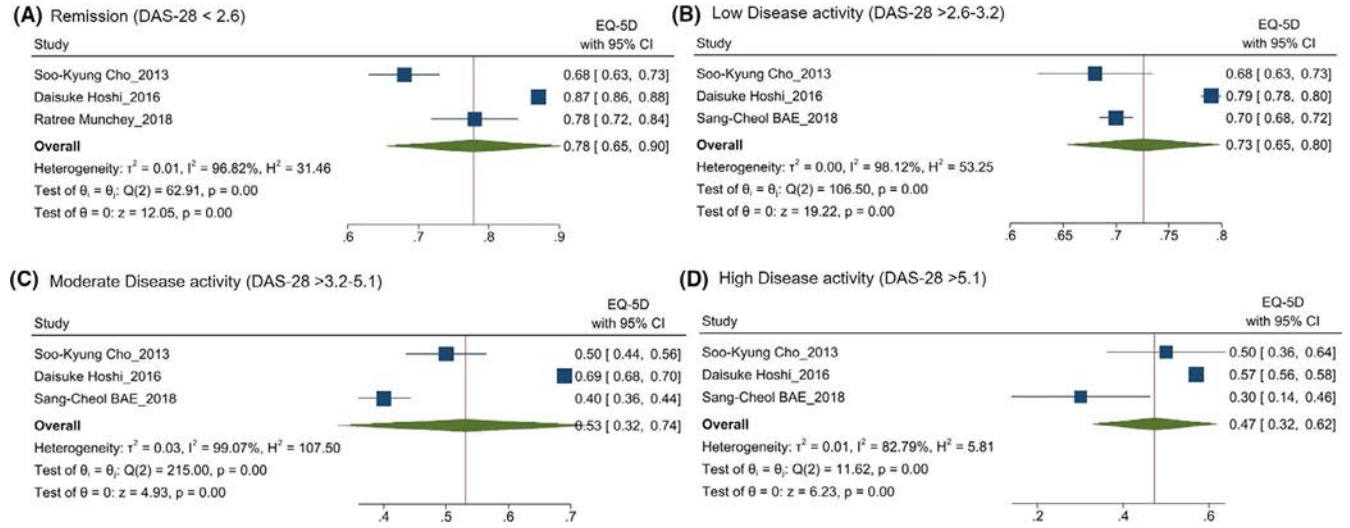


FIGURE 3 Forest plot of EuroQoL 's Five-Dimensional Questionnaire (EQ-5D) utility scores for different disease activities. Pooled mean EQ-5D utility scores in RA for (A) no (remission), (B) low, (C) moderate and (D) high disease activity based on Disease Activity Score of 28 joints (DAS-28)

EQ-5D value may not be truly representative of the disease activity of that sample. From the measures of dispersion reported in the individual studies, it is observed that the participants in the sample might not be homogenous for particular disease activities. However, few studies have recorded separate EQ-5D values for no (remission), low, moderate, or high RA disease activity within the included studies. It is also presumed that these values for disease activity would have been derived from a homogeneous population. Therefore, we conducted a separate meta-analysis of these EQ-5D values specific to different grades of disease activity. The utility of remission and low activity disease states were comparable, with the lowest utility value in patients with high disease activity.

EQ-5D has the advantage of being able to quantify quality-adjusted life years (QALY), which is important for conducting cost-utility analysis. Although SF-36 is often used to measure QALY, the simplest and most commonly used HR-QoL tool is EQ-5D.[73] In other countries, there are several CUAs that depend on EQ-5D to evaluate the cost-effectiveness of RA treatment strategies.[74,75]

In our study, the differences in utility values observed across the countries could be attributed to the difference in disease activities within the population. However, the studies were insufficient to pool EQ-5D values for various disease activities unique to a country when the DAS-28 score was not reported in all the studies. The inter-country differences could also be owing to the socio-cultural and other variations prevailing across countries. However, a recent study found that there is no significant correlation between cultural variables and differences in health utilities across countries.[76] Further, the majority of the studies were from Japan, thus the need to be cautious while using the overall pooled estimates for developing Asian countries; however, individual country-based pooled estimates could be used. Further, concerning the EQ-5D scores, the degree of heterogeneity was very high, making it less accurate for meta-analysis, which could be seen as an important limitation. The majority of the included studies did not mention the diagnostic criteria for RA. Lastly, as most of the included studies were single-arm interventional studies without control groups, the risk of bias for such studies was high.

In summary, study results will be a source of evidence while conducting cost-utility studies in Asia for the RA utility values. Future studies on health utilities in RA should consider disease activity as an important factor and aim at precise utility estimates for disease activity.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Haridoss M: conceptualization, data curation, formal analysis, original draft; Bagepally BS: conceptualization, data curation, formal analysis, inputs on original draft investigation, methodology, software, review and editing; Meena kumari N: data curation, formal analysis, review and editing.

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SUPPORTING INFORMATION

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

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APPENDIX A

Search Results:

	PubMed	Hits
Population	Search ("Arthritis, Rheumatoid"[Mesh] OR "Rheumatoid Arthritis" OR Rheumatoid)	153 167
Outcome	Search (EQ-5D OR "Euroqol 5 Dimension" OR Euroqol OR "utility score" OR "utility value")	10 528
P & O	Search ("Arthritis, Rheumatoid"[Mesh] OR "Rheumatoid arthritis" OR Rheumatoid) AND (EQ-5D OR "Euroqol 5 Dimension" OR Euroqol OR "utility score" OR "utility value")	291
	Scopus	**Hits**
P & O	(TITLE-ABS-KEY (*"Rheumatoid arthritis"* OR *rheumatoid*) AND TITLE-ABS-KEY (*eq-5d* OR *"Euroqol 5 Dimension"* OR *euroqol* OR *"utility score"* OR *"utility value"*))	389
	Embase	**Hits**
P & O	'rheumatoid arthritis':ti,ab,kw AND ('european quality of life 5 dimensions questionnaire':ti,ab,kw OR 'eq 5d score':ti,ab,kw OR 'eq 5d':ti,ab,kw OR euroqol:ti,ab,kw OR 'utility score':ti,ab,kw OR 'utility value':ti,ab,kw)	711

Section/topic	#	Checklist item	Reported on page #
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Yes, 1
Abstract			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Yes, 2
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Yes, 3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Yes, 4
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (eg, Web address), and, if available, provide registration information including registration number.	Yes, 4
Eligibility criteria	6	Specify study characteristics (eg, PICOS, length of follow-up) and report characteristics (eg, years considered, language, publication status) used as criteria for eligibility, giving rationale.	Yes, 4
Information sources	7	Describe all information sources (eg, databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Yes, 4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Yes, 4 & Appendix A
Study selection	9	State the process for selecting studies (ie, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Yes, 4
Data collection process	10	Describe method of data extraction from reports (eg, piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Yes, 5 & 6
Data items	11	List and define all variables for which data were sought (eg, PICOS, funding sources) and any assumptions and simplifications made.	Yes, 5 & 6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Yes, 6 & 7
Summary measures	13	State the principal summary measures (eg, risk ratio, difference in means).	Yes, 6 & 7

(Continues)

APPENDIX A (Continued)

Section/topic	#	Checklist item	Reported on page #
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (eg, I^2) for each meta-analysis.	Yes, 6 & 7
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (eg, publication bias, selective reporting within studies).	Yes, 6 & 7
Additional analyses	16	Describe methods of additional analyses (eg, sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Yes, 6 & 7
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Yes, 7, Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (eg, study size, PICOS, follow-up period) and provide the citations.	Yes, 7 & 8 Tables 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Yes, 7-8 & Sup fig 3 & 4
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Yes, 7-8, Table 1, Fig. 2-3
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Yes, 9-11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Yes, 9-11 Table 2 Supl Fig 2-4
Additional analysis	23	Give results of additional analyses, if done (eg, sensitivity or subgroup analyses, meta-regression [see Item 16]).	Yes, 8-11 & supl. Figs 1
Discussion			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (eg, healthcare providers, users, and policy makers).	Yes, 11
Limitations	25	Discuss limitations at study and outcome level (eg, risk of bias), and at review-level (eg, incomplete retrieval of identified research, reporting bias).	Yes, 13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Yes, 13
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (eg, supply of data); role of funders for the systematic review.	Yes, 14

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ORIGINAL ARTICLE

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Out-of-pocket spending among a cohort of Australians living with gout

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Abstract

Objective: To measure the direct and indirect out-of-pocket (OOP) costs borne by Australians with gout.

Methods: A cross-sectional, Australia-wide, web-based survey was conducted over 12 months between May 2017 and April 2018. Participants were recruited via advertisements in doctors' clinics and healthcare organizations' websites, and social media platforms such as Facebook and Twitter. Survey questions collected information about participants' OOP spending on direct medical and non-medical gout-related healthcare costs. Participant demographics, gout status, healthcare sought, workdays lost to due gout and health-related quality of life were also collected.

Results: Seventy-nine patients with gout completed the survey; 70 (89%) were male, and on average were 56 (SD 16) years of age and had gout for 14 (SD 12) years. For this cohort, the median total OOP direct medical cost was AU$200 per year (interquartile range [IQR]: AU$60-AU$570). Sixty (76%) people with gout reported being affected by gout during work; however, only 0.25 (IQR: 0-3) days of work (approximately $60) were lost due to gout in a year. Nine percent (n = 7) of participants experienced cost-related treatment attrition and 33% reported economic hardship (n = 26). Participants who experienced economic hardship or cost-related treatment attrition had higher median total gout-related direct costs than those who did not.

Conclusion: In Australia, gout has an OOP financial cost and reduces work productivity. The presence of cost-related treatment attrition among people with gout indicates that financial costs may be a significant barrier to seeking treatment for a subset of patients with gout.

KEYWORDS

cost of illness, gout, out-of-pocket costs

Nicholas Nathan and Amy D. Nguyen contributed equally to this paper.

1 | INTRODUCTION

Gout is a chronic disease of the joints characterized initially by recurrent inflammatory reactions or flares in response to the formation of urate crystals in joint spaces in individuals with high serum uric acid (sUA) concentrations.[1] It affects 0.08% (95% uncertainty interval [UI]: 0.07-0.08) of the global population,[2] and its global prevalence rose by 26% (95% UI: 25.2-27.7) between 2005 and 2015.[3] The prevalence of gout in Australia has been rising since 1968,[4] and between 2008 and 2013, at least 1 in 100 patients visiting general practitioner (GP) clinics in Australia had gout.[5] Gout usually presents as an acute flare, typically characterized by a hot, red, swollen and extremely painful joint with a reduced range of motion.[1] Over time, poor control of sUA concentrations can lead to the subcutaneous deposits of monosodium urate crystals, called tophi.[6,7] Patients with these tophi can have chronic pain, frequent gout flares and irreversible joint damage leading to joint deformities and long-term functional impairment.[1,6,8]

In the treatment of disease, both the patient and the physician have a role to play and in the case of gout, excellent guidance for physicians is available, for example from The European League Against Rheumatism.[9] However, from the patients' perspective there are factors preventing the optimal management of disease, such as the inability to afford treatment.[10,11] Cost-related treatment attrition (CRTA), which is when a patient fails to seek healthcare due to high costs, has previously been reported with other chronic illnesses in Australia.[10,12,13] A 2015 study of Australian patients with gout showed that cost was a factor preventing patients from seeking treatment for gout.[14] In order to investigate patients' access to successful treatment for gout, the overall cost borne by patients as a part of gout treatment must be measured. Furthermore, gout is a disabling chronic disease associated with work production losses[15] and patients in Australia pay proportionally more out-of-pocket (OOP) for healthcare than most OECD member countries.[16] Therefore, the aim of this study was to measure the direct and indirect costs to understand the extent of costs borne by Australian patients with gout.

2 | MATERIALS AND METHODS

2.1 | Study design

This was a cross-sectional study consisting of an open, web-based survey created using WorldApp KeySurvey (Appendix S1). It was a voluntary survey posted on a public website that was specifically created to host the survey without any other advertisements. The survey was in English and ran from May 2017 to April 2018. It included 63 questions which elicited information about participants' demographics, medical history, gout severity, quality of life, household financial status and healthcare expenditure. Elements of this survey were based on a questionnaire used in a cross-sectional study examining chronic kidney disease in Sydney in 2013,[13] the Australian "45 and Up" study,[17] the Brief Illness Perception Questionnaire,[18] and the Health Assessment Questionnaire – Disability Index which has been validated for gout.[19] Two questions from the gout-specific Work Productivity and Activity Impairment Questionnaire (WPAI)[15,20] were used as an indication of absenteeism and presenteeism. It was decided that this study would use a 12-month timeframe instead of 7 days as in the WPAI questionnaire, due to the infrequent nature of gout flares and concerns that many participants might not have had an episode of gout in the 7 days before they completed the survey. The survey questions were developed by a rheumatologist, health economist, qualitative researcher and medical student. It was pilot-tested with a gout patient and a clinical researcher at St Vincent's Hospital Sydney. Ethics approval was obtained from The University of New South Wales, Human Research Ethics Advisory Panel D: Biomedical (#HC16956). All data collected were held only by the researchers for the sole purpose of estimating the direct and indirect OOP costs (OOPC) borne by Australians with gout.

2.2 | Recruitment

GP clinics (n = 189), rheumatologists (n = 222) and pharmacies (n = 61) from every state in Australia were sent invitation letters via email and fax with information on the study and flyers for posting in practices/stores. Rheumatologists were identified from the Australian Rheumatologist Association (ARA) website.[21] A random sample of GPs and pharmacies were identified from every state in Australia via online search engines.

Non-profit organizations, including Arthritis Australia, the Australasian Rheumatology Association (ARA),[22] the Agency for Clinical Innovation,[23] MOVE muscle bone & joint health (currently known as Musculoskeletal Australia),[24] the Pharmaceutical Society of Australia[25] and Primary Health Networks[26] across Australia, publicized the study online and in print. Social media including Facebook and Twitter were also used to publicize the study. Advertisements were also placed on online classifieds sections such as Gumtree[27] and Backpage.[28]

Inclusion criteria comprised patients who were 18 years old and above, who were living in Australia and who had suffered at least 1 episode of gout in their lifetime. Participants self-reported their gout diagnosis and status. Participants provided informed consent before filling out the survey, and could opt to be placed in a draw to win a $200 voucher upon survey completion.

2.3 | Data analysis

Survey data were automatically placed in a Microsoft Excel data sheet. Surveys that provided primary outcome measures (ie, direct and indirect medical and non-medical costs) were included in the analysis. Duplicate entries were identified via participants' personal information. Survey completeness rate was 63%.

Total direct cost was calculated by summing the total OOP expenditure from various categories, including medical, transport and

TABLE 1 Participants' demographics and background characteristics[a]

	N = 79
Mean age, y, ± SD[b]	55.5 ± 15.7
Sex, n (%)	
Male	70 (89)
Female	9 (12)
Mean BMI, kg/m^2 ± SD	30.8 ± 7.01
BMI, kg/m^2, n (%)	
Underweight, <18.5	0 (0)
Normal, 18.5-24.9	18 (23)
Overweight, 25.0-29.9	22 (28)
Obese, ≥ 30.0	39 (49)
Comorbidities, n (%)	
Hypertension	40 (51)
Obesity	28 (35)
Hypercholesterolemia	27 (34)
Diabetes	12 (15)
Cardiovascular disease	11 (14)
Cancer	5 (6)
Chronic respiratory conditions	4 (5)
Others	7 (9)
ASGC Remoteness Area, based on participants' residential postal code, n (%)[c]	
RA1 – Major cities	54 (68)
RA2 – Inner regional	9 (11)
RA3 – Outer regional	12 (15)
RA4 – Remote	1 (1)
RA5 – Very remote	0 (0)
Participants' annual gross household income, n (%)[c]	
Under $20 000	5 (8)
$20 000-$29 999	1 (2)
$30 000-$39 999	2 (3)
$40 000-$49 999	4 (7)
$50 000-$59 999	4 (7)
$60 000-$69 999	3 (5)
$70 000-$79 999	7 (12)
$80 000-$89 999	6 (10)
$90 000-$99 999	4 (7)
$100 000 or more	24 (40)

Abbreviations: ASGC, Australian Standard Geographical Classification; BMI, body mass index; SD, standard deviation.

[a]Percentages may not add up to 100% due to rounding.

[b]n = 77, missing data for age = 5. **n = 76, missing data for ASGC Remoteness Area = 3.

[c]n = 60; participants who would rather not report = 13, participants who did not know their annual household income = 6.

home-care expenses due to gout. Direct costs are reported as totals for medical and non-medical costs. Absenteeism or productivity loss due to absence from work was determined by the number of workdays missed due to gout over the past 12 months. We valued absenteeism by multiplying the average number of workdays missed by the average adult weekly earning in November 2017 in Australia.[29] The extent of presenteeism or productivity loss while at work due to impairment caused by gout was determined by asking participants how much they felt that gout affected their work performance while they were at work on a scale of 1 to 10, with 1 being not affected at all, and 10 as the most severely affected. We could not value presenteeism with this measure because we did not capture the actual hours worked by participants over the past year.

Economic hardship was defined as the inability to pay for essentials and dissaving practices. Dissaving is spending an amount greater than the disposable income, resulting in using up savings, taking loans or incurring debt.[13] CRTA was defined as the inability to afford doctor consultations, medications, blood tests and other medical care for gout.

All data were processed with IBM SPSS Statistics for Macintosh, Version 25.0. (Released 2017. IBM Corp.). Descriptive analyses were performed and data are presented as the median (interquartile rang [IQR]) and mean (SD) for non-normal and normal distributed data, respectively.

Total direct cost was compared between participants who reported CRTA and those who did not, with non-parametric Mann-Whitney *U* and Kruskal Wallis tests and values were considered significant if $P < .05$, at 95% confidence interval (95% CI).

3 | RESULTS

3.1 | Participants' demographics and characteristics

Of the 131 responses received, 79 were included in the analysis (Appendix S2). Seventy (89%) of these participants were male with a mean age of 56 years (SD 16) (Table 1). Of the 60 participants who reported their annual household income, 32 (53%) were earning less than the median annual Australian gross household income level of $88 000.[30] The majority of participants were residing in major metropolitan cities (68%). The three most common comorbidities were hypertension (51%), obesity (35%) and hypercholesterolemia (34%). The mean body mass index (BMI) of participants was 30.8 kg/m^2 and 77% were either overweight or obese (ie, BMI ≥ 25 kg/m^2).

3.2 | Gout status

The average age at diagnosis of gout was 42 years (SD 16) and patients had been suffering from gout for an average of 14 years (SD 12) (Table 2). The majority of participants (83%) reported having at least 1 gout flare in the past 12 months. Of these, 15 (19%) had more than five flares in the past 12 months. Almost all (94%) reported the gout flares in the feet, ankle or toes, and 10% had tophi.

3.3 | Medical therapies used for gout

Only 38 participants (48%) were on urate-lowering therapy and 13 (17%) were using "natural substances" such as "tart of cherry" and "celery seed extract" (Table 3). The most common reasons for not taking medication for gout was forgetting to take them (33%), experiencing side effects (18%) and not wanting to take medication (15%). Eighteen participants (23%) reported that they were compliant in taking their gout medication, while 7 (9%) reported they were not taking medication due to their inability to afford them. The majority (92%) of the participants had visited a GP and 16 (20%) had seen a specialist doctor for their gout in the last 12 months. Outpatient/ambulatory doctor consults were the most common form of medical care used, while hospitalizations were rare.

3.4 | Total direct costs

A small number of participants reported extremely high direct costs relating to their gout, greatly inflating the calculated mean cost of their condition. The median annual total direct OOPC of gout was AU$200 (IQR: AU$60-AU$650) and mean total direct OOPC was AU$666 (range AU$0-AU$7088) (Table 4). The three highest categories contributing to mean yearly expenditure were prescription medication (AU$207), non-prescription medication (AU$87) and traditional Chinese medication (AU$84). These accounted for 57% or AU$378 of the mean total direct costs (Figure 1). The median direct medical costs totaled $200 (IQR: AU$60-$570), and median non-medical costs totaled $0 (IQR: AU$0-$15). Medical costs include all medicines, consultations with health professionals, imaging, pathology tests, medical procedures and hospital admissions. The main non-medical direct costs reported were transport and home assistance (Figure 1).

3.5 | Absenteeism and presenteeism

Absenteeism, or work days missed due to gout, over the last 12 months, had a median value of one-quarter of a workday lost (IQR: 0-3 days) (Table 4), which is valued at approximately $60. In terms of presenteeism, 60 participants (76%) reported they were affected in some capacity, with 14 (18%) being severely affected (Table 4).

3.6 | Economic hardship and cost-related treatment attrition

Overall, seven participants (9%) experienced CRTA and 26 participants (33%) reported economic hardship. All participants who experienced CRTA could not pay for their gout medication (Appendix S3). Participants who reported CRTA spent more than those who did not (AU$770 [IQR: AU$280-$5550] and AU$185 [IQR: AU$52.50-$607.50], respectively; $P = .013$) (Appendix S4). Twenty-one (27%) drew on savings and 10 (13%) increased the balance owing on their credit cards. Almost 10% reported not being able to afford either dental appointments, medical consultations, medications, utility bills or minimum payments on credit cards (Appendix S3). Those who reported facing economic hardship also reported a higher total direct cost compared to those who did not (AU$440 [IQR: AU$200-$1727.50] and AU$150 [IQR: AU$44-$485], respectively; $P < .05$) (Appendix S4).

TABLE 2 Participants' gout status[a]

	N = 79
Mean age at diagnosis of gout, y ± SD[b]	41.6 ± 13.4
Mean duration of gout diagnosis, y ± SD[c]	14.3 ± 11.7
Number of acute gout attacks in the past 12 months, n (%)	
0 attacks	13 (16)
1-2 attacks	30 (38)
3-5 attacks	21 (27)
>5 attacks	15 (19)
Duration of most recent attack, n (%)	
1-2 d	21 (27)
3-4 d	24 (30)
5-7 d	20 (25)
7-14 d	6 (8)
>14 d	8 (10)
Joints affected, n (%)	
Feet, ankle and toes	74 (94)
Legs	20 (25)
Hands, wrist and fingers	9 (11)
Arms	8 (10)
Others (eg neck)	1 (1)
Presence of tophi, n (%)[d]	
Yes	8 (10)
No tophi present	71 (90)

Abbreviation: SD, standard deviation.

[a]Percentages may not add up to 100% due to rounding.

[b]n = 74, missing data for mean age at diagnosis of gout = 5.

[c]n = 73, missing data for mean duration of gout diagnosis = 6.

[d]n = 78, missing data for presence of tophi = 1.

4 | DISCUSSION

This study is the first to examine the cost of gout to Australian patients. The median total direct cost of gout was found to be AU$200 per year and participants missed a median of 0.25 workdays per year. The majority (76%) of participants reported their gout affected them at work, and 9% of participants experienced CRTA.

Expenditure on gout medication accounted for 66% of the mean direct medical costs. This high expenditure on medicines is

TABLE 3 Types of medical care and assistance sought for gout and participants access to them[a]

	N = 49
Medication taken for gout, n (%)	
Allopurinol	36 (46)
Colchicine	21 (27)
Febuxostat	1 (1)
Probenecid	1 (1)
Indomethacin	8 (10)
Meloxicam	2 (3)
Other (eg tart of cherry, celery seed extract)	13 (17)
Reasons for not taking medication for gout, n (%)	
Forget to take them	26 (33)
Side effects	14 (18)
Do not want to take them	12 (15)
Cost	7 (9)
Other reasons	31 (39)
Self-reported compliance with gout medication,[b] n (%)	18 (23)
Healthcare providers consulted, n (%)	
General practitioner	73 (92)
Specialist	16 (20)
Pharmacist	8 (10)
Dietician	6 (8)
Physiotherapist	6 (8)
Other (cardiologist, naturopath)	3 (4)
Activities for which participants needed help regularly in the past month, n (%)	
Shopping	22 (28)
Getting around (eg walking, running errands)	19 (24)
Gardening	18 (23)
Housework	18 (23)
Preparing meals	11 (14)
Personal care (eg bathing, using the toilet)	5 (6)
Medical care (eg taking medication)	4 (5)

[a]Percentages may not add up to 100% due to rounding.

[b]Responses to the survey questions: "Are you currently taking medication for your gout?" and "If there are times that you do not take your medications, what is/are the reason/s for this?"

consistent with previous Australian studies.[31,32] In our study, almost all the OOPC was associated with direct medical costs, with non-medical costs accounting for less than 1% of total direct cost (Figure 1). As such, interventions to reduce the direct cost burden of gout must be aimed at reducing medical costs (ie, medications and consultations). A likely reason for high OOPC despite pharmaceutical subsidies through Australia's Pharmaceutical Benefits Scheme (PBS) for prescription medicines, would be purchasing of non-prescription medication (not subsidized)[33] which was the second highest category of OOPC reported by the survey respondents. While medication such as analgesics and anti-inflammatory medications are commonly used to treat gout flares and can be accessed without a prescription, we also found that other therapies such as "tart of cherry", for which there is evidence of effectiveness,[34] and "celery seed extract", are also being purchased. Furthermore, while consultation fees with GPs and specialists are subsidized through Australia's universal health insurance scheme (Medicare), practitioners can charge more than the agreed Medicare schedule fee and patients must pay OOP to cover this cost. To mitigate the burden of OOPC to patients, strategies such as promoting low-cost, effective non-pharmacological approaches (eg, dietary control and lifestyle measures),[35] expanding the subsidization of effective low-cost medications, educating patients about the benefits and risks of non-prescribed medications, reviewing the subsidy policies of specialist services and utilizing more efficient models of care rather than a reliance on specialist services, could be explored.

Comparisons with the other cost-of-gout studies conducted in countries other than Australia reveal several findings. The mean gout-related OOPC experienced was US$876 in the United States,[36] while in Spain, the mean total direct cost was €2228 and the mean total indirect cost was €68.[37] Studies that have estimated gout-related costs based on medical claims report a wide range of values from US$332[38] to US$9748[39] and US$12 620.[36] These costs are all substantially higher than our reported value of AU$200 (US$144) per year for Australian patients. Comparisons with other countries is difficult, largely because funding arrangements differ considerably. For instance, in Australia, there are highly subsidized medicines and services available where the government bears the bulk of costs through a universal health insurance scheme, as compared to countries such as the US where this is not the case. In contrast to the study by Wu et al[36] in the US, we found fewer people reporting absenteeism. This may also reflect differences in employment conditions between the two countries. A systematic review of the economic burden of gout by Rai et al[40] also reported a wide range of costs experienced by patients with gout, which was dependent on factors such as age and employment status. Our study has extended this literature base by capturing the impact of gout on productivity while at work (presenteeism) in addition to time off work (absenteeism), postulated to be an important factor in costing a chronic condition such as gout.[41] Our findings highlight that like many other rheumatic diseases,[42] the ability to work is affected for most people with gout and should therefore be accounted for when estimating the cost burdens arising from gout. Further work is therefore needed to value gout-related presenteeism, as well as capture the impact of other production losses such as on unpaid work, not captured in our study.

Both the OOPC and absenteeism data were skewed with a small number of participants reporting very high OOPC or large numbers of workdays missed due to gout. These findings suggest that people with gout can have extremely different experiences, with a few

TABLE 4 Direct and indirect costs[a]

	N = 79
Total direct cost, AU$,	
Median (IQR)	200 (60-650)
Mean	666
Minimum, maximum	0, 7088
Direct, medical cost,[b] AU$	
Median (IQR)	200 (60-570)
Mean	604
Minimum, maximum	0, 7088
Direct, non-medical cost,[c] AU$,	
Median (IQR)	0 (0-15)
Mean	62
Minimum, maximum	0, 1000
Absenteeism, workdays,	
Median (IQR)	0.25 (0-3)
Mean	3.2
Minimum, maximum	0, 65
Presenteeism,[d] n (%)[e]	
Not affected	19 (24)
Mildly affected	19 (24)
Moderately affected	26 (33)
Severely affected	14 (18)

Abbreviation: IQR, interquartile range.

[a]Percentages may not add up to 100% due to rounding.

[b]Medical costs = medications, consultations, hospital admission, surgery, imaging, tests, medical equipment, procedures and ambulance services

[c]Non-medical cost = transport, home assistance, home modification, special food

[d]Presenteeism scores were from 1-10; not affected = 1, and extremely affected = 10. Categories were defined as such – Not affected (score = 1), Mild (score = 2-4), Moderate (score = 5-7), Severe (score = 8-10).

[e]n = 78, missing data = 1.

individuals experiencing larger costs. Further examination of the contextual and other factors contributing to these widely differing experiences is required to inform the design of strategies to assist people with gout, particularly those severely impacted.

CRTA has been identified among Australian patients with chronic kidney disease[13] and chronic obstructive pulmonary disease.[12] CRTA was first noted among Australian patients with gout in 2016[14]; however, CTRA for gout has not been quantified. In our study, almost 10% of participants reported not seeking medical treatment for their gout due to the inability to afford treatment and medication for gout. It is possible that because patients are unable to afford gout medications or to attend consultations with health professionals, the measured direct medical cost incurred by gout patients was also reduced. All the patients who had reported CRTA in our study were unable to afford gout medications. A serious concern is that if the trend of gout severity and rising OOPC, are coupled with CRTA, gout patients could become trapped in a cycle of an inability to financially afford treatment that could control or eliminate the manifestations of the disease, resulting in worsening disease, and greater costs to treat the worsening disease. It has been found that controlling sUA and gout flares would reduce the costs associated with gout and improve the overall quality of life of gout patients.[43] Therefore, further work to needed to make treatment more accessible for gout patients.

The data regarding CRTA in this study adds to recent literature on Australians forgoing medical care and medications because of costs.[10,12,13,32,44,45] Given the rising costs of healthcare and OOP expenditure by patients, for example increasing co-payment for subsidized pharmaceuticals,[16] it would be important to keep assessing the level of CRTA among Australian patients over time. Regarding economic hardship and CRTA, it has been recommended that health providers should be more involved in identifying patients at risk and referring them to appropriate services.[13] Australian GPs' effectiveness in assessing gout patients' economic hardship or CRTA has not been explored. There could be greater awareness raised among health providers, about the potential for economic hardship and CRTA among patients with gout and other chronic diseases.

4.1 | Limitations

Survey responses were dependent on participants' recall and memory. Poor memory or an inaccurate recollection of costs by the participants could render the data inaccurate. This issue was addressed by choosing a recall period to minimize this effect. Recall of costs and impacts over 1 year was considered long enough to provide an appropriate representation of costs associated with intermittent gout flares and not too long to tax the participants' memory; however, this recall period has not been validated. Our study results are more representative of Australians dwelling in or near major cities in New South Wales, even though advertising approaches were designed to capture a geographically heterogeneous population. This may impact the generalizability of the findings to other states/territories and regional areas of Australia. Broader recruitment and larger numbers would enable the determination of the variability in the cost of gout between states/territories and regional areas of Australia. Also, the gift card incentive may have been a motivation for completion of the survey, and therefore biased our sample. The survey of absenteeism and presenteeism in this study, while drawn from a validated survey, was via self-report and therefore subjective and may not accurately reflect productivity loss at the workplace, particularly as the meaning of productivity at work may differ among individuals.[46]

5 | CONCLUSIONS

This study offers a cross-sectional view into the experience of an individual with gout residing in Australia. It uncovers some of the

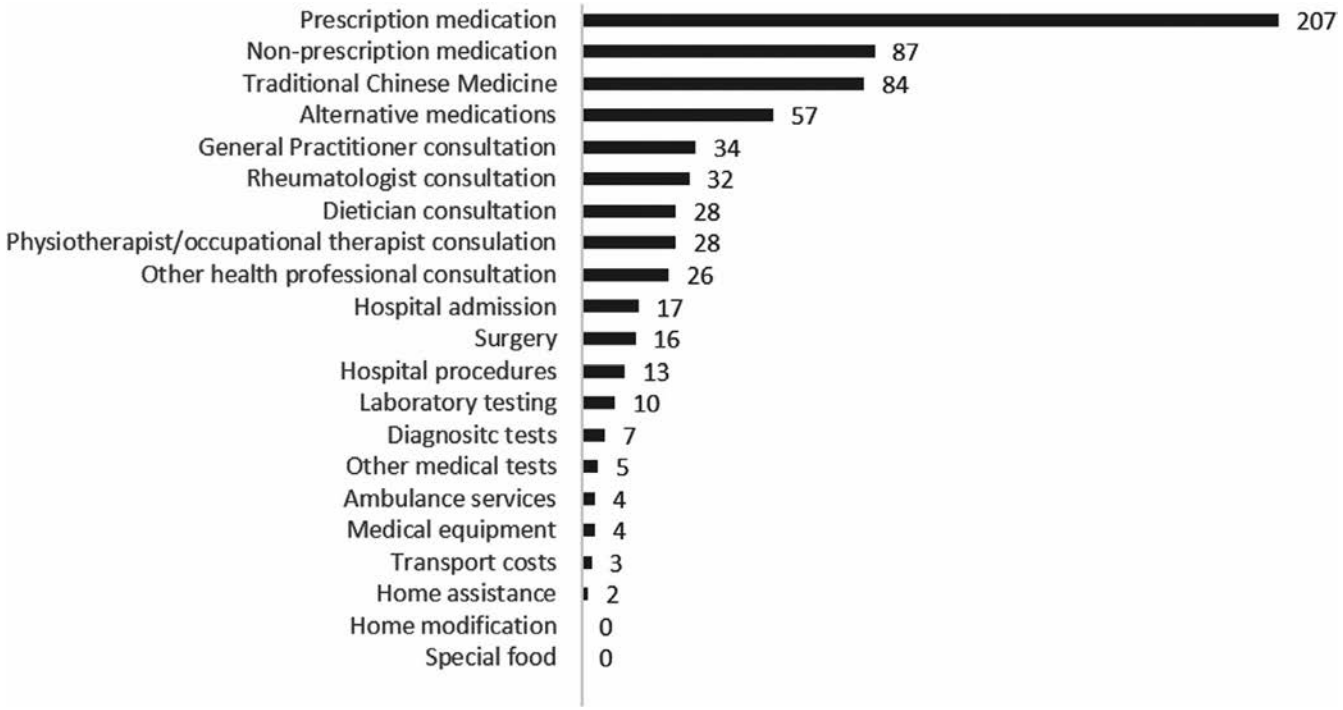

FIGURE 1 Participants' mean direct gout-related medical and non-medical costs over 12 mo, by category (AU$)

key challenges that gout patients face in relation to managing their gout. It has identified the cost of medication as a key contributor to the OOPC experienced by gout patients and has discovered that OOPC is a barrier to treatment for certain individuals. A greater understanding of how people prioritize treatment of their gout relative to other comorbidities will enhance our understanding of how participants view their gout and make financial healthcare decisions.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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MiR-221-5p is involved in the regulation of inflammatory responses in acute gouty arthritis by targeting IL-1β

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Abstract

Aim: Gout is caused by the accumulation of deposited monosodium urate (MSU) crystals in the joints. Recent studies have shown that interleukin-1β (IL-1β) is a key inflammatory mediator of acute gouty arthritis (AGA), and its level is regulated by microRNAs (miRNAs). The purpose of this study was to study the role of miR-221-5p in the pathogenesis of AGA.

Methods: One hundred patients with AGA and 94 healthy individuals were recruited. The expression of serum miR-221-5p was determined by quantitative real-time polymerase chain reaction. The receiver operating curve (ROC) was applied for diagnostic value analysis. A luciferase reporter assay was performed to confirm the interaction of miRNA and the 3′-untranslated region (UTR) of IL-1β. Enzyme-linked immunosorbent assay was used to detect serum and proinflammatory factors.

Results: miR-221-5p had lower expression in the serum of AGA patients. The area under the curve was 0.884, the sensitivity was 82.0%, and the specificity was 80.9%. Serum miR-221-5p was negatively correlated with the expression levels of visual analog scale and IL-1β. Cell experiments showed that overexpression of miR-221-5p significantly inhibited the expression of inflammatory factors tumor necrosis factor-α, IL-8, and IL-1β, while down-regulation of miR-221-5p was the opposite. Luciferase analysis showed that IL-1β was the target gene of miR-221-5p.

Conclusions: This study confirmed that miR-221-5p regulates the production of inflammatory cytokines during the pathogenesis of AGA. These results suggested that miR-221-5p could be used as a potential therapeutic target for the treatment of AGA.

KEYWORDS

acute gouty arthritis, IL-1β, inflammatory, MiR-221-5p, THP-1 cells

1 | INTRODUCTION

Gout attacks can be caused by hunger, trauma, surgery, ingestion of high-purine foods, excessive alcohol consumption, and medications that affect urate concentrations.[1] It is divided into 3 clinical stages: acute gouty arthritis (AGA), intercritical gout, and chronic gout. AGA is an acute inflammation caused by the precipitation of urate crystals in joints.[2] It is one of the most common types of auto-inflammatory arthritis, characterized by a sudden onset and significant pain that resolves spontaneously within a week.[3,4] Interleukin-1β (IL-1β) is a central cytokine in the initiation of the acute inflammatory response, which plays a key role in the pathogenesis of gout,[5] especially its role in the pathology of AGA. Tongfengshu capsule, a Chinese patent medicine, is composed of radix et rhizoma rhei palmati, semen plantaginis, rhizoma alismatis, radix achyranthis bidentatae and radix stephaniae tetrandrae;

it has been used for the treatment of AGA with the involvement of IL-1β and tumor necrosis factor (TNF)-α regulation, suggesting the potential role of IL-1β and TNF-α in the progression of AGA.[6] However, the mechanism of action of IL-1β in AGA is unclear.

MicroRNAs (miRNAs) are evolutionary conserved non-coding small RNA molecules that act as negative post-transcriptional gene regulators.[7] Since a single miRNA molecule can target 100s of messenger RNAs (mRNAs), the abnormal expression of miRNA is related to the occurrence of many diseases.[8] Recent research suggests that miRNAs may be involved in the development of arthritis.[9,10] For example, the expression level of miR-155 in patients with gout arthritis is significantly higher than that in healthy individuals, and the overexpressed miR-155 can promote the production of monosodium urate (MSU)-induced inflammatory cytokines by reducing SHIP-1 levels.[9] Considering the important role of miRNAs in inflammatory diseases, especially AGA, more studies on miRNAs are urgently needed.[11]MiR-221-5p has been widely reported to be aberrantly expressed in various metabolic diseases and involved in the diseases progression. For example, miR-221-5p is identified to be involved in the progression of diabetes.[12] Another study also confirmed that miR-221-5p participates in the development of osteoarthritis. In addition, after constructing a miRNA gene pathway network, miR-221-5p is identified to be enriched in various metabolic pathways.[13] Up to now, the molecular mechanism of miR-221-5p in AGA has been unclear.

In summary, miR-221-5p is critical for human cell inflammation and AGA. However, the functional role of miR-221-5p in AGA is not yet clear. Therefore, the purpose of this study was to study the role of miR-221-5p in the pathogenesis of AGA.

2 | MATERIALS AND METHODS

2.1 | Subject and sample collection

One hundred AGA patients and 94 healthy individuals matched in age and gender participated in the study. The 100 AGA patients recruited excluded the following conditions: (a) infection (b) tumor (c) rheumatoid arthritis. Five milliliters of peripheral blood samples were collected from each subject and immediately centrifuged. Subsequently, the serum samples were stored at −80°C for further analysis. All controls had no history of systemic inflammation or tumor. Clinical data including erythrocyte sedimentation rate (ESR), serum uric acid (SUA), visual analog scale (VAS), serum creatinine (SCR), age, gender, body mass index (BMI), leukocyte count, neutrophils count, and lymphocyte count were recorded in all participants.

The protocol of this study was approved by the Ethics Committee of Qingdao Municipal Hospital (no. 201710), and written informed consent was collected from each participant.

2.2 | Cell culture and transfection

The human monocyte THP-1 cell line was cultured in Roswell Park Memorial Institute 1640 medium (Life Technologies) and cultured in a 37°C, 5% CO_2 constant temperature incubator. THP-1 cells at 1.5×10^6/mL were incubated in 96-well plates. The THP-1 cells were stimulated for 3 hours with 0.5 μmol/L phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) the day before stimulation. Then, the cells were stimulated with 250 μg/mL MSU crystals (Invitrogen) for 24 hours, causing inflammation, and presenting a variety of features of AGA. In order to regulate the expression level of miR-221-5p, cells were transfected with miR-221-5p mimic, miR-221-5p inhibitor, or their negative control (miR-NC), which was produced by Ribo Bio. Liposome 2000 (Invitrogen) was used for transfection according to the manufacturer's instructions.

2.3 | Total RNA extraction and quantitative real-time polymerase chain reaction assay

Total RNA was extracted using TRIZOL reagent (Invitrogen). The miRNA bulge loop was reverse transcribed using the PrimeScript RT Reagent Kit (TaKaRa). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to detect gene expression using SYBR premix ExTaq M. II commercial kit (Takara) and the Applied Biosystems 7900 Real Time PCR System (Applied Biosystems). PCR parameters were as follows: 95°C for 3 minutes, followed by 40 cycles of 95°C for 10 seconds, 60°C for 20 seconds, and 72°C for 1 second. The relative gene expression was normalized to that of the internal control U6 according to the comparative delta CT ($2^{-\Delta\Delta Ct}$) method.

2.4 | Evaluation of inflammatory cytokines

The concentrations of IL-1β, IL-8, and TNF-α proteins in THP-1 cell culture supernatant were determined using an enzyme-linked immunosorbent assay kit (UK Abeam) in accordance with the manufacturer's instructions. Each sample was analyzed 3 times.

2.5 | Luciferase reporter assay

The putative binding sites of miRNAs in the 3′-UTR (untranslated region) of the human IL-1β gene transcript were predicted by Target Scan ((http://targetscan.org/), and then verified by luciferase reporter gene experiment. Cells were co-transfected with miR-221-5p mimic or inhibitor, and miR-221-5p wild type (WT) or mutant seed region (MUT) of IL-1β 3′-UTR. Lipofectamine 2000 (Invitrogen) was used for cell transfection. Relative luciferase activity was measured by the dual luciferase reporting system (Promega) according to the manufacturer's instructions. The fluorescent activity of renal cells was used as an internal reference.

2.6 | Statistical analysis

In our study, all statistical analyses were performed with Prism 6 (GraphPad Software, San Diego, CA, USA) and IBM SPSS 20

statistical software. The data were expressed as mean and standard deviation (SD). The differences between the 2 groups were compared by Student's *t* test or one-way analysis of variance. Receiver operating characteristic (ROC) curves were used to determine the specificity and sensitivity of the diagnostic value of miR-221-5p for AGA. Correlation analysis was performed using Pearson correlation coefficients.

3 | RESULTS

3.1 | Clinical characteristics of different patient groups

Table 1 reports the main characteristics of the study population and laboratory results. A total of 194 individuals were included, the age range was 27-65 years. Among them, 94 subjects were healthy controls (44 males/ 50 females), and 100 AGA patients (48 males/ 52 females). There was no difference in age, gender distribution, BMI, ESR, and lymphocytes count between the groups ($P > .05$). There were significant differences in SUA, leukocyte count, neutrophils counts ($P < .001$). The VAS score in patients was 6.16 ± 2.36.

3.2 | The expression level of miR-221-5p and its correlation with VAS in AGA

We first studied the serum levels of miR-221-5p in healthy controls and AGA groups. The results of the study are shown in Figure 1A. The expression level of miR-221-5p in the serum of patients with AGA (0.61 ± 0.23) was significantly lower than that of the healthy control group (1.00 ± 0.25) ($P < .05$). The results indicated that miR-221-5p may be a key biomolecule for AGA and play an important biological role in its disease progression. In addition, in order to further explore the relationship between miR-221-5p and AGA, we also made a correlation between VAS and miR-221-5p. As shown in Figure 1B, serum miR-221-5p was negatively correlated to VAS ($r = -.7671$, $P < .0001$) in AGA patients. We concluded that miR-221-5p might be associated with the occurrence and severity of AGA.

TABLE 1 Baseline characteristics of the subjects

Characteristics	Controls (n = 94)	AGA (n = 100)	*P* value
Age, y	46.5 (11.8)	43.2 (11.7)	.796
Gender, male/female, n	44/50	48/52	.868
BMI, kg/m^2	20.72 (1.73)	21.05 (1.64)	.553
ESR, mm/h	4.68 (2.81)	4.99 (3.01)	.517
SUA, μmol/L	185.15 (8.20)	227.44 (12.36)	<.001
VSA, μmol/L	—	6.16 (2.36)	—
SCR, μmol/L	89.34 (26.48)	87.32 (26.27)	.6
Leukocyte count, 10^9/L	7.06 (1.83)	25.92 (8.73)	<.001
Neutrophils count, 10^3/mL	4.49 (1.44)	31.19 (9.05)	<.001
Lymphocytes count, 10^9/L	2.55 (0.94)	2.29 (0.86)	.125

Note: Data are expressed as n or mean and standard deviation.

Abbreviations: AGA, acute gouty arthritis; BMI, body mass index; ESR, erythrocyte sedimentation rate; SCR, serum creatinine; SUA, serum uric acid; VSA, visual analog scale.

3.3 | Diagnostic value of miR-221-5p in patients with AGA

Receiver operating curve curves were drawn based on the expression level of miR-221-5p in AGA patients and the control group to evaluate the diagnostic value of miR-221-5p in AGA patients. As shown in Figure 2, the miR-221-5p expression may be used to distinguish AGA patients from healthy individuals. The area under the curve (AUC) was 0.884, cut off value was 0.800, sensitivity was 82.0%, specificity was 80.9%. The results of this study confirmed the diagnostic value of miR-221-5p in differentiating AGA patients from healthy individuals.

3.4 | Effect of miR-221-5p on inflammatory responses in THP-1 cells

As shown in Figure 3A, the transfection of miR-221-5p mimic/inhibitor had a significant effect on the expression of miR-221-5p ($P < .001$), transfection with miR-221-5p mimic significantly increased the expression of miR-221-5p, while transfection with miR-221-5p inhibitor had the opposite effect. As shown in Figure 3B-D, the expression levels of TNF-α, IL-8, and IL-1β in the MSU group were significantly increased compared with the control group. Compared with the MSU group, the expression levels of inflammatory factors were significantly decreased in the miR-221-5p mimic transfection group ($P < .001$), and significantly increased in the miR-221-5p inhibitor group ($P < .001$).

3.5 | MiR-221-5p directly targets IL-1β in THP-1 cell

miRNAs were known to function by inhibiting the expression of their target genes. According to the Target scan analysis results, the binding sites of miR-221-5p in IL-1β are shown in Figure 4A(a). Luciferase reporter assay results showed that miR-221-5p mimic significantly inhibited luciferase activity of IL-1β WT 3′-UTR (Figure 4A(b), $P < .001$), while miR-221-5p inhibitor significantly increased luciferase activity. In addition, the luciferin activity of the mutant group was not affected by transfection with miR-221-5p mimic or miR-221-5p inhibitor. As shown in Figure 4B, the correlation between miR-221-5p expression and target gene IL-1β in AGA patients was also analyzed. The results showed that the expression of miR-221-5p in AGA patients was negatively correlated with IL-1β level ($r = -.6762$, $P < .0001$).

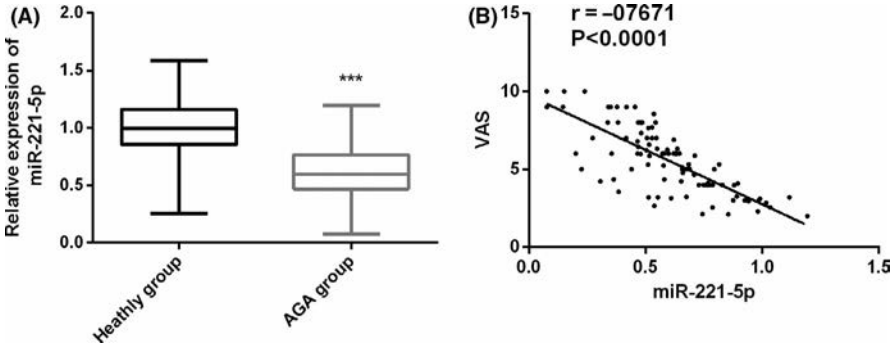

FIGURE 1 The expression level of miR-221-5p and its correlation with visual analog scale (VAS) in acute gouty arthritis (AGA) patients. A, The expression level of miR-221-5p in the serum of patients with AGA was decreased compared with the control group (***$P < .001$). B, a negative correlation between serum miR-221-5p and VAS was found ($r = -.7671$, $P < .0001$)

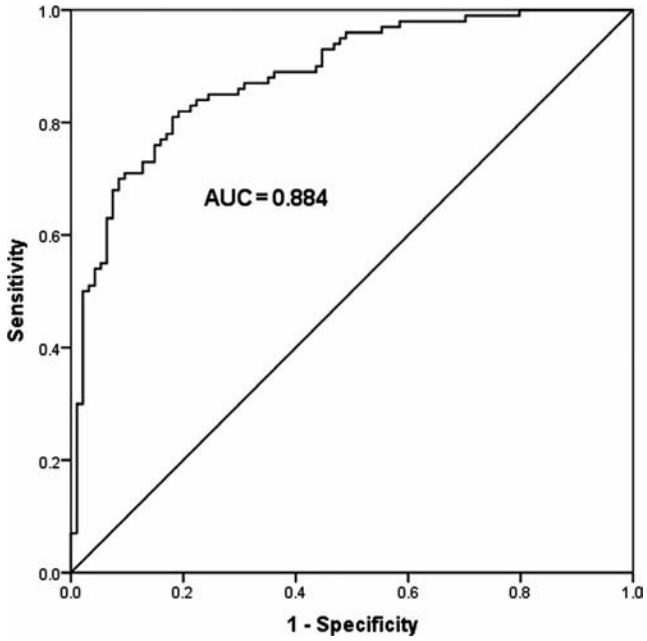

FIGURE 2 The receiver operating characteristic (ROC) curve was used to analyze the diagnostic value of miR-221-5p in acute gouty arthritis (AGA). The area under the curve (AUC) was 0.884, sensitivity was 82.0%, specificity was 80.9%

4 | DISCUSSION

Gout is a common metabolic disease and AGA is one of the important complications.[14] AGA is a group of clinical syndromes caused by MSU crystal deposition on bone, joints, and subcutaneous tissues, which is the most common initial symptom of gout.[15] It is worth noting that while hyperuricemia has been classically associated with gouty arthritis, asymptomatic hyperuricemia is frequently found in metabolic syndrome, diabetes mellitus, chronic kidney disease, and osteoarthritis.[16,17] Osteoarthritis is the most common form of arthritis overall, and gout and osteoarthritis frequently coexist in the same patient.[18] Further, one study has confirmed the low expression of miR-221-5p in osteoarthritis.[19] Therefore, its role in AGA attracts our interest. In our study, it was proposed that the expression of miR-221-5p in AGA patients was significantly lower than in healthy subjects, which was consistent with the results reported in osteoarthritis, indicating the association of miR-221-5p with AGA.[19]

Serum miRNA is stable in stored samples,[20] and it is more practical as a biomarker and easier to isolate than specific cell types of miRNAs, especially in AGA. Recent studies have suggested that miRNAs including miR-155 and miR-146a may be involved in the development of AGA in humans. For instance, a study has confirmed that miR-155 was upregulated in synovial fluid mononuclear cells (SFMCs) from patients with AGA.[21] Another study confirmed that miR146a plays a negative regulatory role in AGA in humans.[22] However, the application of miR-221-5p in AGA has not been reported. In addition, a large number of studies have confirmed that VAS is an important indicator for the clinical assessment of AGA.[23] Considering the dysregulation of miR-221-5p in AGA patients, we further studied the correlation between miR-221-5p and VAS, and the results showed that miR-221-5p was negatively correlated with VAS. Meanwhile, accurate and timely diagnosis and monitoring of treatment outcomes are critical to AGA patient prognosis. To solve this problem, reliable AGA biomarkers are urgently needed. Many studies have shown that miRNAs can be a biomarker useful for diagnosis and prognosis.[24] In view of the significant correlation between miR-22-5p and VAS, we further evaluated the ability of serum miR-221-5p to differentiate AGA from healthy individuals by establishing ROC curves. High sensitivity and specificity of AUC was detected, demonstrating that miR-221-5p had the ability to distinguish AGA patients from healthy controls.

Acute gouty arthritis is one of the most painful inflammatory conditions.[25] Therefore, the onset of AGA is accompanied by all the characteristics of an acute inflammatory response. These include intimal hyperplasia, infiltration of neutrophils, mononuclear phagocytes, and lymphocytes. miRNAs are confirmed to be central players in pathways associated with MSU-induced inflammation and gouty arthritis. Further, another study has confirmed that miRNA inhibited the expression of IL-1β induced by MSU in THP-1 cells, such as miR-488 and miR-920.[26] In the present study, THP-1 cells were stimulated with MSU crystals to mimic inflammation features of AGA, and the cell experiments demonstrated that miR-221-5p overexpression inhibited the release of inflammatory cytokines, including IL-1β, IL-8 and TNF-α. The abnormal expression of miRNAs can affect specific targets and pathways, leading to the phenotype of auto-inflammatory disease, which is also supported by some in vivo studies. For example, the researchers have found that miR-488 and miR-920 can directly target the 3′-UTR of IL-1β in gouty arthritis.[26] It is demonstrated that IL-1β can be involved in the

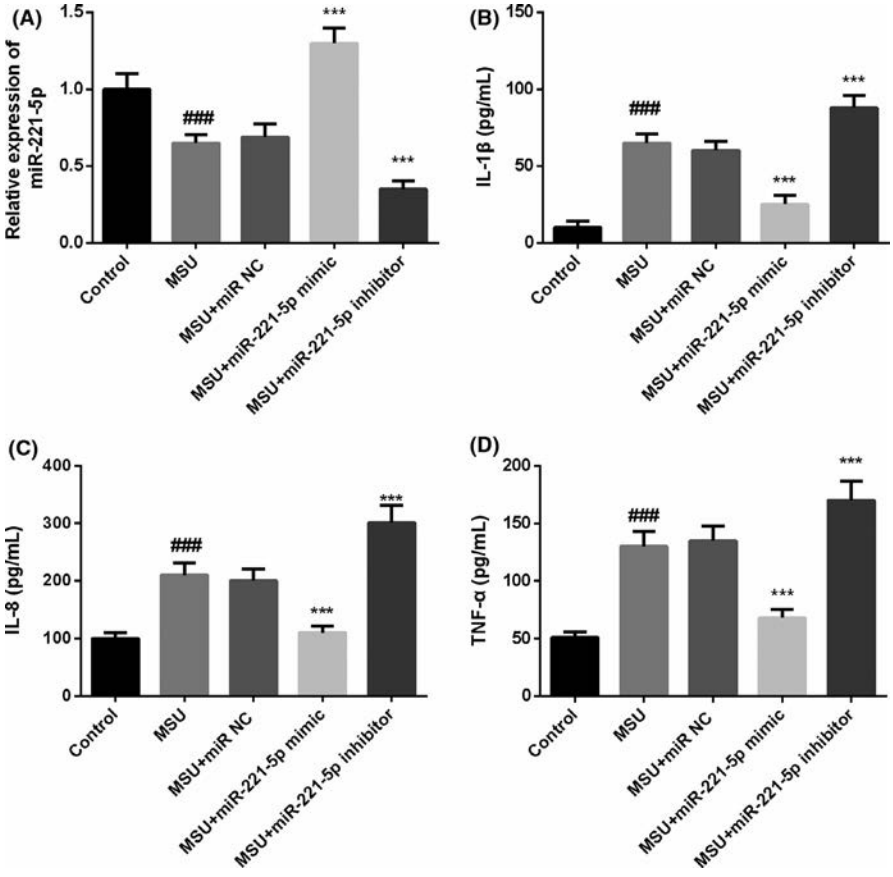

FIGURE 3 Effect of miR-221-5p on inflammatory responses in THP-1 cells. A, The transfection of miR-221-5p mimics significantly increased the expression of miR-221-5p, whereas the expression was downregulated significantly after miR-221-5p inhibitors were transfected. B-D, Effects of miR-221-5p on inflammatory cytokines in THP-1 cell models treated with monosodium urate (MSU). $^{###}P < .001$, compared with the control group. $^{***}P < .001$, compared with the MSU group

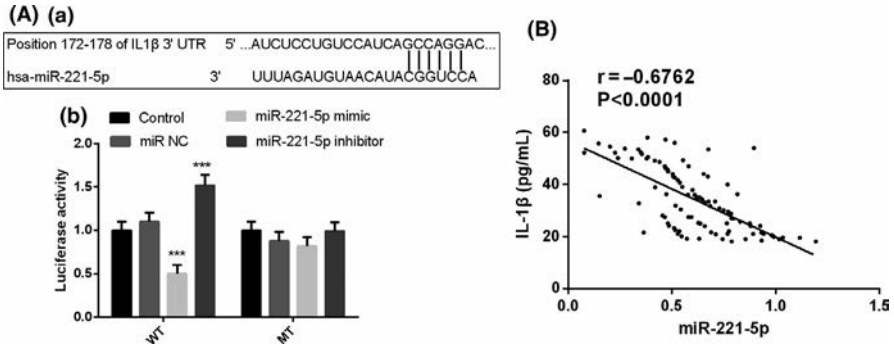

FIGURE 4 Interleukin (IL)-1β was the target gene of miR-221-5p. A, (a) The binding site of miR-221-5p in IL-1β. (b) miR-221-5p mimic significantly inhibited luciferase activity of wild type (WT) 3′-UTR (untranslated region) of IL-1β and miR-221-5p inhibitor significantly increased its luciferase activity. Further, the luciferin activity of the mutant type group was not affected by the transfection of miR-221-5p mimic or miR-221-5p inhibitor. $^{***}P < .001$. B, Correlations of miR-221-5p expression with the IL-1β expression ($r = -0.6762$, $P < .0001$)

treatment of AGA patients as a target gene. The identification of miRNAs possibly targeting IL-1β in gouty arthritis is another major goal for the future.[27] Therefore, targeting miRNAs may be an effective option for treating auto-inflammatory disease in the future.[28] In our results, the luciferase reporter assay analysis showed that IL-1β may be involved in the AGA process as a target gene of miR-221-5p. All evidence supported our conclusion that miR-221-5p might be involved in the development of AGA and inhibit inflammatory responses via targeting IL-1β. However, only part of the role of miR-221-5p in THP-1 cells is studied in this research. In the future, we can further verify its cell function and explore the specific mechanism of the role of miR-221-5p in the development of AGA patients. In addition, the study population should be expanded to better verify the current study effect.

5 | CONCLUSION

It is commonly known that auto-inflammation plays a pivotal role in the pathology of AGA. However, the exact etiology and pathogenesis are poorly understood. In general, we found that miR-221-5p was downregulated in patients with AGA. ROC curves were drawn based on the expression level of miR-221-5p and the diagnostic value of miR-221-5p in distinguishing AGA patients from healthy people was

confirmed. Therefore, it has potential as a therapeutic or biomarker for AGA. miR-221-5p may participate in the development of AGA patients by acting on target gene IL-1β to inhibit the release of inflammatory factors in THP-1 cells.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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ORIGINAL ARTICLE

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Clinical impact of musculoskeletal ultrasound on rheumatoid arthritis in routine care

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Abstract

Objective: To evaluate the impact of musculoskeletal ultrasound (MSUS) in the management of rheumatoid arthritis (RA) patients and to investigate factors affecting treatment strategy by the referring rheumatologist.

Methods: Prospective study of RA patients evaluated at a MSUS clinic over a 6-month period. Data extraction included demographics, current treatment and MSUS findings. Pre- and post-MSUS follow-up of 3 months data were analyzed. Patients were classified into 2 groups based on the decision of the referring rheumatologist to change the treatment after the MSUS examination. Comparisons between groups were performed in a univariate analysis. We used logistic regression models to investigate factors associated with changes in clinical management.

Results: A total of 64 RA patients were included. Mean age was 61.9 years and 83.6% were female. Main referral indication was assessment of disease activity (89%). Overall, MSUS led to subsequent therapeutic actions by the referring rheumatologist in 41 (64.1%) patients, and to a change in the clinical impression of the complaint that generated the referral in 7 (11.5%) patients. The detection of power Doppler (PD), the 28 swollen joint count and the presence of radiographic erosions were significantly associated with a subsequent clinical action. In the multivariate analysis only PD remained significant (odds ratio = 3.29; 95% CI: 1.05-10.26).

Conclusion: Disease activity evaluation is the most common indication for MSUS examination, with the presence of PD the factor most frequently associated with changes in therapeutic management. This study highlights the impact of MSUS, especially the use of PD, to support treatment decisions in RA routine care.

KEYWORDS

assessment, Doppler, rheumatoid arthritis, routine care, treat-to-target, ultrasound

1 | INTRODUCTION

The use of musculoskeletal ultrasound (MSUS) in rheumatoid arthritis (RA) has increased considerably in recent years mainly because of low cost, patient-friendly format easily accessible in the clinic and a great number of publications supporting its use in the management of patients with RA.[1-3] MSUS has higher sensitivity versus clinical examination to detect synovitis,[4] and may be useful to support diagnosis, to guide treatment decisions and to define remission.

Regardless of the increasing use in routine care, little information about the impact of MSUS on therapeutic decision in routine care is available[5-8] and it has not yet been established if its use would change treatment decisions within a treat-to-target (T2T) strategy or whether it would lead to better outcomes in RA patients.[9] In addition, 2 major studies failed to show any advantage of MSUS to be incorporated in a T2T strategy in which its incorporation leads to more intense treatment with no clinical benefit for the patients.[10,11] However, MSUS may be useful in routine care when clinical uncertainty about level of disease activity and when tapering is considered in clinical remission, although the benefit in these 2 scenarios remain to be established.[12]

Our aim was to determine the impact of MSUS in routine clinical management of RA patients and to investigate if MSUS findings are associated with subsequent clinical actions by the referring rheumatologist.

2 | METHODS

2.1 | Patients

We conducted a prospective study of consecutive RA patients evaluated at a rheumatology MSUS outpatient clinic between July 2019 and January 2020 at an academic rheumatology center. The MSUS outpatient clinic is run by a rheumatologist with experience in the use of MSUS and is scheduled 3 days a week.

We included consecutive patients with RA according to the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria,[13] 18 years or older who were referred to the MSUS clinic during a 6-month period. The study was performed in routine daily practice conditions and all patients were unselected, so the sample truly represents the clinical workload undertaken in the study period.

2.2 | Data collection

An independent data collector extracted the following variables from the electronic health record: reason for referral, demographics (age, gender); duration of RA since diagnosis; comorbidities; radiographic bone erosions; laboratory data including rheumatoid factor (RF), anti-cyclic citrullinated peptide (ACPA) antibody, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP); and 28 tender and swollen joint counts. We also collected information about treatment, specifically changes in conventional synthetic (csDMARD) or biological (bDMARD) disease modifying antirheumatic drugs and steroid therapy at the follow-up after the MSUS evaluation. Patients were stratified into 2 groups based on the clinical impact of the MSUS visit: (a) patients with no change in therapy by the referring rheumatologist after MSUS evaluation; and (b) patients with change in therapy after MSUS evaluation (including changes in dose of current treatment, intensification or decrease in therapy or interventional procedures based on the MSUS results).

2.3 | MSUS assessment

All MSUS examinations were performed by the same evaluator (JMC), who was unaware of the physical exam by the referring rheumatologist. The exam was performed using an Esaote MyLab 8 (Esaote, Genoa) with a high frequency (8-15 MHz) transducer. Synovitis and tenosynovitis were evaluated in gray (GS) and Power Doppler (PD) scales using a semi-quantitative scale from 0 to 3 according to EULAR guidelines.[14] PD mode was only assessed if GS mode showed pathologic findings (grades 1-3). For each patient, PD vascularity and GS abnormalities at the requested scanned joints and tendons were categorized as positive when at least 1 site was positive for that finding, or negative when no site was positive for that finding. PD findings were considered positive when at least 1 joint or tendon showed abnormal PD vascularity (grade 1 or higher). MSUS scans usually last an average of 15 to 20 minutes, but US guided injections may require extra time. A standardized MSUS report form was returned to the physicians who would then decide on the clinical management and/or changes in therapy.

2.4 | Statistical analysis

Statistical analysis was performed using SPSS software (version 20). The results of descriptive analyses were presented using frequencies and percentages for qualitative variables and as median or mean values for continuous variables. Between-groups comparisons were made using Chi-square test or Fisher's exact test to analyze differences between proportions; Student's *t* test was used for comparison between means. We used multivariate logistic regression models to investigate factors associated with a change in clinical management. All statistical tests were 2-sided; *P* values <.05 were considered to indicate a statistically significant result.

2.5 | Ethics approval

This study was performed in accordance with the ethical standards of the responsible committee on human experimentation and the Helsinki Declaration of 1975, as revised in 1983. Research ethics

committee approval for the study protocol was obtained from the local ethics committee prior to commencing the study.

3 | RESULTS

3.1 | Demographics and clinical assessment

In the period from July 2019 to January 2020, 64 RA patients referred to the US clinic were included into the study. They represented 14% of the activity performed in this 3 day-a-week MSUS clinic. Demographic, clinical data, MSUS findings and treatment are presented in Table 1. Mean (SD) age was 61.9 (11.4) years, 51 (83.6%) were female and mean (SD) time from diagnosis was 8.5 (9.1) years. Regarding antibodies status, 8 patients (12.5%) were RF positive, 8 (12.5%) ACPA positive, and 36 (56.3%) were positive for both RF and ACPA. Prior to the MSUS assessment, 37 patients (57.8%) were on csDMARDs (46.9% methotrexate, 9.4% leflunomide, 1.6% sulfasalazine and 10.9% hydroxychloroquine), 19 (29.7%) on bDMARDs, and 36 (53.3%) on prednisolone. Based on physical examination (28 tender and swollen joint count), 42 (65.6%) and 39 (60.9%) patients had at least 1 tender or swollen joint, respectively, and the mean (SD) number of tender and swollen joints were 2.3 (3.2) and 1.8 (2.8), respectively.

3.2 | MSUS referral patterns and evaluation findings

The most frequent reason for referral by the rheumatologist in our MSUS clinic was disease activity evaluation in 57 patients (89%), followed by evaluations of suspected soft tissue disorders in 5 (7%), and US guided injection in 3 (4%). Figure 1 shows the frequency of the regions scanned by ultrasound in the study sample. Mean (SD) number of scanned areas by patient was 4 (2.9) and 56 (74.7%) examinations included more than 1 anatomical region. The most frequent MSUS finding were synovitis in 37 (57%) patients, followed

TABLE 1 Demographic and clinical characteristics of rheumatoid arthritis patients

	Total N = 64	Change in clinical management n = 41 (64.1%)	No change in clinical management n = 23 (35.9%)	*P*
Demographic variables				
Age	60 ± 12.4	61.2 ± 12.9	62.6 ± 8.9	.6
Gender				
Female	54 (84.4%)	37 (90.2%)	17 (73.9%)	.08
Time (y) from diagnosis	8.5 ± 9.1	8.4 ± 9.9	8.7 ± 7.6	.9
Smoking				
Non-smoker	35 (54.7%)	19 (46.3%)	16 (69.6%)	.1
Smoker	13 (20.3%)	11 (26.8%)	2 (8.7%)	
Former smoker	16 (25%)	11 (26.8%)	5 (21.7%)	
Radiographic erosions	30 (46.9%)	23 (57.5%)	7 (30.4%)	<.05
Time from diagnosis (y)	8.5 ± 9.1	8.4 ± 9.9	8.7 ± 7.6	.8
Treatment				
csDMARD	37 (57.8%)	22 (53.7%)	15 (65.2%)	.4
bDMARD	20 (31.2%)	12 (29.3%)	8 (34.8%)	.6
Prednisone	36 (56.3%)	25 (61%)	11 (47.8%)	.3
Prednisone dosage (mg/d)	3.7 ± 4.6	4.5 ± 5.5	2.1 ± 2.5	.06
28 tender joint count	2.3 ± 3.2	2.6 ± 3.8	1.7 ± 2.4	.2
28 swollen joint count	1.8 ± 2.8	2.5 ± 3.4	1 ± 1.6	<.05
Laboratory data				
ESR (mm/h)	27.4 ± 19.8	26.1 ± 15.2	32 ± 26.8	.3
CRP (g/L)	0.9 ± 1.4	0.9 ± 1.4	0.9 ± 1.7	.8
RF (IU/mL)	165.3 ± 432.8	134.6 ± 244.1	235.2 ± 680.9	.5
ACPA (IU/mL)	730.7 ± 965.6	601.5 ± 780.9	1080.4 ± 1,241.9	.07
MSUS examination				
US PD findings	39 (60.9%)	30 (73.2%)	9 (39.1%)	<.05

Abbreviations: ACPA, anti-cyclic citrullinated peptide antibody; bDMARD, biological disease modifying antirheumatic drugs; CRP, C-reactive protein; csDMARD, conventional synthetic disease modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; PD, power Doppler; RF, rheumatoid factor; US, ultrasound.

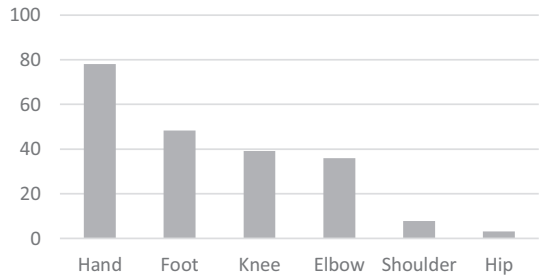

FIGURE 1 Frequency of joint sites scanned by musculoskeletal ultrasound

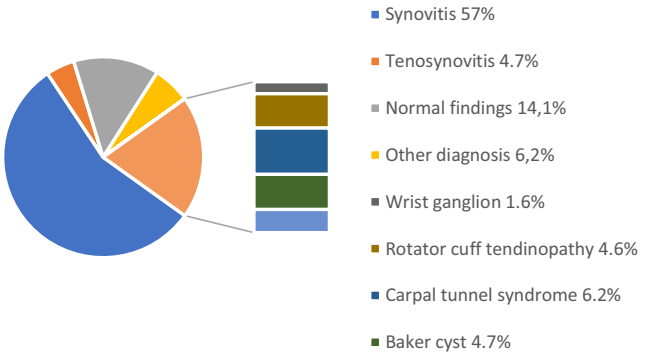

FIGURE 2 Frequency of musculoskeletal ultrasound findings

by soft tissue disorders in 12 (19%) patients (including Baker cyst, common extensor tendon of the wrist tendinopathy, wrist ganglion, rotator cuff tendinopathy and carpal tunnel syndrome). Only 9 patients (14.1%) had normal MSUS findings (Figure 2). Overall, inflammatory findings including at least 1 joint or tendon showing PD signal grade 1 or higher was found in 39 (60.9%) patients. Agreement between physical examination and MSUS findings to detect synovitis was poor (kappa 0.22). However, MSUS findings led to a change in the underlying diagnosis and/or in the clinical impression of the chief complaint that generated the referral in only 7 (11.5%).

3.3 | DMARD and steroid use in relation to MSUS findings

MSUS findings led to a subsequent therapeutic action by the referring rheumatologist in 41 (64.1%) patients. In a total of 28 patients (43.8%) therapy was initiated and/or escalated, including 5 (7.8%) initiations and/or dose increments of corticosteroids, 18 (28.1%) initiations and/or dose increments of csDMARDs, 2 (3.1%) initiations of bDMARDs and 11 (17.2%) articular or periarticular corticosteroid injections. On the other hand, 19 (29.7%) suspended or decreased therapy, including 14 (21.9) dose reductions of corticosteroids, 9 (14%) dose reductions of csDMARDs and 1 (1.6%) cessation of bDMARDs. In the univariate analysis, the detection of PD (73.2% vs 39.1%; $P < .05$), the 28 swollen joint count (2.5 ± 3.4 vs 1 ± 1.6; $P < .05$) and the presence of radiographic erosions (57.5% vs 30.4%; $P < .05$) were significantly associated with a subsequent clinical action (Table 1). Moreover, PD was significantly more frequent among patients who escalated and/or initiated therapy compared with patients who did not (78.6% vs 47.2%; $P < .05$). In the multivariate analysis only the detection of PD signal in the MSUS examination remained significantly associated with a change in clinical management (odds ratio [OR] = 3.29; 95% CI; 1.05-10.26) (Table 2).

4 | DISCUSSION

Medical history and a physical examination are the key components for diagnosis and management of RA.[15] However, there is a widespread adoption of MSUS in routine care in part supported by its contribution to diagnostic process,[16] guiding treatment and evaluate response,[17] predicting structural damage and flares,[18,19] and evaluating remission and adjusting treatment tapering.[20,21]

According to the ACR/EULAR classification criteria,[13] a patient with synovitis can be classified as having RA if a certain number of joints with synovitis are detected or if bone erosions are present. However, clinical examination and conventional radiography (CR) are neither sensitive nor accurate enough to detect disease activity and structural damage in early disease.[22-24] Thus, US-detected synovitis in clinically unaffected joints may be used to increase the number of involved joints to satisfy the fulfillment of the classification criteria. Moreover, the use of MSUS to monitor patients considered to be in remission can help predict those likely to suffer subsequent joint damage and flare-up of disease.[25] However, the EULAR recommendations for the use of imaging of the joints in the clinical management of RA[26] identified the lack of strong evidence to support the role of MSUS and the urgent need for standardization and dedicated trials.[9]

Our study adds to the existing literature by detailing real-life data of how PD findings may provide relevant information that highly influences subsequent therapy changes of the referring rheumatologist in a routine clinical setting.

We found that the diagnostic impact of MSUS in RA patients is relatively low. We considered impact to a change in the underlying diagnosis and/or in the clinical impression of the main complaint, only present in 7 (11.5%) patients. Contrary to our expectation, MSUS evaluation did not substantially change the physician impression in most RA patients. A possible explanation is that most of the patients have an established RA with only 34.4% of patients fulfilling the recent onset RA criteria.[27] In these patients, as diagnosis was already established, the main indication for MSUS was inflammatory activity assessment rather than differentially excluding other forms of arthritis.

In our study, around 4 anatomical regions were evaluated at each MSUS, with 74.7% of patients having more than one area scanned, not including the routine scanning of the contralateral joint region for comparison. This trending use of MSUS may be likely influenced by a polyarticular involvement of RA compared to other conditions, but it also suggests that rheumatologists rely on US to assess disease

TABLE 2 Independent factors associated with a change in clinical management based on logistic regression model

	P	Odds ratio	95% CI	
			Lower	Upper
28 tender joint count	0.12	1.27	0.94	1.71
Radiographic erosions	0.18	2.28	0.69	7.62
US PD synovitis/ tenosynovitis	0.04	3.29	1.05	10.26

Abbreviations: PD, power Doppler; US, ultrasound.

activity and gain information of the global state of inflammation. This MSUS exam pattern by rheumatologists differs with the traditional one by radiologists, in which more than 90% of examinations include just one anatomical area.[28]

On the other hand, the impact of MSUS on treatment decisions was high. The presence of PD - including PD synovitis or tenosynovitis at any location - was statistically more frequent in patients who change treatment compared with those who did not (OR = 3.29; 95% CI: 1.05-10.26). To date, there are no specific recommendations about the minimal grade of PD synovitis requiring treatment, or how big the discrepancy between clinical and MSUS assessment should be to trigger therapeutic intervention (either increase, change or suspend therapy). Interestingly, although the proportion of swollen joints by physical examination and PD findings by MSUS was similar, the level of agreement between both techniques was poor (kappa 0.22). Only the presence of PD signal was significantly associated with a change in clinical management by the referring rheumatologist. These data suggest that, in routine care, clinicians usually give more weight to MSUS findings to guide treatment decisions when disagreement between MSUS and clinical examination exist.

The strength of our study was the examiner being blinded to clinical data and rheumatologists being unaware of the present study when managing their patients. Additionally, the evaluations were performed in a routine care context, increasing the applicability of our results. However, several limitations of the study should be noted. The generalizability of our findings was limited due to the relatively small study sample and a single referral center. We did not have a control group in which treatment decisions would have been based only on clinical and laboratory findings without the use of MSUS exam. Although no inter-reader reliability analysis has been undertaken, the fact that all examinations were performed by a single independent rheumatologist adds reliability to the results obtained. Our results may be also confounded by the lack of strong evidence to support MSUS to guide clinical decisions in a T2T strategy in RA patients. Therefore, it is likely that different physicians put different weights into the MSUS data while reevaluating their intentions to change treatment actions after reviewing the MSUS report. Due to the design of our study undertaken in real clinical practice conditions, feasibility of MSUS assessment could not be evaluated. MSUS is a time-consuming examination so it would be desirable to elucidate which patients should be scanned, from an efficiency point of view.

We conclude that the presence of PD in the MSUS examination highly impacts the management of RA patients in the setting of our longitudinal study. Further studies should evaluate its role on disease activity assessment into a T2T strategy in these patients.

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CONFLICT OF INTERESTS

None declared.

ETHICS APPROVAL

The research protocol has been approved by the Research Ethics Committee of Hospital General Universitario Gregorio MArañón, and all patients gave informed written consent for their participation in the study.

PATIENT CONSENT FOR PUBLICATION

Not required.

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ORIGINAL ARTICLE

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Large fiber peripheral neuropathy in systemic sclerosis: A prospective study using clinical and electrophysiological definition

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Abstract

Aim: The reported prevalence of peripheral neuropathy in systemic sclerosis (SSc) is variable between 0.01% to 28%, probably due to differences in sample size, study design and population. Our aim is to determine the prevalence of large fiber peripheral neuropathy in SSc and to identify any contributing factors.

Method: A prospective cross-sectional study of 60 SSc patients were evaluated for large fiber neuropathy using the modified clinical Total Neuropathy Score (cTNS) and nerve conduction study (NCS) of the upper and lower limbs. A combination of clinical (cTNS score ≥ 2) and NCS criteria (≥2 abnormal nerves including 1 sural [symmetrical polyneuropathy] and NCS abnormalities consistent with individual nerves/nerve roots [focal neuropathy]) was used to diagnose peripheral neuropathy.

Results: The majority had limited cutaneous subset (75%). Mean age was 55.73 (SD ± 13.04) years and mean disease duration was 8.61 (SD ± 8.09) years. Twenty-two (36.7%) had combined clinical and NCS criteria for peripheral neuropathy, 14 (23.3%) with symmetrical polyneuropathy and 8 (13.3%) with focal neuropathy. Symmetrical polyneuropathy patients had significantly lower hemoglobin levels (11.2 vs. 12.35 g/L; $P = .047$). Serum vitamin B_{12} levels were normal, therefore excluding vitamin B_{12} deficiency. No other associations were found for both polyneuropathy and focal neuropathy with demography, co-morbid diseases and SSc disease factors such as Raynaud's phenomenon and modified Rodnan skin score.

Conclusion: Large fiber neuropathy is common in SSc patients, which could contribute to non-lethal burden in SSc with sensory loss and muscle weakness. Apart from lower hemoglobin in polyneuropathy, there were no associations with disease-specific features or co-morbid diseases.

KEYWORDS

peripheral neuropathy, polyneuropathy, scleroderma, sensory, systemic sclerosis

Jasmin Raja and Tharshannia Balaikerisnan have equally contributed to this research study and are co-first authors.

1 | INTRODUCTION

Peripheral neuropathy is an under-recognized clinical feature in systemic sclerosis (SSc) and may be due to the disease itself or to co-morbid diseases and non-SSc-related disorders.[1,2] Patterns of peripheral nerve involvement can be either focal (or multifocal) or diffuse and symmetrical (polyneuropathy). Pure sensory polyneuropathy is the most frequently associated neuropathy in SSc.[3,4] Other patterns of peripheral polyneuropathies observed were mixed sensory and motor polyneuropathy and mononeuritis multiplex.[1,2] Peripheral nerve manifestations in SSc can involve large and small fibers in a non-length-dependent manner instead of compression. The methods of assessment could include quantitative sensory testing which is a sensitive test in addition to clinical neurological examination and nerve conduction study (NCS).[2] It is unknown whether neuropathy in patients with SSc is a primary or secondary event. A few possible pathophysiological mechanisms contributing to peripheral neuropathy have been postulated. First is the hypothesis of involvement of vascular dependent neuropathy due to vasculitis or vessel wall deterioration of the vasa nervorum. Nerve biopsy lesions had shown increased connective tissue and clusters of myelinated fibers as well as microangiopathic changes in the nerve (endoneurial, perineurial and epineurial) vessels. Alterations of vasa nervorum with intimal proliferation and adventitial edema were also observed.[5] Less commonly, biopsy-proven vasculitis and mononeuritis multiplex were seen in few cases.[5-7] Second, direct nerve compression damage by calcinosis or edema in the early phase of disease and fibrosis in the advanced stage of disease may occur.[6] This can also contribute to focal entrapment neuropathies seen in SSc, commonly carpal tunnel syndrome.[8-10] Third, the presence of anti-neuronal antibodies linked to SSc could suggest an immune-mediated mechanism directed toward the peripheral nerve.[1]

A few studies have investigated and reported the presence of cutaneous nerve involvement in SSc, involving myelinated, unmyelinated sensory and autonomic nerve fibers.[11,12] Ultrastructural modifications of peripheral nervous system in the skin of SSc patients were also studied based on morphological findings examined on transmission electron microscopy and concluded that the peripheral nerve damage in SSc has been shown to evolve from the early to the advanced phase, especially in the diffuse subset.[13]

The reported prevalence of peripheral neuropathy in SSc varies widely between 0.01% to 28%, and is probably due to relatively small sample sizes and retrospective nature of some of the previous studies as well as the different definitions used to diagnose neuropathy.[2-4,14-16] A recent prospective study of SSc patients found a high prevalence of neuropathy (28%), but in the majority of patients, neuropathy was not thought to be related to SSc. In the study, the majority of patients were Caucasians, 15% African Americans and only 5% Asians. Neuropathy was found to be associated with African American ethnicity.[15] There have been few studies of SSc and peripheral neuropathy in other populations. Malaysia is an Asian country with a multi-ethnic population comprising of Malays, Chinese, Indians and other races. The objective of this study was to determine the prevalence of neuropathy in SSc patients and to look for any associated demographic and clinical factors in these patients.

2 | MATERIALS AND METHODS

This was a prospective cross-sectional study conducted from January 2017 until August 2018 at the University of Malaya Medical Center (UMMC), Kuala Lumpur. The study was approved by the UMMC medical ethics committee (Institutional Review Board reference no: 20161227-4700) and all participants provided written informed consent. Eighty-six patients who fulfilled the 2013 American College of Rheumatology (ACR) / European League Against Rheumatism (EULAR) criteria were identified from the SSc database of the Rheumatology Unit, UMMC.[17] Of these, 60 patients were enrolled into the study. Of the remaining 26 patients, 12 had been lost to follow-up, 10 were deceased and 4 refused consent for the study. There were no exclusion criteria.

All patients were evaluated by a single investigator using a set of standard interview questions and detailed clinical examination. Modified Rodnan skin score (mRSS) was performed on all scleroderma patients at the time of NCS. Skin score was also specifically assessed at both hands. Additional information was obtained from patients' medical records. Presence of diabetes mellitus was from past medical history and medications. For the rest without history of diabetes, the evaluation was done using fasting blood sugar (FBS). Interstitial lung disease (ILD) was defined as significant lung fibrosis involving more than 20% of the lungs based on high resolution computed tomography of thorax and forced vital capacity (FVC) predicted value of either <70% or deterioration of FVC predicted value 5% to 10% based on pulmonary function test. Pulmonary arterial hypertension (PAH) was defined as mean pulmonary artery pressure (mPAP) ≥25 mm Hg and pulmonary capillary wedge pressure (PCWP) <15 mm Hg diagnosed by right heart catheterization. Small intestinal bacterial overgrowth (SIBO) was defined as symptoms suggestive of presence of excessive bacteria in the small intestine such as abdominal bloating, loose stool and flatulence, which were relieved with a course of antibiotics.

We used modified Total Neuropathy Score (cTNS), a validated tool for peripheral neuropathy that encompasses signs, symptoms and objective testings. It includes the components motor, sensory and autonomic symptoms as well as objective neurological signs of muscle power, reflexes and pin sensitivity.[18] The sensory symptoms included paresthesias (tingling), numbness and neuropathic pain (burning, aching and stabbing). The severity of symptoms and signs were scored on a scale of 0 to 4, from none to most severe. Patients who scored ≥ 2 were labeled as clinically defined neuropathy.

All patients then underwent NCS using the Synergy on Nicolet® EDX system (Natus Medical Incorporated, USA), carried

TABLE 1 Comparisons on clinical characteristics between those with and without polyneuropathy

		No polyneuropathy n = 46 (76%)	Polyneuropathy n = 14 (24%)	*P* value
Demographics	Mean age (y)	53.6 ± 13.1	62.6 ± 10.7	**.024**
Gender, n (%)	Female	41 (68.3)	14 (23.3)	.329
	Male	5 (8.4)	0 (0)	
Race, n (%)	Malay	15 (32.6)	4 (28.5)	.936
	Chinese	27 (58.6)	9 (64.2)	
	Indian	3 (6.5)	1 (7.1)	
	Eurasian	1 (2.1)	0 (0)	
BMI kg/m^2 (mean)		23.3 ± 5.9	21.0 ± 5.1	.176
SSc duration (mean)		8.8 ± 8.77	8.00 ± 5.51	.748
Skin variables, n (%)				
	Diffuse SSc	10 (21.7)	5 (35.7)	.720
	Limited SSc	36 (78.2)	9 (64.3)	.309
Diffuse vs limited SSc		0.479		
mRSS score (mean)		10.13 ± 8.00	8.36 ± 4.23	.431
Biological variables				
Hemoglobin (mean) (g/L)		12.35 ± 1.32	11.2 ± 1.25	**.007**
MCV > 97 (fL)		4 (8.69)	3 (21.4)	.159
Creatinine (mean) (μmol/L)		62.09 ± 51.2	86.57 ± 93.22	**.027**
FBS (mean) (mmol/L)		4.93 ± 1.16	6.11 ± 1.92	.012
ESR (mean) (mm/h)		33.7 ± 20.42	44.23 ± 19.6	.106
Serum vitamin B_{12} (mean) (pmol/L)		NA	484.2 ± 328	
Serum folate (mean) (nmol/L)		NA	28.08 ± 16.81	
SSc autoantibodies, n (%)				
	Anti-ScL-70	15 (37.5)	4 (28.5)	.660
	Anti-centromere	4 (9.3)	1 (7)	.804
	Anti-SSA	10 (21.7)	2 (14.2)	.472
	Anti-SSB	8 (17.4)	1 (7)	.401
	Anti-RNP	11 (23.9)	4 (28.5)	.860
	Anti-Ro52	4 (8.7)	3 (21.4)	.226
	Anti-PM-ScL100	0	0	0
	Anti-PM-ScL75	3 (21.4)	1 (7)	.336
	Anti-RP155	2 (4.3)	1 (7)	.700
	Anti-fibrillin	3 (6.5)	3 (21.4)	.182
Comorbidities, n (%)				
	Diabetes mellitus	4 (8.7)	3 (21.4)	.337
	Hypertension	12 (26.1)	5 (35.7)	.484
	Chronic or ESRF	4 (8.7)	4 (28.5)	.086
	Thyroid disease	2 (4.3)	1 (7)	.556
Clinical features, n (%)				
	Digital ulcer	4 (8.7)	8 (57.1)	.617
	Raynaud's phenomenon	26 (56.5)	9 (64.2)	.706
	Inflammatory arthritis	24 (52.2)	8 (57.1)	.770
	Myositis	9 (19.6)	3 (21.4)	1.000
	Telangiectasia	19 (41.3)	6 (42.8)	.730

(Continues)

TABLE 1 (Continued)

		No polyneuropathy n = 46 (76%)	Polyneuropathy n = 14 (24%)	*P* value
	Calcinosis	7 (15.2)	3 (21.4)	.685
	ILD	21 (45.7)	12 (85.7)	.030
	PAH	4 (8.6)	1 (7)	.524
	GERD	25 (54.3)	8 (57.1)	1.000
	SIBO	2 (4.3)	1 (7)	.142
Treatments ever received, n (%)				
	Azathioprine	13 (28.3)	3 (21.4)	.740
	Prednisolone	20 (43.5)	5 (35.7)	.760
	Methotrexate	4 (8.7)	0 (0)	.564
	Mycophenolate mofetil	6 (13)	2 (14.2)	1.000
	Hydroxychloroquine	18 (39.1)	5 (35.7)	1.000
	IVIG	1 (2.17)	0 (0)	.233
	Cyclophosphamide	0 (0)	1 (7)	.233
	Proton pump inhibitor	17 (37)	2 (14.2)	.189

Note: Values are presented in ± SD or the number (%) unless indicated otherwise. On univariate analyses, age, lower hemoglobin and higher creatinine levels were associated with polyneuropathy (bold).

Abbreviations: BMI, body mass index; ESR, erythrocyte sedimentation rate; ESRF, end stage renal failure; FBS, fasting blood sugar; GERD, gastroesophageal reflux disease; ILD, interstitial lung disease; IVIG, intravenous immunoglobulin; MCV, mean corpuscular volume; mRSS, modified Rodnan skin score; NA, not available; PAH, pulmonary arterial hypertension; SIBO, small intestinal bacterial overgrowth; SSc, systemic sclerosis.

out by a neurologist trained in electrophysiology. The following nerves were studied bilaterally: median, ulnar (motor, sensory and F wave), common fibular, posterior tibial (motor and F wave), radial and sural nerves (sensory) as well as the soleus H reflex. Skin temperature was kept at least 32 degrees Celsius. Normal reference values were previously obtained from a study of 160 healthy volunteers.[19] NCS parameters were considered abnormal if they were above the 95th percentile of reference values for latency and below the 5th percentile for amplitude and velocity. NCS-defined polyneuropathy was when there were abnormal parameters in at least 2 nerves, including a sural nerve.[20] NCS-defined focal neuropathy was when there were NCS findings consistent with abnormalities of individual nerves or nerve roots, other than sural. It is recognized that NCS measures the fastest conducting, predominantly large nerve fibers and may be normal in the subset of small fiber polyneuropathy.[20] However, skin biopsy evaluation was not performed in this study for the assessment of small fiber polyneuropathy. Following previously published recommendations for research in polyneuropathy, we required both clinical and NCS criteria for polyneuropathy to be fulfilled to make a diagnosis of definite large fiber symmetrical polyneuropathy.[10] Similarly, the correlation of clinical symptoms and NCS-defined focal neuropathy was required before a diagnosis of definite focal neuropathy was made.

Statistical analyses were performed using IBM SPSS software version 24. Continuous variables were described with mean and standard deviation. Categorical variables were reported in frequency and percentage. Associated factors of symmetrical polyneuropathy and focal neuropathy were analyzed separately, as both subtypes are likely to have different pathophysiological mechanisms. Clinical associations with peripheral neuropathy were tested using Pearson Chi-square, Fisher's exact, independent Student's *t* test, Mann-Whitney *U* test and Kruskal Wallis, where appropriate. To determine factors independently associated with polyneuropathy, multiple logistic regression analysis was used. Mean differences were reported with their corresponding 95% confidence intervals (CI). A *P* value of $< .05$ was taken as statistically significant.

3 | RESULTS

Of 60 SSc patients, 55 (91.7%) were women. Age range was between 20 and 84 years (mean 55.7; SD ± 13.04). Thirty-six (60%) were Chinese, 19 (31.7%) Malays, 4 (6.7%) Indian and 1 (1.7%) Eurasian (mixed European and Asian) (Table 1). Disease duration (from first non-Raynaud's symptoms) ranged from 1 to 44 years (mean 8.61; SD ± 8.09). The majority of SSc patients (46, 76%) had limited cutaneous subtype while 14 (23%) were diffuse cutaneous subtype. Twelve (20%) had overlap with other connective tissue diseases which fulfilled the respective ACR or EULAR criteria, namely systemic lupus erythematosus (SLE) (8), Sjögren's syndrome (4), rheumatoid arthritis (1), dermatomyositis (2) and polymyositis (1). The most common non-neurological clinical manifestations were interstitial lung disease (33, 55%), inflammatory arthritis (32, 53.3%), Raynaud's phenomenon (30, 55.6%) and gastroesophageal reflux disease (33, 55%). Associated scleroderma-antibody seropositivity frequencies were as follows: anti-ScL-70

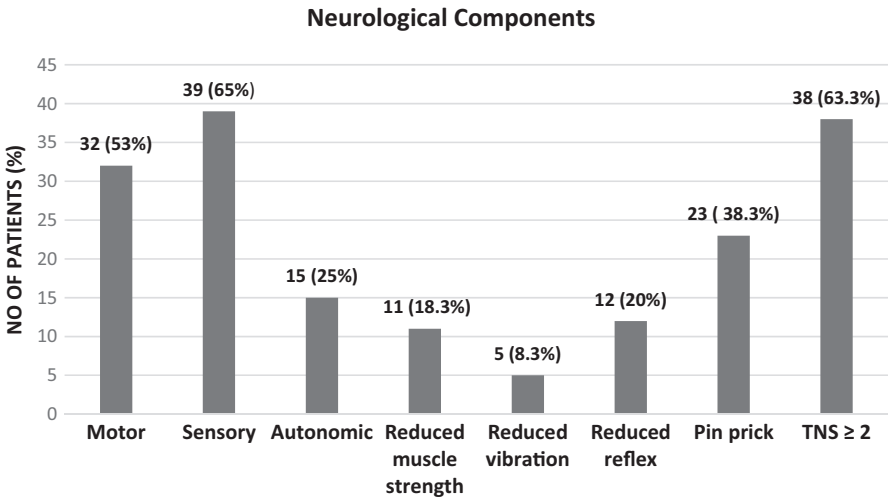

FIGURE 1 Neurological components of clinical Total Neuropathy Score (cTNS)

(19 patients, 31.7%), anti-RNP (15, 25%), anti-centromere (5, 8.3%), anti-fibrillin (6, 10%), anti-RP155 (3, 5%), anti-PMScL-75 (4, 6.7%) and 1 (1.7%) each for anti-RP11, anti-Ku, anti-Th/To and anti-Nor90. Co-morbid diseases included systemic hypertension (17, 28.3%), chronic or end stage kidney disease (8, 13.3%), diabetes mellitus (7, 11.6%) and thyroid disease (3, 5%).

3.1 | Neuropathy manifestations of SSc

Sensory symptoms, typically numbness of the distal extremities (39 patients, 65%) were more common than motor symptoms (muscle weakness in 32 patients, 53%) while autonomic symptoms (postural hypotension, urinary incontinence, erectile dysfunction and digestive dysfunction) were seen in 15 (25%). Twenty-three (38.8%) patients had impaired pin sensitivity, 12 (20%) had abnormal reflexes and 11 (18.3%) had mild to moderate muscle weakness. Based on modified cTNS score of ≥2, 38 (63.3%) patients had clinical neuropathy (Figure 1).

3.2 | Nerve conduction studies (NCS)

Seventeen (28.3%) had NCS-defined polyneuropathy, while 12 (21.4%) had NCS-defined focal neuropathy. The latter included common fibular neuropathy (5 patients), median nerve entrapment at the wrist (carpal tunnel syndrome, 4 patients), lumbosacral radiculopathy (2 patients) and posterior tibial neuropathy (1 patient). Three patients had both NCS-defined polyneuropathy and focal neuropathy (all of whom with median nerve entrapment).

Fourteen (23.3%) had clinical and NCS-defined polyneuropathy and were diagnosed as having definite symmetrical polyneuropathy. Comparisons of NCS parameters between those with and without polyneuropathy are summarized in Table 2. An additional 8 patients (13.3%) had clinical neuropathy and NCS-defined focal neuropathy. Hence, the overall prevalence of peripheral neuropathy in SSc is 36.6%.

3.3 | Associated factors of large fiber polyneuropathy in SSc

Table 1 shows the comparison of clinical and laboratory characteristics between those with and without definite polyneuropathy. There were no associations of polyneuropathy with SSc disease markers such as skin fibrosis (mRSS), specific organ manifestations, Raynaud's phenomenon or vasculopathy, SSc-specific autoantibodies, treatment or co-morbid diseases such as diabetes mellitus and kidney disease.

On univariate analyses, there were associations with age, lower hemoglobin and higher creatinine levels with polyneuropathy. On multivariate analysis, only lower hemoglobin level was significantly associated with polyneuropathy (11.2 vs. 12.35 g/L; $P = .047$). However, there was no significant difference in the frequency of anemia (defined as <12 g/L) between the groups; 8 of 14 (57.1%) in the polyneuropathy group versus 27 of 46 (58.7%) in the non-polyneuropathy group. Serum vitamin B_{12} levels were within the normal range (mean 484.2 pmol/L, SD ± 328.8) in all patients with polyneuropathy. There was also no correlation between high MCV (>97 fL) and polyneuropathy. Conversely, although serum iron levels were not routinely measured, there were only 2 patients with polyneuropathy who had hypochromia and microcytosis (normal range MCV 77-97 fL and MCH 27-32 pg). The 12 patients with focal neuropathy neither had active inflammatory arthritis nor active skin disease, although tethering of skin was present in these patients, mainly in the hands (Table 3). No SSc disease factors or co-morbid diseases were found to be associated with focal neuropathy, suggesting that the latter is likely to be due to local causes, such as focal nerve compression.

4 | DISCUSSION

The reported prevalence of peripheral neuropathy in rheumatic diseases is higher than in the general population, which is estimated to be about 2.4%.[21] In rheumatoid arthritis, the prevalence of peripheral

TABLE 2 Comparison of NCS values between systemic sclerosis patients with and without polyneuropathy[a]

NCS parameters	No polyneuropathy	Polyneuropathy	*P* value
Mean ulnar SAP amplitude (μV)	14.6 ± 6.0	9.8 ± 5.8	.012
Mean ulnar SNCV (m/s)	45.9 ± 8.0	41.8 ± 4.9	.007
Mean ulnar CMAP amplitude (mV)	9.1 ± 2.4	8.3 ± 2.2	.187
Mean ulnar MNCV (m/s)	57.7 ± 4.8	51.9 ± 3.0	<.0001
Mean sural SAP amplitude (μV)	13.5 ± 9.0	3.2 ± 3.1	<.0001
Mean sural SNCV (m/s)	46.3 ± 8.7	29.7 ± 16.6	<.0001
Mean common fibular CMAP amplitude (mV)	3.9 ± 2.4	2.4 ± 1.6	.042
Mean common fibular MNCV (m/s)	45.8 ± 9.0	36.5 ± 11.3	<.0001
Mean posterior tibial CMAP amplitude (mV)	9.5 ± 3.8	5.6 + 2.8	<.001
Mean posterior tibial MNCV (m/s)	46.9 ± 8.4	36.0 ± 12.0	<.001

Abbreviations: CMAP, compound muscle action potential; MNCV, motor nerve conduction velocity; NCS, nerve conduction study; SAP, sensory action potential; SNCV, sensory nerve conduction velocity.

[a]Cutoff values for abnormality in our electrodiagnostic laboratory are as follows: ulnar SAP amplitude < 5.2 μV; ulnar SNCV < 45.8 m/s; ulnar CMAP amplitude < 4.9 mV; ulnar MNCV < 52.2; sural SAP amplitude < 5.2 μV; sural SNCV < 38.1 m/s; common fibular CMAP amplitude < 2.4 mV; common fibular MNCV < 41.2 m/s; posterior tibial CMAP amplitude < 5.1 mV; posterior tibial MNCV < 39.8 m/s.

neuropathy is 33% whereas in SLE and primary Sjögren's syndrome the reported prevalence is 25% and 27%, respectively.[22-24] There have been fewer studies on peripheral neuropathy in SSc, with varying sample sizes and definitions for peripheral neuropathy (not all used objective NCS criteria), hence the wide range in its reported prevalence in SSc.[2,15,16] A systematic review of the literature gave an estimated prevalence of peripheral neuropathy in SSc at 14.5%, and suggested that peripheral nervous system involvement may be quite common.[25]

In this study, the prevalence of peripheral neuropathy in SSc of 36.7% was higher than prevalence reported in previous studies.[2,15,25] Polyneuropathy was found in 23.3% and a further 13.3% had focal neuropathy. Neuropathy symptoms and signs in SSc patients were more often sensory rather than motor or autonomic, a similar finding to other studies.[16,25] We applied a stricter definition for definite peripheral neuropathy (combining both clinical and NCS criteria) and found that a majority of patients with clinically defined neuropathy had normal NCS. The significance for this group of patients could be that symptoms/signs are subjective and less specific for neuropathy but also raise the possibility of small fiber polyneuropathy (in which NCS would be normal) in some patients.[16,26]

A lower hemoglobin level was an independent associated factor of large fiber polyneuropathy. However, the difference in the means between those with and without polyneuropathy was slight, only 1.15 g/L and anemia (if a cut-off of <12g/L was used) was not significantly associated with polyneuropathy. Low hemoglobin levels in SSc can occur due to occult gastrointestinal bleeding from peptic ulcer disease or gastric antral vascular ectasia, malabsorption, malnutrition or small bowel bacterial overgrowth. Vitamin B_{12} deficiency in particular, due to either malabsorption or malnutrition, can predispose patients to both lower hemoglobin levels and polyneuropathy.[27] However, all our polyneuropathy patients had normal serum vitamin B_{12} levels. Similarly, although serum iron levels were not measured, only 2 patients with polyneuropathy had hypochromia and microcytosis. Furthermore, iron deficiency is not usually a cause for polyneuropathy. A lower hemoglobin level could be non-specifically associated with the disease.

In the 8 patients (33.3%) with definite focal neuropathy, the underlying cause is likely nerve compression rather than focal vasculitis as there were no clinical features to suggest the latter that could present as acute onset of neuropathic pain and weakness.[11,15] Furthermore, there were no associations with SSc disease activity markers. Specifically, there was no association with Raynaud's to suggest vasculopathy or mRSS to suggest skin edema/fibrosis as a cause for nerve fiber compression ($P = .647$). Similar findings were reported in another study, but the evaluation of peripheral neuropathy was in both feet only.[4]

The exact pathophysiological mechanism of neuropathy in SSc is unknown. This study suggests that large fiber neuropathy is common in SSc, but its association with underlying disease factors especially skin changes are unclear. However, small fiber peripheral neuropathy has been observed in both affected and normal skin in SSc.[2,11] In a more recent study of skin biopsy samples of SSc patients, none of the 11 patients complained of sensory disturbances. However, significant loss of epidermal nerve fibers (ENF) density was seen with correlation with dermal vasculature extension in both affected skin

TABLE 3 Focal (entrapment) neuropathy and skin score details

Patient	Focal neuropathy	Skin score R hand	Skin score L hand	Skin score R foot	Skin score L foot	Skin score R and L legs and thighs
1	Bilateral CTS	1	2			
2	L CTS	1	2			
3	R CTS	1	1			
4	Lumbosacral radiculopathy	2	2			
5	Posterior tibial neuropathy	2	2	N/A	N/A	N/A
6	Common fibular neuropathy	1	1	N/A	N/A	N/A
7	Common fibular neuropathy	2	2	1	1	0
8	Common fibular neuropathy	3	3	1	1	0
9	Common fibular neuropathy	3	3	N/A	N/A	N/A
10	Common fibular neuropathy	3	1	N/A	N/A	N/A
11	R CTS	1	1			
12	Lumbosacral radiculopathy	0	2			

Abbreviations: CTS, carpal tunnel syndrome; L, left; R, right.

and apparently normal skin with more severity in the clinically involved skin.[11] A follow-up study of immunohistochemistry of cutaneous innervation was done whereby there was expansion of dermal vascular bed with significant increase in ENF density following prostacyclin analog, Iloprost infusion. This finding suggests tissue oxygenation reverts axonal terminal degeneration, therefore small fiber neuropathy in SSc is a reversible process induced by local ischemia.[26]

A previous study of 60 SSc patients reported a prevalence of 28% for neuropathy, which the authors conclude are often unrelated to the disease.[15] Peripheral neuropathy was associated with being male, African American ethnicity, diabetic as well as some disease characteristics such as limited cutaneous scleroderma and positive U1 RNP antibodies. However, there were some differences from our study in which their subjects with neuropathic symptoms but normal NCS were also included; so as not to exclude patients with possible small fiber polyneuropathy. Unlike that study, we did not find any differences in the prevalence of polyneuropathy among our 3 main ethnic groups, nor did we find any association with diabetes mellitus or other co-morbid diseases.

There are several limitations in this study. A combined clinical and NCS criteria made the diagnosis of large fiber peripheral neuropathy more precise but at the expense of excluding small fiber neuropathy in our patients. Future studies which include assessment for small fiber neuropathy would be important. In addition, we did not routinely screen for causes of low hemoglobin in all our patients, for example serum iron levels. As such, the significance of the association with lower hemoglobin with polyneuropathy could not be elucidated further.

In conclusion, large fiber peripheral neuropathy is common in SSc and should be recognized. It contributes to the non-lethal burden in SSc with sensory loss and muscle weakness which can lead to increased risk of foot ulceration and falls. There was an association of polyneuropathy with low hemoglobin level, but not with specific SSc disease markers or other co-morbid diseases.

AUTHORS CONTRIBUTION

JR – designed the study, collected data, interpreted the results and wrote the manuscript. TB – collected data, interpreted the results and wrote the manuscript. LP – performed the nerve conduction study (NCS). GKJ – designed the study, performed NCS, interpreted the results on NCS and wrote the manuscript.

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ORIGINAL ARTICLE

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A patient-centered knowledge translation tool for treat-to-target strategy in rheumatoid arthritis: Patient and rheumatologist perspectives

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Abstract

Aim: Implementation of treat-to-target (T2T) for rheumatoid arthritis (RA) presents many challenges and an evidence-practice gap has emerged. This study assessed clinician and patient barriers to the implementation of an RA-T2T strategy and developed a knowledge translation (KT) tool for use in "real-life" clinical settings.

Methods: Surveys of patients and rheumatologists measured agreement with RA-T2T recommendations and use in daily practice. Patient knowledge and perceptions were assessed as was clinician willingness to alter practice and barriers to RA-T2T using visual analog scales. An electronic KT-tool was developed and a two-phase usability trial undertaken to assess use in clinical interactions.

Results: Ninety-one percent of patients had no prior knowledge of RA-T2T but agreed with the recommendations showing mean level agreement scores (8.39-9.54, SD 2.37-1.54). Ninety percent were willing to try RA-T2T, 49% felt their treatment could be improved and 28% wanted more involvement in treatment decisions. Rheumatologists agreed with RA-T2T recommendations (7.30-9.27, SD 2.59-0.91). Barriers to implementation identified by rheumatologists included time, appointment availability and perceived patient reluctance to escalate medications. Usability experiences with the KT-tool were tracked and clinicians reported it was easy to use (100%), resulted in a discussion of RA-T2T (73%) and a target being set for 63% of consults. Patients reported they read (92%) and understood (87%) the information in the KT-tool, and that a target was set in 62% of interactions.

Conclusions: RA-T2T uptake in clinical practice may be improved through understanding local clinician and patient barriers and an implementation strategy utilizing a patient-driven KT-tool.

KEYWORDS
implementation, knowledge translation, rheumatoid arthritis, treat-to-target

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a common incurable chronic inflammatory joint disease affecting 1%-2% of the population and is associated with significant disability, decreased work capacity and reduced life expectancy.[1] Treat-to-target (T2T) regimens in RA reduce disease burden, joint damage, disability[2-4] and mortality.[5] While an RA-T2T approach clearly improves outcomes, implementation in clinical practice is challenging and an evidence-practice gap has likely emerged.[6,7]

Within an RA-T2T approach, monitoring disease activity requires the use of a validated composite disease activity measure as the "target". Clinical trials and systematic reviews confirm RA measures such as the simplified disease activity score (SDAI) and the clinical disease activity index (CDAI) accurately reflect and discriminate well between low, medium and high disease activity, are sensitive to change and are feasible to use in clinical practice.[8] Disease activity scores have been utilized as part of multiple adjustment protocols in RA-T2T clinical trials worldwide, demonstrating the strong evidence for the target in T2T regimens.[2-4,9,10]

A key over-arching principle in RA-T2T is a shared decision-making process between patient and clinician.[11] Enlisting patients in this way as "partners" is crucial, as evidence shows that RA patients are often poorly adherent in terms of medications, with various demographic, psychological and clinical factors as predictors.[12] RA studies also show discordance between an individual patient and a rheumatologist's rating of disease activity, and that each approaches decisions to escalate medications very differently.[13] Patient-centered management empowers patients to engage in the management of their own health care with consideration of their preferences and values. Knowledge translation (KT) tools can improve a patient's disease-specific knowledge, including treatment options, and simplify and support discussions between clinicians and patients.[14] KT-tools may be one solution to close evidence-practice gaps.[15]

In this study we aimed to assess patient awareness, understanding and knowledge of RA-T2T and to identify perceived needs, barriers to implementation and willingness for involvement in RA-T2T. Additionally we investigated clinician attitudes toward, barriers to use and acceptance of RA-T2T. Clinicians and patients participated in a two-phase usability study of an electronic patient-centered KT-tool for RA-T2T to facilitate a shared decision-making process.

2 | METHODS

2.1 | Surveys and participants

Two cross-sectional surveys were undertaken in parallel: A survey of Australian patients with known RA was undertaken to evaluate awareness, understanding, and perceived need of RA-T2T, barriers to implementation, willingness for involvement in RA-T2T and the use of a KT-tool during a clinical visit. Demographics and RA disease characteristics were also collected. This survey was administered through a public rheumatology clinic at Princess Alexandra Hospital and through Southern Rheumatology, a private rheumatology clinic in Brisbane, Australia. Eligible patients over the age of 18 with an existing diagnosis of RA were recruited via a rheumatologist. The second survey investigated RA-T2T knowledge of Australian rheumatologists and specifically their attitude and agreement with RA-T2T, self-reported practice and acceptability of a patient-centered approach and use of a KT-tool. The survey was distributed through the Australian Rheumatology Association. Agreement was measured using visual analog scales (1 Fully disagree to 10 Fully agree) for RA-T2T recommendations and for use in daily practice (1 Never to 5 Always). Participants provided informed consent and the study was approved by the Metro South Hospital and Health Service and The University of Queensland Human Research Ethics Committees.

2.2 | KT-tool design

A patient-centered KT-tool for RA-T2T was developed to be utilized and assessed at the point of care. Tool development was based on theoretical frameworks including Graham et al.[16] knowledge to action framework, systematic reviews of both clinical and patient decision support tools in RA and the survey data. The electronic KT-tool was administered via a tablet including introductory information for RA-T2T based on the patient version of the RA-T2T recommendations and principles.[17] Guiding information allowed the electronic calculation of a target, including a patient global score completed by the patients while waiting for review in the clinic. The KT-tool included a prompt for the patient and rheumatologist to calculate the disease activity score (CDAI or SDAI) electronically during the consultation so they could view an output indicating the level of disease activity (visually and quantitatively). A final prompt was provided for the patient and rheumatologist to make a shared decision about setting a target, ongoing treatment and review interval (Appendix S1).

2.3 | KT-tool usability study

To test acceptability, applicability and feasibility of the patient-centered KT-tool a 2-phase usability study was undertaken. The first phase was a "think-aloud" interview with a small sample.[18] Participants were encouraged to say what they were thinking as they used the KT-tool, allowing researchers to identify problems and barriers the user might experience while using the tool, as well as identifying useful components and thoughts on included content. The researcher conducting the interview used a series of neutral prompts and general comments were also noted. Each think-aloud interview was audio-recorded, notes taken and analyzed using an instant data analysis method described by Joe et al.[19] A list of all usability issues identified was compiled and then ranked based on severity: (a) critical – unable to complete task; (b) severe – significant delay or frustration in task completion; and (c) cosmetic – minor issues. Frequency with which the issue arose was recorded. Issues

were aggregated into larger themes using an inductive approach to identify any major themes relating to usability. Changes to improve the usability of the KT-tool were made based on the results of this phase and a significantly revised tool was used in the second phase usability study.

The second phase of the usability study involved providing the revised KT-tool to patients and rheumatologists in a single arm exploratory study in a clinical environment as recommended for feasibility studies.[20] RA patients (n = 40) attending the public rheumatology clinic at Princess Alexandra Hospital were recruited. Inclusion criteria included known RA patients aged over 18 years seeing 1 of 7 rheumatologists. Patients completed a short evaluation immediately after the clinic appointment to assess use of the KT-tool, including whether a target was set, if their RA management changed according to the target and when they would be reviewed. Participating rheumatologists completed a 6-item assessment at the end of each consultation involving the KT-tool, to report if and how they used it.

2.4 | Statistical analysis

Analysis from the survey data included description of demographics and characteristics of patients and rheumatologists. A 10-point visual analog scale (1 = fully disagree to 10 = fully agree) measured the level of agreement with RA-T2T recommendations and a 5-point scale (1 = never to 5 = always) measured application in daily clinical practice. Levels of agreement with survey questions were quantified as low 0%-50%, moderate 51%-75% and high 76%-100%. Univariate linear regression assessed whether variables were predictive of agreement with RA-T2T recommendations 4, 5 and 6, and their use in clinical practice, specifically between age, gender, years practicing as a rheumatologist and access to a rheumatology nurse. The residual and Q-Q plots tested assumptions for linear modeling. The linear models were developed using "stats" package in R. Thematic analysis of the free text from the survey identified the primary clinician barriers to implementation of RA-T2T. Quantitative data from the usability study were analyzed using summary statistics. Standard statistical analyses employed GraphPad Prism version 7.02.

3 | RESULTS

3.1 | Patient survey

One hundred and sixteen patients consented to the survey with 111 complete surveys. Seventy percent (n = 78) of patients attended a public rheumatology clinic and 30% (n = 33) a private clinic. RA patients were aged between 18 and 75 and 71% (n = 79) were female. Forty-eight percent (n = 53) of patients reported having had RA for more than 10 years and 84% (n = 93) reported visiting their rheumatologist 2-4 times per year (Table 1).

Based on surveyed patient-perception of their RA treatment, 56% (n = 63) stated they understood their RA treatment very well or extremely well. Twenty-seven percent (n = 30) indicated they would like to be more involved in treatment decisions; 54% (n = 60) felt their RA treatment was working well or extremely well and 49% (n = 55) stated it could be improved. When asked if they had ever heard of RA-T2T, 91% (n = 101) responded no. After reading the RA-T2T recommendations patients reported high mean levels of agreement (8.39-9.54; SD 1.14-2.37; Figure 1A). Sixty-eight percent (n = 75) of patients thought that an RA-T2T plan for their RA was extremely or very important, 28% (n = 31) important and 4% (n = 5) somewhat important, and 90% (n = 100) stated they would be willing to try an RA-T2T approach.

When patients were asked what they would be willing to change as part of an RA-T2T management plan they indicated with moderate agreement that they were willing to have further blood tests, have joint counts performed, use a patient-reported outcome (PRO) for shared decision-making and slightly less willing to schedule more visits or spend more time discussing RA-T2T (Figure 2C).

TABLE 1 Characteristics of patients in the rheumatoid arthritis treat-to-target (RA-T2T) survey n = 111

Female, n (%)	79 (71)
Age, n (%)	
18-24 y	3 (3)
25-34 y	4 (4)
35-44 y	6 (5)
45-54 y	23 (21)
55-64 y	38 (34)
>65 y	37 (33)
Years living with rheumatoid arthritis, n (%)	
<1 y	5 (4)
1-5 y	33 (30)
6-10 y	20 (18)
11-20 y	31 (28)
>20 y	22 (20)
Average review with rheumatologist, n (%)	
1 visit/y	7 (6)
2 visits/y	42 (38)
3-4 visits/y	51 (46)
5-6 visits/y	9 (8)
>6 visits/y	2 (2)
Care in public rheumatology clinic, n (%)	78 (70)

3.2 | Rheumatologist survey

Eighty-five rheumatologists responded with 73 completed surveys. Rheumatologists undertaking the surveys were aged between 25 and 74 years; 42% (n = 31) were female, and 62% (n = 45) had more than 10 years of practice as a rheumatologist. Rheumatologists had a mix of work roles including full or part time in private or public practice and in university settings (teaching and/or research; Table 2).

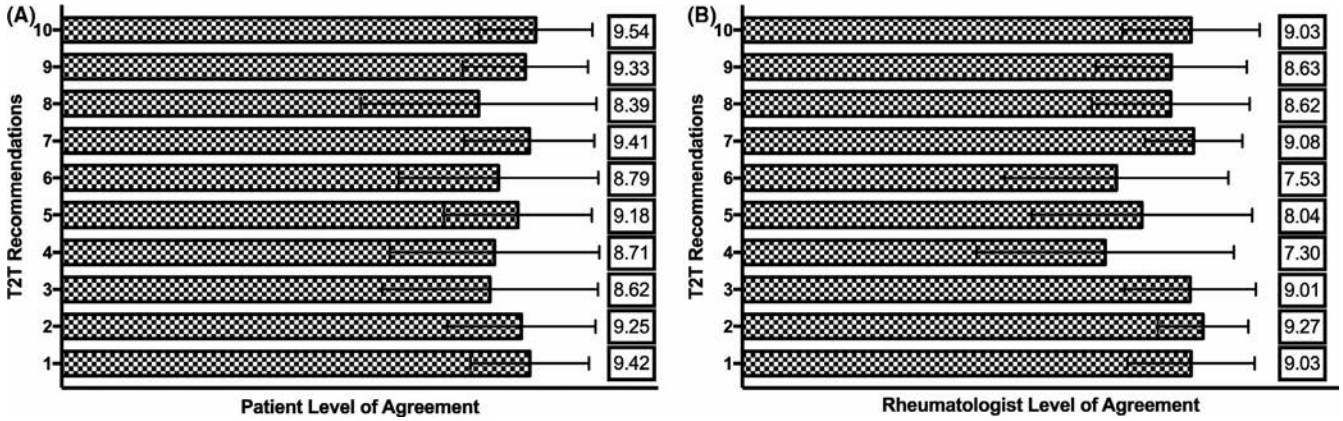

FIGURE 1 Level of agreement with rheumatoid arthritis treat-to-target (RA-T2T) recommendations measured by visual analog scale (1 fully disagree to 10 fully agree) with mean and standard deviation by patients (Panel A) and rheumatologists (Panel B)

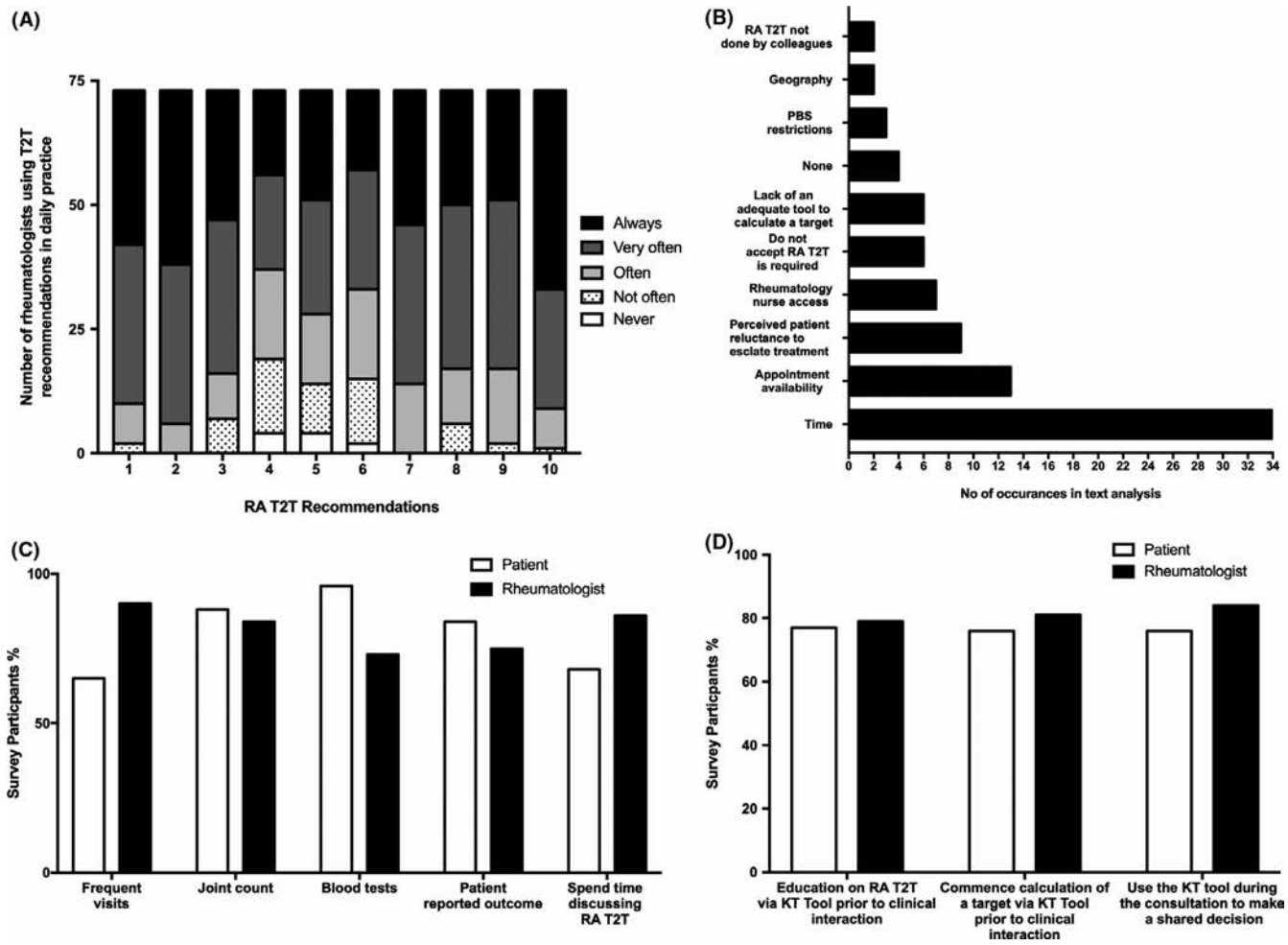

FIGURE 2 Rheumatologists (n = 73) use of rheumatoid arthritis treat-to-target (RA-T2T) recommendations in daily clinical practice measured by visual analog scale (1 never to 5 always; Panel A). Free text analysis of survey question of rheumatologists (n = 73) identifying thematic barriers to the implementation of RA-T2T by text occurrence (Panel B). Percentages of patients (n = 111) and rheumatologists (n = 73) willing to undertake frequent visits, joint counts, blood tests, use a patient-reported outcome or spend time discussing RA-T2T during a clinic appointment (Panel C) and willingness to utilize a knowledge translation (KT)-tool for education, target calculation and shared decision making (Panel D)

The mean level of agreement scores with the 10 RA-T2T recommendations ranged from 7.30 to 9.27 (SD 0.91-2.59). The lowest level of agreement was with recommendation 4, 7.30 (SD 2.59), "The use of a validated composite measures of disease activity, which includes joint assessments, is needed in routine clinical practice to guide treatment decisions", and recommendation 6, 7.53 (SD 2.25),

"Measures of disease activity must be obtained and documented regularly, as frequently as monthly for patients with high/moderate disease activity or less frequently for patients in sustained low disease activity or remission" (Figure 1B).

The use of RA-T2T recommendations in daily clinical practice was explored and 49% (n = 36) of rheumatologists reported they always or very often use a disease activity score in daily practice to guide treatment decisions with a further 25% (n = 18) using them often (recommendation 4; Figure 2A). Fifty-five percent (n = 40) of rheumatologists reported they always or very often obtain and document a measure of disease activity, more or less frequently dependent on disease activity in routine clinical practice, with a further 25% (n = 18) using them often in daily practice (recommendation 6; Figure 2A).

While age of the rheumatologist, years practicing as a rheumatologist and access to a rheumatology nurse did not predict a lower level of agreement for recommendations 4, 5 or 6, male gender predicted a lower level of agreement (R^2 = .138 P = .001) with recommendation 4 and use in clinical practice (R^2 = .08 P = .015).

Eighty-eight percent (n = 64) of rheumatologists stated that in daily practice they always or very often involve the patient in setting the treatment target and the strategy to reach this target (recommendation 10; Figure 2A). Forty-two percent (n = 31) of rheumatologists felt that RA-T2T is necessary for every RA patient. When rheumatologists were asked what they would be willing to do further as part of an RA-T2T management plan, they indicated that they would be willing to schedule more visits, perform joint counts, order further blood tests, use a PRO for shared decision-making and spend time discussing RA-T2T (Figure 2C). Sixty-four percent (n = 47) indicated they would be willing to calculate a disease activity score for every patient.

Thirty-three percent (n = 24) of those surveyed reported having a nurse in the clinic in which they worked. Rheumatologists indicated that nurses can adequately perform joint counts (n = 55, 75%), calculate disease activity scores (n = 64, 88%) and provide information and education regarding RA-T2T (n = 67, 92%).

In the survey, rheumatologists were asked the open-ended question: In your opinion and taking into consideration where and how you currently practice as a rheumatologist, what is the biggest barrier to utilizing a T2T approach in RA? Free text analysis of the answers identified 10 themes related to barriers to implementation of RA-T2T, with the most common (identified by text occurrence) being time, appointment availability and perceived patient reluctance to escalate medications (Figure 2B).

3.3 | KT-tool

Rheumatologists and patients were asked about their willingness to use a KT-tool in the clinic to stimulate a shared decision-making process for RA-T2T. Both rheumatologists and patients reported high levels of agreement with: (a) the patient reading educational information on RA-T2T via the KT-tool prior to the visit (rheumatologist 79% and patient 77%); (b) the use of the KT-tool immediately before the consult to allow the patient to complete a patient global assessment (rheumatologist 81% and patient 76%); and (c) the use of the KT-tool during the clinic consultation to make a shared decision regarding a target and treatment (rheumatologist 84% and patient 76%; Figure 2D).

3.4 | Usability testing - interviews

A convenience sample of 9 adults with RA participated in usability interviews to identify problems and barriers with the KT-tool. Participants were predominantly female (n = 7), with a median age of 59 (range 43-83 years). Usability interviews identified 19 issues. After initial changes were made to the KT-tool to address these issues, a further 9 usability issues were identified through further interviews with another 4 participants. Across the 28 usability issues identified, 4 were classed as critical, 12 as severe and 12 as cosmetic (Table 3).

The most common usability issue identified in interviews related to the disease activity score calculation and use, including the patient global disease activity scale (9 usability issues). Further usability issues included general formatting, communication and language. Changes made in response to these included information about how to provide a score, consistent terminology relating to the score, color coding to visually reinforce the scale direction, and multiple options for providing a score (dragging a slider, clicking on numbers, and clicking on scale dash lines; Table 3).

3.5 | Usability trial of RA-T2T KT-tool

Across 10 clinics, a total of 40 patients and 7 rheumatologists participated in the usability trial with a median of 7 patients having a clinical interaction with each rheumatologist.

3.5.1 | Patient experience with and perceptions of the KT-tool

Thirty-eight patients completed a brief online survey noting their experiences with the KT-tool immediately after a consultation. Patient participants were mostly female (n = 26, 68%) with a mean age of 61 ± 13 (SD) and a median of 10 (range 1-40) years living with RA; 24% of these had RA for <10 years. From this survey, patients read (92%) and understood (87%) the information on the KT-tool and entered a global disease activity score in most cases (95%). Patients thought the KT-tool was used to set a target in 62% of clinical interactions, and a target of remission or low disease activity in 13 (34%; Table 4). Patient demographic variables, perceptions and experiences with the KT-tool were not associated with age, gender or years living with RA.

TABLE 2 Characteristics of rheumatologists in the rheumatoid arthritis treat-to-target (RA-T2T) survey n = 73

Female, n (%)	31 (42)
Age, n (%)	
25-34 y	11 (15)
35-44 y	21 (29)
45-54 y	18 (25)
55-64 y	14 (19)
>65 y	9 (12)
Practicing as a rheumatologist, n (%)	
<5 y	11 (15)
5-10 y	9 (12)
10-20 y	15 (21)
>20 y	30 (41)
Rheumatology trainee	8 (11)
Clinical practice setting, n (%)	
Private practice full time	16 (22)
Public hospital full time	9 (12)
Private practice part time	30 (41)
Public hospital part time	35 (48)
Academic /university	19 (26)
Rheumatology nurse in clinic, n (%)	24 (33)

3.5.2 | Rheumatologist experience with and perceptions of the KT-tool

The participating rheumatologists completed tracking sheets noting their experiences with the KT-tool and reported that they discussed RA-T2T with 29 patients (73%). A target was set using the KT-tool for 25 patients (63%). Rheumatologists used the tool and felt it was easy to use in all 40 patients (100%), but its use extended the length of the consultation for half the patient consultations (n = 20, 50%). The tool "interfered" with the consultation for 5 patients and helped somewhat/very much to involve the patients in treatment decisions in 18 (45%).

4 | DISCUSSION

RA-T2T has been proven to improve outcomes for people living with RA; however, increasing the use of RA-T2T strategy in routine clinical settings remains a significant goal. This study investigated patient and physician barriers to the implementation of an RA-T2T strategy in Australia. To our knowledge it is the first to collect evidence to inform the development and usability testing of a KT-tool within the clinical consultation to stimulate both the use of the RA-T2T framework and to promote shared decision-making between patient and rheumatologist.

The data from this study indicated that most (91%) patients had never heard of an RA-T2T approach; however, when they were provided information, they reported high levels of agreement with the majority stating they would be willing to try the approach. This is supported by a previous report indicating that a patient version of the original RA-T2T principles and recommendations was endorsed with high levels of agreement by patients.[17] Additionally in 2 further studies from Canada and New Zealand which sought patient perspectives on RA-T2T, both demonstrated high levels of agreement with the principles and recommendations.[21,22]

Rheumatologists in this survey agreed highly with RA-T2T recommendations with the exception of the routine use of a validated composite disease activity score. Forty-nine percent of rheumatologists reported they always or very often used a disease activity score in daily practice to guide treatment decisions. Research across the world replicates these findings, including a multinational survey of 1901 rheumatologists representing 34 countries.[23] Further specific analysis of RA-T2T in clinical environments in the USA found that RA-T2T aspects, such as a disease activity score, were recorded in only 35.7% of appointments,[24] reinforcing that significant implementation of RA-T2T in clinical settings has not occurred despite broad agreement and endorsement in practice guidelines, including the American College of Rheumatology and European League against Rheumatism guidelines.[25,26]

To further explore potential implementation barriers, we investigated patient and rheumatologist willingness to change as part of an RA-T2T strategy and the perceived clinical or structural barriers. Patients indicated they were less willing to schedule more visits or spend additional time in the consultation discussing RA-T2T. This is in keeping with previous research which shows that patients value flexibility of visit frequency above other parameters.[21] While RA-T2T strategy often requires frequent reviews to ensure therapy adjustment, models of care such as tele-rheumatology that allow for patient preferences including appointment flexibility should be considered moving forward.[27]

Rheumatologists described structural barriers of time and appointment availability and also a perceived reluctance of patients to escalate treatment. The logistical barriers of time and available appointments may speak to the local environment in Queensland and more broadly in Australia where there are only 0.014 rheumatologists per 1000 population.[28] Although this study was undertaken in an urban area, the lack of specialist rheumatology access is even more pronounced for patients living in regional areas of Australia. However, patient-driven undertreatment identified by the rheumatologists in this study has also been identified in other larger RA-T2T studies. Analysis of the TRACTION trial revealed the barrier to treatment adjustment in 37.1% of visits was patient preference[29] while a further cluster-randomized trial in RA-T2T found that patient preference was the reason for the non-acceleration of treatment in 52% of clinical interactions.[30]

Underpinning RA-T2T is the concept of a shared decision-making process between patient and clinician.[11] In this study 88% of rheumatologists stated that in daily practice they always or very often involved the patient in setting the RA treatment target and the strategy to reach this target. In contrast only 9% of patients were aware of RA-T2T. Research suggests that the perception of and actual

TABLE 3 Usability issues identified in 1st round of usability interviews n = 9

Issue	Detail	Severity	Frequency	Changes made
Formatting	Text sits right at top of page when iPad held vertically	1	1	Lock orientation to horizontal
	Whiteness of background created glare	1	2	No change
	Font too small for body text	1	2	Enlarged font
	Too much text for one screen	1	1	Split over 2 screens
	Crashes if press "return"	3	2	Debug
	Not clear that output not input	2	2	Remove output boxes
	Did not understand to press "next" to go to next page	1	2	No change
Language	Some language difficult for lay audience	1	1	Rewrite text
	Not familiar with abbreviations and don't remember meaning across screens	1	2	Remove all abbreviations except RA, CDAI, SDAI
	Define RA abbreviation	1	1	Add text to clarify
Communication	GDS Concept not defined, referred to as "score" and "estimation"	1	1	Definition and consistent terminology
	CDAI/SDAI not understood	1	3	Define terms and add pop-up for further use
	Patients tried to enter detail designed for rheumatologist completion	2	3	Add text to clarify
	CRP did not know what this was and unclear that for rheumatologist to enter	2	3	Add pop-up text to clarify term and text re who to enter
	If CRP not entered, SDAI output shows "requires CRP", which was not understood	2	2	Remove output boxes
	Confusion why categories of remission, low, moderate and high different size	1	2	Add text re clinical meaning of categories
	Desire to track changes over time	1	1	Add text to prompt discussion with clinician
	Did not understand concept as a whole	2	1	No change
	Did not understand concept shared decision making	2	1	No change
Disease activity score calculation and use	Expected 10 to indicate good health rather than other way around	3	1	Added color coding to visually reinforce scale direction
	Unclear how to estimate, whether to consider physical impacts, psychological impacts, medicines, etc.	2	1	Add info re how to score
	Tried to enter score by clicking numbers rather than on scale	2	1	Allow score selection by clicking on numbers
	Patients did not realize the score presented was what they had entered previously	2	3	Add text to clarify
	Expected poor health to be on left of screen and good health on right	3	3	Switch scale to vertical
	Tried to enter score by clicking dash lines rather than on scale or number	2	3	Allow score selection by clicking on lines
	Try to enter scores designed for rheumatologist completion	3	3	Change title and add stop sign
	Tried to re-enter score already completed	2	2	Show as output differently to input
	Interpreted scale as output rather than input	2	1	No change

Note: Severity rated as 1, 2 and 3 (critical).

shared decision making can be discordant between patient and clinician and in RA a low to moderate level of shared decision making has been identified.[31]

This study indicated the need for patient awareness/education of RA-T2T and additionally for the timely use of a disease activity score within the clinical consult. Therefore a KT-tool was developed to address these issues while also prompting a shared decision. Both rheumatologists and patients indicated high levels of willingness to use a KT-tool before and during a clinical visit, and initial usability testing identified multiple issues allowing refinement of the tool for use. Patients and rheumatologists tracked their usability experience with the KT-tool with both reporting targets were set within

TABLE 4 Patient experiences and perceptions of the tool (n = 38)

Patient responses to knowledge translation tool use during clinic appointment	n (%)
Read the info on the iPad about T2T*	
Yes in full	34 (91.9)
Yes somewhat	3 (8.1)
No	0
Information on the iPad about RA-T2T was easy to understand	
Yes	35 (92.1)
No	3 (7.9)
Understood the info on the iPad about T2T	
Yes in full	33 (86.8)
Yes somewhat	5 (13.2)
No	0
Entered a global disease activity score	
Yes	36 (94.7)
No	1 (2.6)
Not sure	1 (2.6)
Rheumatologist used score to set a treatment target*	
Yes	23 (62.2)
No	6 (16.2)
Not sure	8 (21.6)
Target was set during the clinic appointment using the knowledge translation tool	
Remission	7 (18.4)
Low disease activity	6 (15.8)
Other	2 (5.3)
Not sure	18 (47.4)
No target was set	5 (13.2)
Change to RA management plan as a result of setting a target	
Yes	12 (31.6)
No	21 (55.3)
Not sure	5 (13.2)

RA-T2T, rheumatoid arthritis treat-to-target.

*n = 37 responded to this survey question.

approximately 60% of visits. Clinicians reported it was easy to use and that it prompted a discussion of RA-T2T. Patients reported that the information on the tool was read and understood and that they easily entered a global disease activity score.

KT-tools have been utilized in other chronic diseases and may improve patient knowledge, support discussion and decision making between clinicians and patients and encourage patient-centered care.[15,32] These attributes may assist in overcoming some of the implementation challenges associated with treating to target in RA, particularly in association with strategies such as learning collaboratives, recently demonstrated to have a positive impact in RA-T2T.[33] While further investigation including a randomized trial is now required to assess the capacity of the co-created RA-T2T KT-tool to alter physician and/or patient behavior and improve clinical outcomes, our initial data indicate the potential utility of the instrument.

Limitations in this study include the small sample size of both patients and rheumatologists and that patients were recruited from only 1 Australian city. Current treatment details (including disease-modifying antirheumatic drugs) was not collected and the patient group was mostly treatment experienced. This may have influenced patient survey results and indeed applicability of the results to newly diagnosed or treatment-naïve patient cohorts. The level of health literacy was not assessed in this patient group and therefore the KT-tool will require further testing for both use in people with variable levels of health literacy and indeed other cultural settings. The general limitations of survey research are further relevant to this study and include that the data were from a single point in time, there is potential for accuracy of recall issues and participation bias. However, the results were strengthened by participants from both the private and public settings and good participation rates.

RA-T2T uptake in routine clinical practice may be improved through understanding local clinician and patient barriers and a point-of-care implementation strategy incorporating an electronic, patient-driven KT-tool. Further work should focus on strategies to overcome residual barriers, and testing tools that may assist clinicians and patients in real-life clinical practice to implement RA-T2T.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Excess mortality persists in patients with rheumatoid arthritis

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Abstract

Objectives: To investigate the causes and risk of death in a large cohort of Korean patients with rheumatoid arthritis (RA).

Methods: Patients in the Hanyang BAE(Bae registry of Autoimmune diseases for Epidemiology) RA cohort who fulfilled the American College of Rheumatology criteria were analyzed. A total of 2355 patients were enrolled from October 2001 to December 2015. Mortality data were derived by linking with data from the Korean National Statistical Office. Standardized mortality ratio was estimated by dividing observed deaths by expected number of deaths in the general population.

Results: Over the observation period, 225 deaths were reported. Total age- and sex-adjusted standardized mortality ratio was 1.65 (95% confidence interval 1.44-1.87). The most common cause of death was malignancy (40 cases; 17.8%), followed by respiratory disease (38 cases; 16.9%) and cardiovascular disease (32 cases; 14.2%). Mortality rate and causes of death differed according to year and age of RA onset. Compared with survivors, individuals who died were more likely to be male, smokers, diagnosed with RA at an older age, and to have long disease duration, higher erythrocyte sedimentation rate and C-reactive protein, higher rheumatoid factor positivity rate, more severe radiographic damage, and more comorbidities.

Conclusion: The mortality rate of patients with RA remains higher than that of the general population. Therefore, to improve the survival of patients with RA, attention should be paid to the management of comorbidities as well as to the RA itself.

KEYWORDS

cohort studies, mortality, rheumatoid arthritis

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease in which the abnormal immune system causes arthritis. RA has a prevalence of 0.5%-1% of the world's population.[1] Although RA is characterized by joint symptoms, other organ systems can also be involved. Patients with RA have a higher mortality risk than the general population. Previous studies confirmed that the mortality rate of individuals with RA is 1.29- to 2.03-fold higher than that of the general population.[2-8] Different countries have different causes of death. In western studies, cardiovascular disease was the main cause of death, followed by malignancy and respiratory disease.[3-5,9,10] However, in Japan, malignancy was the main cause of death, followed by respiratory and cardiovascular disease.[6]

Treatment of patients with RA has improved over time. In a meta-analysis that analyzed 11 long-term observational studies from

1955 to 1995, the mortality rate of patients with RA remained higher than that of the general population, but decreased slightly over the past few decades.[11] The introduction of biologics and a treat-to-target strategy over the past few decades has improved outcomes of patients with RA, including improved disease activity and reduced mortality.[12-14]

However, few studies have investigated the effect of changes in treatment strategy on mortality and changes in mortality overall. Although mortality study methods vary widely, few studies combine sufficient clinical information with death data from the National Statistical Office in a long-lasting cohort comprising many patients.

With the increased overall survival rate of patients with RA, the need to reassess actual life expectancy using recent mortality data in a large cohort has increased. The purpose of our study is to update the mortality and causes of death in Korean patients with RA and to compare the mortality rate with that of the general population.

2 | MATERIALS AND METHODS

2.1 | Study population

This study included 2355 patients with RA who were consecutively enrolled and prospectively followed in the Hanyang BAE (Bae registry of Autoimmune diseases for Epidemiology) RA Cohort[2] between October 2001 and December 2015. The BAE RA Cohort is composed of Korean patients with RA who are over 19 years old, fulfill the 1987 revised American College of Rheumatology criteria,[15] and were treated at Hanyang University Hospital for Rheumatic Diseases, a tertiary referral center. Informed consent was obtained from all participants. This study was approved by the Institutional Ethics Review Board of Hanyang University Hospital (IRB File No. HYUH 2001-06-001). Evaluated parameters include sex, age at disease onset, disease duration, body mass index, comorbidity, and smoking. Disease-specific variables were collected at baseline: rheumatoid factor (RF), anticitrullinated protein antibodies (ACPA), and radiographic stage.[16] A Korean version of the Health Assessment Questionnaire (K-HAQ) was also used.[17]

2.2 | Mortality data

The Korean National Statistical Office (KNSO) maintains a civil registry for death including age at death, cause of death, and date and time of death. In Korea, deaths are reported to local government administrative offices according to Family Register Law and Statistics Law. Local government administrative offices send the reported death data to the KNSO. The transmitted data are reviewed, and causes of death are classified and counted according to the sixth version of the Korean Classification of Disease. Deaths of patients in the cohort were confirmed by linking their national register number to the national death registry of the KNSO. Causes of death were coded by the KNSO using the 10th version of the International Classification of Diseases.

2.3 | Subgroup analysis

Subgroup analysis was conducted according to year of RA onset based on the 1990s and 2005. The reason for this was that rheumatology began professionally since 1990, and biologics have been used since 2005. Subgroup analysis was conducted according to age of RA onset to compare the standardized mortality ratio (SMR) and causes of death between patients with early and late onset of disease. According to previous studies, early-onset and late-onset RA were divided at 60 years old.[18,19] In another study, patients with early-onset disease before the age of 40 years had a high risk of death from cardiovascular disease.[3] Therefore, classification was performed according to age at diagnosis of 0-39, 40-59, and ≥60.

2.4 | Statistical analysis

Patient characteristics are expressed as means (standard deviation) or numbers with proportions. All-cause death risk in the cohort was compared with the mortality of the general population from 2001 to 2015. The person-years at risk for each patient were calculated by subtracting the date of enrollment in the cohort from the earlier of two exit dates (date of death or end of observation period, 31 December 2015). Expected numbers of deaths were calculated by multiplying person-years at risk by age (in 5-year intervals) and sex-specific mortality rate. The SMR was estimated by dividing the observed deaths by the expected number of deaths for an age- and sex-matched general population. Confidence intervals (CI) were calculated based on the Poisson distribution. Generalized linear model and Cox proportional hazards model were used for assessing the impact of comorbidities and biologics usage on increase in mortality. These analyses were conducted by adjusting age, sex, and date of diagnosis. All Cox regression results were presented as hazard ratios (HRs) with 95% CIs. All statistical analyses were performed using SAS statistical software (release 9.4, SAS, Cary, NC, USA). *P* values less than 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Baseline characteristics in patients with RA sorted by death

The demographic and clinical characteristics of analyzed patients are summarized in Table 1. A total of 2355 patients were enrolled. The enrolled patients included a high proportion of women (88.7% vs 11.3%). The mean age at RA onset was 41.8 ± 12.8 years, and disease duration was 18.1 ± 10.4 years.

TABLE 1 Comparison of demographic and clinical characteristics of analyzed patients

Variables	Total (n = 2, 355)	Patients who died (n = 225)	Survivors (n = 2, 130)	*P* value
Male sex	265 (11.3)	42 (18.7)	223 (10.5)	<0.001
Age at onset, years	41.8 ± 12.8	49.7 ± 12.7	41.0 ± 12.5	<0.001
Disease duration[a], years	18.1 ± 10.4	20.7 ± 10.9	17.8 ± 10.3	<0.001
Health assessment[b]				
K-HAQ	0.9 ± 0.7	1.4 ± 0.8	0.9 ± 0.7	<0.001
Body mass index, kg/m^2	22.3 ± 3.2	21.9 ± 3.4	22.3 ± 3.2	0.062
Smoking				
Current/Ex-smoker	376 (16.1)	54 (25.1)	322 (15.2)	<0.001
Laboratory findings[b]				
ESR, mm/h	41.5 ± 26.2	48.6 ± 26.1	40.8 ± 26.1	<0.001
CRP, mg/dL	1.8 ± 2.6	2.6 ± 3.5	1.7 ± 2.5	<0.001
RF positive	1972 (84.3)	213 (94.7)	1759 (83.2)	<0.001
ACPA positive	1804 (86.1)	128 (88.9)	1676 (85.9)	0.382
Radiographic stage[c]				
Stage I + II	1391 (59.2)	99 (44.5)	1292 (60.8)	<0.001
Stage III + IV	959 (40.8)	125 (55.8)	834 (39.2)	
Comorbidity[b]				
Hypertension	330 (14.0)	63 (28.0)	267 (12.5)	<0.001
Diabetes mellitus	106 (4.5)	24 (10.7)	82 (3.8)	<0.001
Angina pectoris	6 (0.3)	1 (0.4)	5 (0.2)	0.453
Myocardial infarction	2 (0.1)	-	2 (0.1)	1.000
Hepatitis B	32 (1.4)	5 (2.2)	27 (1.3)	0.382

Note: Data are presented as mean ± SD or number (%).

Abbreviations: ACPA, anticitrullinated protein antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; K-HAQ, K-Health Assessment Questionnaire; RF, rheumatoid factor.

[a]From disease onset to two exit dates (date of death or end of observation period, 31 December 2015).

[b]These variables represent data at time of enrollment.

[c]Radiographic stages were classified according to Steinbrocker stage.

Over the observation period, 225 deaths were reported (183 women, 42 men). Compared with survivors, patients who died were more likely to be male (18.7% vs 10.5%, $P < 0.001$), and they had an older age of RA onset (49.7 ± 12.7 vs 41.0 ± 12.5, $P < 0.001$). Also, disease duration was longer in patients who died compared with survivors (20.7 ± 10.9 vs. 17.8 ± 10.3, $P < 0.001$). Activity limitation measured by K-HAQ at enrollment was associated with increased mortality (1.4 ± 0.8 vs 0.9 ± 0.7, $P < 0.001$). Erythrocyte sedimentation rate and C-reactive protein levels at enrollment were higher in patients who died (48.6 ± 26.1 vs 40.8 ± 26.1, 2.6 ± 3.5 vs 1.7 ± 2.5, $P < 0.001$). Also, RF positivity at enrollment was higher in patients who died (94.7% vs. 83.2%, $P < 0.001$). In the radiologic stage, the proportion of Stage III + IV was higher in patients who died (55.8%), and the proportion of Stage I + II was higher in survivors (60.8%). Patients who died were more likely to be current smokers or ex-smokers (25.1% vs 15.2%, $P < 0.001$) and had more comorbidities, including hypertension (28.0% vs 12.5%, $P < 0.001$) and diabetes mellitus (10.7% vs 3.8%, $P < 0.001$).

3.2 | Causes of death in patients with RA

The major causes of death in patients with RA are listed in Table 2. The most common cause of death was malignancy (40 cases; 17.8%), followed by respiratory disease (38 cases; 16.9%: including six from interstitial lung disease [ILD] and 22 from pneumonia), cardiovascular disease (32 cases; 14.2%: including 21 from ischemic heart disease [IHD] and 11 from cerebrovascular disease), musculoskeletal disease (21 cases; 9.3%), and infection (18 cases; 8.0%). There were 17 deaths (7.6%) from unknown cause.

TABLE 2 Major causes of death in patients with rheumatoid arthritis

Cause of death	2001-2007[a] (n = 56)	2008-2015 (n = 169)	Total (n = 225)
Malignancy	12 (21.4)	28 (16.6)	40 (17.8)
Respiratory disease	10 (17.9)	28 (16.6)	38 (16.9)
ILD	2 (3.6)	4 (2.4)	6 (2.7)
Pneumonia	4 (7.1)	18 (10.7)	22 (9.8)
Other pulmonary diseases	4 (7.1)	6 (3.6)	10 (4.4)
Cardiovascular disease	10 (17.9)	22 (13.0)	32 (14.2)
IHD	5 (8.9)	16 (9.5)	21 (9.3)
Cerebrovascular disease	5 (8.9)	6 (3.6)	11 (4.9)
Musculoskeletal disease	5 (8.9)	16 (9.5)	21 (9.3)
Infection	2 (3.6)	16 (9.5)	18 (8.0)
Unknown	3 (5.4)	14 (8.3)	17 (7.6)

Note: Data are presented as number (%).

Abbreviations: IHD, ischemic heart disease; ILD, interstitial lung disease.

[a]Originally published in ref. 2. (In a previous study, 57 patients were reported to have died by 2007, but information on one patient death was lacking, and only 56 patients were analyzed.)

3.3 | Subgroup analysis according to year of RA onset

The subgroup analysis according to year of RA onset is shown in Table 3 and compares three subgroups based on the 1990s and 2005. In patients diagnosed before the 1990s, malignancy (21.5%) was the most common cause of death, followed by respiratory disease (17.8%) and musculoskeletal disease (12.1%). In patients diagnosed between 1990 and 2004, cardiovascular disease (18.8%) was the most common cause of death, followed by respiratory disease (14.9%) and malignancy (13.9%). In patients diagnosed after 2005, respiratory disease (23.5%) was the most common cause of death, followed by malignancy (17.6%). Age- and sex-adjusted SMR in patients diagnosed was 1.94 (95% CI 1.57-2.30) before the 1990s, 1.45 (95% CI 1.16-1.73) between 1990 and 2004, and 1.57 (95% CI 0.82-2.32) after 2005.

3.4 | Subgroup analysis according to age of RA onset

Classification was performed according to age at diagnosis of 0-39, 40-59, and ≥60 and is shown in Table 4. The mortality rate in patients diagnosed before age 40 was 4.7%, between 40 and 59 was 10.8%, and over 60 was 27.2%. In individuals <40 years of age at RA onset, malignancy (16.7%) was the most common cause of death, followed by respiratory disease (14.6%) and musculoskeletal disease (12.5%). In individuals between 40 and 59 years at RA onset, malignancy (21.5%) was the most common cause of death, followed by respiratory disease (17.4%) and cardiovascular disease (13.2%). In those aged ≥60 years at RA onset, cardiovascular disease (19.6%) was the most common cause of death, followed by respiratory disease (17.9%) and malignancy (10.7%). Age- and sex-adjusted SMR was similar among the three groups; it was 1.78 (95% CI 1.28-2.29) in patients diagnosed before 40 years of age, 1.57 (95% CI 1.29-1.85) in patients diagnosed between 40 and 59 years, and 1.76 (95% CI 1.30-2.23) in patients diagnosed at over 60 years of age.

3.5 | SMR stratified by age group and sex

Total age- and sex-adjusted SMR was 1.65 (95% CI 1.44-1.87). SMR stratified by age group and sex is listed in Table 5. Subgroup analysis according to age of death showed that the highest sex-adjusted SMR of 2.56 (95% CI 0.00-7.59) was observed in patients aged 30-34 years. When patients classified by age underwent subgroup analysis, sex-adjusted SMR in patients who were 45-54 years old was not higher than that of the general population. However, sex-adjusted SMR in patients aged 30-34, 40-44, and ≥55 years was higher than that of the general population. In subgroup analysis, age- and sex-adjusted SMR of men was 1.20 (95% CI 0.84-1.56) and that of women was 1.81 (95% CI 1.55-2.07).

3.6 | The impacts of comorbidities and biologics usage on mortality

Compared with patients without any comorbidities, in patients with one comorbidity, the adjusted HR was 1.05 (95% CI 0.78-1.41) and in patients with two or more comorbidities, the adjusted HR was 2.01 (95% CI 1.13-3.57; $P < 0.05$). The proportion of survivors in the biologic-experienced patients was higher than in the biologic-naive patients (97.6% vs 89.6%; $P < 0.05$). In the biologic-experienced patients, the adjusted HR was 0.39 (95% CI 0.17-0.88; $P < 0.05$; Table 6).

4 | DISCUSSION

Demographic information and mortality data since 2001 were observed within a single-center large RA cohort. SMR and causes of death in patients with RA were analyzed. In addition to SMR and causes of death for all-cause mortality, a multifaceted analysis was conducted according to year and age of RA onset. Over the observation period, 225 deaths were reported. Total age- and sex-adjusted SMR was 1.65 (95% CI 1.44-1.87). The most common cause of death was malignancy, followed by respiratory disease and cardiovascular disease. The mortality rate and causes of death differed according to year and age of RA onset.

This study is an extension of a previous study conducted by the same institution in 2012. The previous study was conducted with

TABLE 3 Subgroup analysis according to year of rheumatoid arthritis onset

Year of disease onset	1950-1989	1990-2004	2005-2015	Total
Total no. of enrolled patients	543	1141	668	2352[a]
Age at disease onset, y	34.9 ± 11.2	42.5 ± 12.2	46.2 ± 12.8	41.8 ± 12.8
Male sex	39 (7.2)	145 (12.7)	81 (12.1)	265 (11.3)
Total no. of deaths	107 (19.7)	101 (8.9)	17 (2.5)	225
Age at disease onset, y	42.6 ± 10.8	55.0 ± 10.4	63.7 ± 9.9	49.7 ± 12.7
Age at death, y	72.0 ± 9.0	69.0 ± 10.0	69.1 ± 9.7	70.4 ± 9.6
SMR (95% CI)[b]	1.94 (1.57-2.30)	1.45 (1.16-1.73)	1.57 (0.82-2.32)	1.65 (1.44-1.87)
Malignancy	23 (21.5)	14 (13.9)	3 (17.6)	40 (17.8)
Respiratory disease	19 (17.8)	15 (14.9)	4 (23.5)	38 (16.9)
ILD	1 (0.9)	3 (3.0)	2 (11.8)	6 (2.7)
Pneumonia	12 (11.2)	9 (8.9)	1 (5.9)	22 (9.8)
Other	6 (5.6)	3 (3.0)	1 (5.9)	10 (4.4)
Cardiovascular disease	11 (10.3)	19 (18.8)	2 (11.8)	32 (14.2)
Musculoskeletal disease	13 (12.1)	6 (5.9)	2 (11.8)	21 (9.3)
Infection	7 (6.5)	9 (8.9)	2 (11.8)	18 (8.0)
Other heart disease	6 (5.6)	5 (5.0)	2 (11.8)	13 (5.8)
Neurologic disease	5 (4.7)	4 (4.0)	0 (0.0)	9 (4.0)
Endocrine disease	7 (6.5)	3 (3.0)	0 (0.0)	10 (4.4)
Gastrointestinal disease	3 (2.8)	1 (1.0)	0 (0.0)	4 (1.8)
Liver disease	0 (0.0)	1 (1.0)	1 (5.9)	2 (0.9)
Renal disease	2 (1.9)	4 (4.0)	0 (0.0)	6 (2.7)
Hematologic disorder	1 (0.9)	1 (1.0)	0 (0.0)	2 (0.9)
Amyloidosis	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.4)
Accident/Suicide	2 (1.9)	9 (8.9)	1 (5.9)	12 (5.3)
Unknown	7 (6.5)	10 (9.9)	0 (0.0)	17 (7.6)

Note: Data are presented as mean ± SD or number (%).

Abbreviations: CI, confidence interval; ILD, interstitial lung disease; SMR, standardized mortality ratio.

[a]Of 2355 enrolled patients, three patients who did not have information on age at disease onset were excluded from analysis. These three patients did not die.

[b]SMR is adjusted for age and sex.

patients enrolled in the cohort from October 2001 to December 2007, and mortality was calculated from 57 deaths among 1534 enrolled patients. As the number of patients, length of observational period, and number of observed deaths increased, the extension study was expected to show better validity. There was an increase in the average age and total number of deaths among patients due to the increase in cumulative period of cohort enrollment. The previous study SMR was 1.35 (95% CI 1.02-1.74).[2] In this study, total age- and sex-adjusted SMR was 1.65 (95% CI 1.44-1.87). Although the mortality rate was higher than that of the general population, the results are similar to those of patients with RA in other countries.[3-7] Relative excess mortality in RA remained unchanged over time despite substantial improvements in RA management. This shows that excess mortality still occurs in RA.

There was a difference in mortality according to year of RA onset. When age- and sex-adjusted SMR were compared according to year of RA onset, patients diagnosed with RA before the 1990s had the highest SMR. The SMR in patients diagnosed before the 1990s was 1.94 (95% CI 1.57-2.30), whereas SMR for those diagnosed between 1990 and 2004 was 1.45 (95% CI 1.16-1.73), and SMR for those diagnosed after 2005 was 1.57 (95% CI 0.82-2.32; Table 3). Also, there was a difference in mortality according to age at RA onset. According to age at RA onset, the mortality rate was high in patients diagnosed after 60 years of age. The mortality rate in patients diagnosed before 40 years of age was 4.7%, in those diagnosed between 40 and 59 years was 10.8%, and in those diagnosed over age 60 years was 27.2% (Table 4). This is consistent with a study reporting that diagnosis age is a predictor of death.[4,6] According to age of RA onset, age- and sex-adjusted SMR in patients diagnosed before 40 and over 60 were higher than in patients diagnosed between 40 and 59 years. The SMR was 1.78 (95% CI 1.28-2.29) in patients diagnosed before age 40 years, 1.57 (95% CI 1.29-1.85) in patients diagnosed between 40 and 59 years, and 1.76 (95% CI 1.30-2.23; Table 4) in patients diagnosed at over 60 years. The mortality

TABLE 4 Subgroup analysis according to age of rheumatoid arthritis onset

Age of disease onset, y	0-39	40-59	≥60	Total
Total no. of enrolled patients	1028	1118	206	2352[a]
Age at disease onset, y	29.9 ± 6.8	48.5 ± 5.5	64.5 ± 4.0	41.8 ± 12.8
Male sex	71 (6.9)	133 (11.9)	61 (29.6)	265 (11.3)
Total no. of deaths	48 (4.7)	121 (10.8)	56 (27.2)	225
Age at disease onset, y	31.6 ± 6.1	49.7 ± 5.8	65.4 ± 4.3	49.7 ± 12.7
Age at death, y	63.4 ± 12.1	70.6 ± 8.2	76.0 ± 5.3	70.4 ± 9.6
SMR (95% CI)[b]	1.78 (1.28-2.29)	1.57 (1.29-1.85)	1.76 (1.30-2.23)	1.65 (1.44-1.87)
Malignancy	8 (16.7)	26 (21.5)	6 (10.7)	40 (17.8)
Respiratory disease	7 (14.6)	21 (17.4)	10 (17.9)	38 (16.9)
ILD	1 (2.1)	2 (1.7)	3 (5.4)	6 (2.7)
Pneumonia	3 (6.3)	15 (12.4)	4 (7.1)	22 (9.8)
Other	3 (6.3)	4 (3.3)	3 (5.4)	10 (4.4)
Cardiovascular disease	5 (10.4)	16 (13.2)	11 (19.6)	32 (14.2)
Musculoskeletal disease	6 (12.5)	9 (7.4)	6 (10.7)	21 (9.3)
Infection	2 (4.2)	11 (9.1)	5 (8.9)	18 (8.0)
Other heart disease	1 (2.1)	9 (7.4)	3 (5.4)	13 (5.8)
Neurologic disease	1 (2.1)	4 (3.3)	4 (7.1)	9 (4.0)
Endocrine disease	4 (8.3)	5 (4.1)	1 (1.8)	10 (4.4)
Gastrointestinal disease	3 (6.3)	1 (0.8)	0 (0.0)	4 (1.8)
Liver disease	0 (0.0)	1 (0.8)	1 (1.8)	2 (0.9)
Renal disease	2 (4.2)	3 (2.5)	1 (1.8)	6 (2.7)
Hematologic disorder	1 (2.1)	1 (0.8)	0 (0.00)	2 (0.9)
Amyloidosis	0 (0.0)	1 (0.8)	0 (0.00)	1 (0.4)
Accident/Suicide	5 (10.4)	4 (3.3)	3 (5.4)	12 (5.3)
Unknown	3 (6.3)	9 (7.4)	5 (8.9)	17 (7.6)

Note: Data are presented as mean ± SD or number (%).

Abbreviations: CI, confidence interval; ILD, interstitial lung disease; SMR, standardized mortality ratio.

[a]Of 2355 enrolled patients, three patients who did not have information on age at disease onset were excluded from analysis. These three patients did not die.

[b]SMR was adjusted for age and sex.

rate was higher in patients with early onset before age 40 and in those with late onset after age 60.

There were also differences by sex. In subgroup analysis the age- and sex-adjusted SMR of men was 1.20 (95% CI 0.84-1.56) and that of women was 1.81 (95% CI 1.55-2.07; Table 5), whereas patients who died were more likely to be male (18.7% vs 10.5%, $P < .001$; Table 1). This is likely due to differences in age of RA onset and smoking status in male and female patients. Male patients were older at RA onset (47.7 ± 13.4 vs 41.0 ± 12.5, $P < .001$), their C-reactive protein level at enrollment was higher (2.8 ± 3.5 vs 1.7 ± 2.4, $P < .001$), and there were significantly more male smokers than female smokers (85.5% vs 7.4%; Supplementary Table S1). There was no significant difference in RF positivity, ACPA positivity, and comorbidity in male and female patients. The diagnosis rate of RA in male patients has increased (7.2% vs 12.5%; Table 3) since the 1990s. In classification according to age at diagnosis, the diagnosis rate of male patients over 60 was higher than that of male patients less than 60 years of age (9.5% vs 29.6%; Table 4). RA is a more common disease in women,[20] but the mortality rate of men is higher than that of women in all age groups in the general population. This may have affected the SMR of male and female patients in this study.

The causes of death in our study are different from those of western studies,[3-5,9,10] where cardiovascular disease was the most common cause of death, but are similar to those in Japan,[6] where malignancy was the most common cause of death. Different countries have different life expectancy, population composition, and causes of death. These differences may be due to genetic factors, environmental factors, treatment strategies, socio-economic status, health insurance, and health care.[21-23] Malignancy was the most common cause of death in our cohort (40 cases; 17.8%). Even in the general population in Korea, the mortality rate from malignancy was the highest in the latest decade of the KNSO data. In 2015, malignancy-related deaths in the general population was the highest, with 150.8 deaths per 100 000 people. Malignancy-related deaths

have continued to increase. In a previous study, malignancy-related death was the most common cause of death, but malignancy-specific SMR was 0.84 (95% CI 0.43-1.47), which was not higher than for the general population.[2] Therefore, malignancy-related death in patients with RA cannot be considered higher than that of the general population.

TABLE 5 Standardized mortality ratios stratified by age group and sex group

Group	Observed deaths	Expected deaths	SMR (95% CI)
Total	225	131.0	1.72 (1.49-1.94)
Total (age- and sex-adjusted)	225	136.0	1.65 (1.44-1.87)
Sex group (age-adjusted)			
Men	42	35.0	1.20 (0.84-1.56)
Women	183	101.0	1.81 (1.55-2.07)
Age group, y (sex-adjusted)[a]			
30-34	1	0.39	2.56 (0.00-7.59)
40-44	4	1.91	2.10 (0.04-4.15)
45-49	2	3.92	0.51 (0.00-1.22)
50-54	6	6.72	0.89 (0.18-1.61)
55-59	17	9.57	1.78 (0.93-2.62)
60-64	23	14.51	1.59 (0.94-2.23)
65-69	35	23.19	1.51 (1.01-2.01)
70-74	51	29.57	1.72 (1.25-2.20)
75-79	55	26.06	2.11 (1.55-2.67)
80-84	25	13.70	1.82 (1.11-2.54)
≥85	6	5.40	1.11 (0.22-2.00)

Abbreviations: CI, confidence interval; ILD, interstitial lung disease; SMR, standardized mortality ratio.

[a]In 5-year intervals age group, group with 0 deaths per group was excluded.

There were 38 (16.9%) deaths from respiratory disease, the second most common cause of death. According to year of RA onset, respiratory disease (23.5%) was the most common cause of death in patients diagnosed after 2005 (Table 3). However, patients diagnosed after 2005 should be re-evaluated for the number of deaths and causes of death because the observation period in this study was short. Among these, ILD and pneumonia were the most significant causes. ILD is a common recognized complication in RA that can have a remarkable effect on morbidity and mortality.[24] According to age of RA onset, ILD-related deaths comprised 2.1% in patients diagnosed before age 40, 1.7% in patients diagnosed between 40 and 59, and 5.4% in patients diagnosed over age 60 years (Table 4). ILD-related deaths were higher in those patients aged ≥60 years at RA onset than in those aged <60 years at RA onset. In a previous study, ILD-related deaths were 3.6% by 2007, but decreased to 2.7% by 2015 (Table 2). According to a study that confirmed the prevalence of ILD in patients with RA and assessed its effect on mortality, the prevalence of ILD in Korean patients with RA was 1.8%. ILD was significantly associated with increased mortality in patients with RA (HR 7.89, 95% CI 3.16-19.69).[25] Another study in the USA assessed the prevalence and mortality of ILD in patients with RA at 5 years after first diagnosis; 35.9% of patients had died.[26] Hence, ILD is still a major risk factor for mortality in patients with RA.

Infection, including pneumonia, is also a leading cause of death in patients with RA. Infection can be caused by the abnormal immunity of RA itself and by immunosuppression with anti-rheumatic agents. In our study, 18 cases involved infections other than pneumonia (8%; Table 2). Sepsis-specific SMR in a previous study was also high at 5.88 (95% CI 0.15-32.76).[2] In a previous study, pneumonia-related deaths comprised 7.1% by 2007, but increased to 9.8% by 2015. Infection-related deaths (other than pneumonia) comprised 3.6% by 2007, but increased significantly to 8.0% by 2015 (Table 2). Even in the general population in Korea, deaths from pneumonia have increased significantly compared

TABLE 6 Analysis of the impacts of comorbidities and biologics usage on mortality using generalized linear model and Cox proportional hazards model

Variables	Total (n = 2, 355)	Patients who died (n = 225)	Survivors (n = 2130)	*P* value[a]	HR (95% CI)	*P* value[b]
Number of comorbidities						
0	1907	144 (7.6)	1763 (92.4)	0.146	1.00 (referent)	
1	394	68 (17.3)	326 (82.7)		1.05 (0.78-1.41)	0.77
≥2	54	13 (24.1)	41 (75.9)		2.01 (1.13-3.57)	0.02
Biologic treatment[c]						
Biologic-naive	2106	219 (10.4)	1887 (89.6)	0.027	1.00 (referent)	
Biologic-experienced	249	6 (2.4)	243 (97.6)		0.39 (0.17-0.88)	0.02

Note: Data are presented as number (%).

Models are adjusted for age, sex, and date of diagnosis.

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a]Generalized linear model

[b]Cox proportional hazards model

[c]Patients who used one or more biologics during the observation period were categorized as biologic-experienced. Patients who had never used biologics during the observation period were categorized as biologic-naive.

with 10 years ago. According to a meta-analysis study reported in the *Lancet*, the use of low-dose biologics did not increase the risk of infection, but standard-dose biologics had a 1.31 times higher risk, and high-dose biologics increase the risk of infection by 1.90 times.[27] Therefore, treatment for RA should always be chosen with the possibility of infection in mind. Also, vaccination against influenza and *Pneumococcus* should be actively implemented.[28,29] Active diagnosis and treatment of pneumonia and infection may lower the risk of mortality.

Death from cardiovascular disease was the third most common cause of death (32 cases; 14.2%), and IHD was the most common (21 cases; 9.3%) among them. IHD-specific SMR in a previous study was also high at 2.09 (95% CI 0.68-4.88).[2] Higher age of RA onset was associated with higher rate of cardiovascular-related deaths, which was 10.4% in patients diagnosed before 40, 13.2% in patients diagnosed between 40 and 59, and 19.6% in patients diagnosed over age 60. In those aged ≥60 years at RA onset, cardiovascular disease (19.6%) was the most common cause of death. In our study, patients who died were more likely to be smokers and have accompanying hypertension and diabetes mellitus. Cardiovascular-related deaths in RA are a major concern in many countries.[3-5,9,10] The chronic inflammation of RA promotes arteriosclerosis and increases the frequency of cardiovascular-related deaths.[30] A study published in the *Lancet* revealed that proper use of methotrexate reduces mortality from cardiovascular disease to provide substantial survival benefits.[31] According to a Korean study on the effect of disease-modifying anti-rheumatic drugs on cardiovascular risk in patients with RA in Asia, their use has a protective effect on cardiovascular disease.[32] A study in the UK showed that mortality from cardiovascular disease has decreased in recent years.[33] However, cardiovascular disease is still the leading cause of mortality in patients with RA, so it is important to raise awareness of cardiovascular risk and treat these patients more actively.

Past studies have reported on various predictors of mortality in patients with RA, including older age, male sex, high disease activity, higher RF, ACPA-positive rate, high K-HAQ score, long disease duration, and comorbidities.[4,6,34-36] In this study, Korean patients with RA had nearly identical risk factors to those reported from past studies.[4,6,34-36] Compared with survivors, patients who died were more likely to be male, diagnosed with RA at an older age, have long disease duration, have higher erythrocyte sedimentation rate and C-reactive protein, have higher RF-positive rate, have more severe radiographic damage, and were more likely to be smokers. Also, RA patients with multiple comorbidities had increased mortality compared with patients without any comorbidities. The use of biologics can be considered to have an effect on reducing the mortality rate of patients with RA. However, because the sample size of biologics users is small, further evaluation will be required for the mortality rate of biologics users.

Our study has both strengths and limitations. One strength is the long follow-up period of 15 years and inclusion of the largest RA cohort in Korea. Another strength is the accurate evaluation of SMR because information about deaths was obtained by linkage with the KNSO. Absence of full information of medication and lack of cause-specific SMR are limitations of our study.

In conclusion, the mortality rate of Korean patients with RA is 1.65 times higher than that of the general population. The mortality rate of Korean patients with RA is similar to that in western countries. The main causes of death were distinctly different from those of previous studies performed in western countries. Also, the mortality rate and causes of death differed according to year and age of RA onset. With a higher risk of respiratory disease, infection, and cardiovascular disease, RA and comorbidities should be carefully managed to improve patient survival.

AUTHOR CONTRIBUTIONS

SCB contributed to the conception and design of the study. All authors contributed to the acquisition, analysis, or interpretation of data. YKL and JYL were responsible for statistical analysis. YKL and GYA drafted the manuscript, and all co-authors were involved in critically revising it for important intellectual content. SCB had full access to all data in the study and takes responsibility for data integrity and accuracy of data analysis. All authors approved the final version submitted for publication.

CONFLICT OF INTEREST

No conflicting relationship exists for any author.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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The effect of suffering from rheumatoid arthritis, systemic lupus erythematosus, and back pain on sexual functioning and marital satisfaction in Iran

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Abstract

Objective: Sexual functioning is an important component of life quality and musculoskeletal disorders may effect sexual functioning, so, the present study was conducted to evaluate sexual functioning in patients suffering from back pain, rheumatoid arthritis, and systemic lupus erythematosus (SLE).

Methods: This study was conducted on 102 patients with rheumatoid arthritis, 103 patients with back pain, 103 patients with SLE, and 210 people in the control group by the consecutive sampling method. The marital satisfaction questionnaire (Enrich), Arizona Sexual Experience Scale (ASEX) questionnaire, and the General Health Questionnaire (GHQ-28) were completed by all the subjects. Disease severity was determined in each group of patients by Disease Activity Score of 28 joints, the Roland Morris questionnaire, and the SLE Disease Activity Index questionnaire.

Results: The GHQ in rheumatoid arthritis and lupus patients was meaningfully higher than the control group ($P < .05$), while there was no meaningful difference between back pain patients and the control group ($P = .414$). The sexual functioning questionnaire score in all 3 groups showed no statistically meaningful difference with the control group ($P < .05$). Also, the marital satisfaction questionnaire score in all the groups showed no statistically meaningful difference compared to the control group ($P = .791$).

Conclusion: The study has shown that the level of sexual function in participants with back pain and the level of mental health and sexual functioning in patients with rheumatoid arthritis and SLE are significantly lower than healthy people and there is a need for intervention for improving mental health as well as sexual functioning in these patients.

KEYWORDS

back pain, marital satisfaction, rheumatoid arthritis, sexual functioning, systemic lupus erythematosus

1 | INTRODUCTION

A family is a kinship unit consisting of a group of individuals united by blood or by marital, adoptive, or other intimate ties. Although the family is the fundamental social unit of most human societies, its form and structure vary widely.[1] In this study, we only dealt with marital relationships, one of the goals of which is to establish sexual relationships and satisfy sexual needs. Sexual desires and needs have a fundamental role in marital life and few marital relationships can be found viable without having sexual relationships.[2] There are many factors that can negatively affect sexual functioning and marital satisfaction. Previous studies showed that one of these factors is chronic diseases.[3] Back pain and chronic inflammatory diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis can generate degrees of disability and consequently affect social, economic, and sexual life.[4-6] Sexual motivation and desire in these patients are affected meaningfully[6,7] and as a result, these diseases may deteriorate the relationships between couples and affect life satisfaction and quality in these people.[8,9] The prevalence of different sexual disorders has been reported in studies on people such disabilities.[10-13]

A search in resources shows that reviewing sexual disorders in chronic diseases such as rheumatoid arthritis, SLE, and back pain has rarely been conducted, even in Western societies. A search for studies also shows a similar situation in Iran. According to the importance of life quality in these patients and the fact that sexual functioning is considered an important component of life quality, it is necessary to review this subject. So, the present study was conducted in order to evaluate sexual functioning in patients with prevalent musculoskeletal diseases.

2 | METHODS

This study was conducted in a cross-sectional and analytical form and 102 patients with rheumatoid arthritis, 103 patients with back pain, 103 patients with SLE, and 210 people as a control group were evaluated regarding their marital satisfaction and sexual functioning. Sampling was conducted sequentially from the referred people to the authors' office in Kerman city, the capital of the biggest province of Iran. Before entering the study, all individuals were provided with an explanation about the purpose and process of the study, and their informed consent to participate in the study was obtained separately.

Heterosexual married people between 18 and 60 years of age (who live with their spouses) and with rheumatoid arthritis, SLE, and back pain diseases were included into the study and people having major psychiatry disorders were excluded from the study according to American College of Rheumatology indicators. Demographic information was asked from all patients including age, gender, education, living place, number of children, disease time period, sexual variations (tending to anal or oral sex), and age of first sexual intercourse and then the following questionnaires were completed.

Arizona Sexual Experience scale (ASEX) questionnaire includes 5 questions in which extremely easily or extremely strong answer is awarded score 1, very easily or very strong: score 2, somewhat easily or somewhat strong: score 3, somewhat weak or somewhat difficult: score 4, very weak or very difficult: score 5 and never: score 6. The reliability of the ASEX questionnaire was determined using Cronbach's alpha coefficient of .95 and according to McGahuey study, the questionnaire had good validity.[14] In Iran, the reliability and validity of this questionnaire have been proven by Pezeshki and Bayrami.[15]

The General Health Questionnaire (GHQ) has been presented by Goldberg and Hailer (1979) and has 4 subscales where each scale has 7 questions. Cases 1 to 7 from 28 terms of this questionnaire are related to the physical symptoms scale. Cases 8 to 14 review symptoms of anxiety and sleep disorder and cases 15 to 21 are related to the evaluation of social functioning symptoms and finally, cases 22 to 28 measure depression symptoms. For summing scores, A is awarded score 0, B: 1, C: 2, and D: score 3. The score above 6 on each scale and in total the score above 22 represents disease symptoms. The questionnaire used in this research is standard and in Persian and is designed in accordance with the Iranian culture and is standardized in different populations. In one study, this questionnaire was measured simultaneously with a parallel test (The Middlesex Hospital Questionnaire) and the correlation coefficient of the 2 tests was 55 and correlation coefficients between the subtests of this questionnaire showed a total score between 0.72 and 0.87, which proves its high validity. This study was performed on a student statistical population. Also, this test has high reliability since the calculated alpha value for all its items is .90.[16]

The Marital Satisfaction Questionnaire (satisfaction from marital relationships according to Enrich questionnaire) includes 15 questions with options of I totally agree to I totally disagree, with scores of 1 to 5, respectively, and the higher the score, the higher is the satisfaction sign.[17] The correlation coefficient of Enrich's questionnaire with family satisfaction scales is 0.41 to 0.60 and with life satisfaction scales is 0.32 to 0.41 which is a sign of construct validity. All subscales of this questionnaire distinguish between satisfied and dissatisfied couples and this shows that the test has good criterion validity. In Iran, the validity of this questionnaire by retesting at a 1-week interval was 0.94 and its validity was reported by Cronbach's alpha coefficient of .95.[18]

Disease intensity in rheumatoid arthritis was in terms of Health Assessment Questionnaire (HAQ) and its scores were as follows: asymptomatic HAQ = 0, between 0 and 1 was mild intensity, between 1 and 2: moderate and above 2 was intense.[19]

The Ronald Morris questionnaire was used to assess the severity of the disease in patients with low back pain. This questionnaire included 24 questions about functional and routine work that were scored between 0 and 24 and the least functioning was awarded a score 0 and the most functioning scored 24.[20]

The lupus activity indicators, according to the SLE Disease Activity Index (SLEDAI) questionnaire, was used to assess the severity of the disease in patients with SLE, approved by Canada

Toronto University, in which convulsion, psychosis, cerebral organic syndrome, visual disorders, cerebral nerves disorders, lupus headache, CVA, and vasculitis have scores of 8, arthritis, myositis, urinary casts, proteinuria, and hematuria score 4, and rash, alopecia, mucosal lesions, pleurisy, pericarditis and low levels of complement and increased double-stranded DNA (dsDNA) score 2, and fever, thrombocytopenia, and leukopenia score 1.[21] Score 9 shows intense lupus, score 3 is equal to moderate intensity, score 1 mild intensity, and previous records with score 0 show asymptomatic lupus.

Analysis of variance test was used in order to compare the average among groups. Comparing qualitative variables was done by the Chi-squared test. Pearson correlation test was used to discover the relationship between quantitative variables.

3 | RESULTS

There were 518 people in 4 groups entered into the study and the patients and control group demographic information is shown in Table 1.

The GHQ score in rheumatoid arthritis and SLE patients were meaningfully higher showing lower mental health level in this group ($P < .05$), but there is no meaningful difference in this questionnaire score between back pain patients and the control group ($P = .414$).

ASEX score in each of the 3 patient groups was meaningfully higher than the control group which shows weaker sexual functioning in the patient groups ($P < .05$), but the level of marital satisfaction questionnaire score in the patient groups had no statistically meaningful difference compared to the control group ($P = .791$) (Table 2).

Disease intensity in the rheumatoid arthritis group was meaningfully higher in the studied women than men ($P = .003$), but the level of disease intensity had no statistically meaningful relationship with sexual functioning, mental health, education level, and living place ($P > .05$) (Table 3). Furthermore, a weak reverse correlation was seen between disease intensity and marital relationship in the studied patients ($P = .032$, $r = -.214$), but disease intensity had no meaningful correlation with age, the number of children, age of first sexual intercourse, and disease period ($P > .05$).

Disease intensity in the back pain patient group was meaningfully higher in women, people who had a problem in sexual functioning, lower mental health (lower score of the GHQ), lower education level, and their living place was a village ($P < .05$) But there was no statistically meaningful difference in disease intensity in people who had sexual variations with people who did not ($P > .05$) (Table 3). Furthermore, a moderate direct correlation was seen between disease intensity and age of first sexual intercourse, the number of children, age and disease time period in the studied patients ($P < .05$, $r = -0.42$) and disease intensity had a moderate reverse relationship with marital satisfaction ($P < .05$, $r = -.42$).

No statistically meaningful relationship was seen in the group with SLE between disease intensity with gender, education level, living place, sexual variations, mental health, sexual functioning, age, number of children, age of first sexual intercourse, and marital

TABLE 1 Participant demographics

Demographic information	Control group	People with systemic lupus erythematous	People with rheumatoid arthritis	People with back pain	*P* value
Age (average ± SD)	38.8 ± 8.3	37.4 ± 8.1	40.8 ± 8.6	43.9 ± 8.6	<.001
Gender (frequency percentage)					
Woman	(47.1%) 99	(90.3%) 93	(74.5%) 76	(62.1%) 64	<.001
Man	(52.9%) 111	(9.7%) 10	(25.5%) 26	(37.9%) 39	
Education rate					
Illiterate, reading and/or writing	(2.9%) 6	(4.9%) 5	(14.7%) 15	(2.9%) 3	<.001
Elementary	(11.4%) 24	(14.6%) 15	(20.6%) 21	(19.4%) 20	
High school	(53.8%) 113	(56.3%) 58	(43.1%) 44	(53.4%) 55	
Academic	(31.9%) 67	(24.3%) 25	(21.6%) 22	(24.3%) 25	
Living place					
Big city	(66.2%) 139	(66%) 68	(58.8%) 60	(65%) 67	.567
Small city	(22.4%) 47	(25.2%) 26	(23.5%) 24	(24.3%) 25	
Village	(11.4%) 24	(8.7%) 9	(17.6%) 18	(10.7%) 11	
Sexual variations					
Positive	(20.5%) 43	(10.7%) 11	(6.9%) 7	(13.6%) 14	.007
Negative	(79.5%) 167	(89.3%) 92	(93.1%) 95	(86.4%) 89	
No. of children	1.9 ± 1.2	2 ± 1.1	2.7 ± 1.6	2.6 ± 1.2	<.001
Age at first sexual intercourse	27.7 ± 5.9	23.6 ± 5.4	23.6 ± 6.1	27.5 ± 5.9	<.001
Disease time period	-	7.6 ± 5.1	7.6 ± 6.1	5.4 ± 4	.008

TABLE 2 Questionnaire scores in each of the 3 patient groups

Questionnaire	Control group	People with systemic lupus erythematous	People with rheumatoid arthritis	People with back pain
General Health Questionnaire	3.5 ± 2.8	5.4 ± 3.6[a]	4.6 ± 3.6[b]	3.8 ± 3.1
Arizona Sexual Experience Scale	15.1 ± 3.9	16.6 ± 4.7[c]	16.5 ± 4.7[d]	17.2 ± 3.8[e]
Marital satisfaction	35.9 ± 5.8	35.3 ± 6.6	36 ± 7.9	35.5 ± 4.3

[a]Compared to control group: $P = .000$.
[b]Compared to control group: $P = .003$.
[c]Compared to control group: $P = .004$.
[d]Compared to control group: $P = .008$.
[e]Compared to control group: $P = .000$.

TABLE 3 Disease intensity in the groups with rheumatoid arthritis and back pain in terms of background variables

	Disease intensity in the group with rheumatoid arthritis (average ± SD)	*P* value*	Disease intensity in the group with back pain (average ± SD)	*P* value**
Gender				
Man	0.08 ± 6.2	.022	1.42 ± 5.69	.030
Woman	0.06 ± 0.88		6.42 ± 1.69	
Sex variety				
Yes	0.24 ± 0.62	.35	0.44 ± 5.5	.109
No	0.83 ± 0.05		0.17 ± 6.25	
Sexual functioning				
He/she doesn't have problem	0.06 ± 0.76	.200	0.18 ± 5.64	<.001
He/she has problem	0.10 ± 0.92		7.05 ± 0.24	
Mental health				
He/she has problem	0.10 ± 0.96	.068	0.30 ± 7.52	<.001
He/she doesn't have problem	0.06 ± 0.73		5.81 ± 0.16	
Education				
Illiterate	0.15 ± 0.74	.717	0.57 ± 9	<.001
Elementary	0.13 ± 0.90		5.57 ± 0.27	
High school	0.08 ± 0.84		6.03 ± 0.18	
Academic	0.11 ± 0.72		4.94 ± 0.26	
Living place				
Big city	0.07 ± 0.84	.488	0.20 ± 5.77	<.001
Small city	0.12 ± 0.72		6.60 ± 0.24	
Village	0.16 ± 0.93		7.36 ± 0.41	

*Comparison of patients with rheumatoid arthritis with the control group in terms of background variables.
**Comparison of patients with back pain with the control group in terms of background variables.

satisfaction ($P > .05$). But the length of disease period was related to greater disease intensity ($P = .006$; Table 4).

4 | DISCUSSION

The purpose of this study was to investigate the effect of chronic diseases such as rheumatoid arthritis, back pain, and SLE on sexual functioning and marital satisfaction. In this study, in which most of the participants are women, disease intensity in different groups had different relations with demographic variables, but only gender was related to the group with rheumatoid arthritis. In addition to gender, literacy level and living place was also related to patients with back pain. On the other hand, disease intensity also had a negative effect on sexual functioning and mental health, disease period had a relation with disease intensity in the group with lupus and other variables had no relation to disease intensity. In several studies, sexual problems due to rheumatism diseases have been attributed to

TABLE 4 Disease intensity in the group with systemic lupus erythematous in terms of background variables

Variable	Inactive	Mild flare	Moderate flare	Sever	*P* value
Age	38.65 ± 6.82	35.93 ± 7.48	37.7 ± 8.76	39.25 ± 11.48	.603
No. of children	2.15 ± 0.19	2 ± 0.20	1.97 ± 0.22	2.12 ± 0.44	.951
Age at first sexual intercourse	23.25 ± 6.08	22.96 ± 5.52	24.62 ± 5.00	21.12 ± 5.84	.320
Disease time period	8.83 ± 1.24	6.46 ± 0.87	7.44 ± 0.72	13.5 ± 2.36	.006
Marital satisfaction	30.90 ± 3.00	31.40 ± 3.58	31.82 ± 3.33	32.87 ± 5.13	.554
Gender					
Man	(5) 1	(9.4) 5	(10) 4	(12.5) 1	.904
Woman	(95) 19	(90.6) 30	(90) 36	(90) 7	
Education level					
Illiterate	(0) 0	(9.4) 3	(5) 2	(0) 0	.165
Elementary	(30) 6	(9.4) 3	(10) 4	(25) 2	
High school	(35) 7	(59.4) 19	(67.5) 27	(37.5) 3	
Academic	(35) 7	(21.9) 7	(17.5) 7	(37.5) 3	
Living place					
Big city	(70) 14	(71.9) 23	(60) 24	(87.5) 7	.728
Small city	(25) 5	(21.9) 7	(27.5) 11	(12.5) 1	
Village	(5) 1	(6.2) 2	(12.5) 5	(0) 0	
Sexual variations					
Yes	(0) 0	(15.6) 5	(10) 4	(25) 2	.186
No	(100) 20	(84.4) 27	(90) 36	(75) 6	
Mental health					
He/she has problem	(55) 11	(40.6) 13	(27.5) 11	(50) 4	.186
He/she doesn't have problem	(45) 9	(59.4) 19	(72.5) 29	(50) 4	
Sexual functioning					
He/ she has problem	(25) 5	(37.5) 12	(55) 22	(37.5) 3	.140
He/she doesn't have problem	(75) 15	(62.5) 20	(45) 18	(62.5) 5	

symptoms such as pain, morning stiffness, tiredness, disability, and hip and knee joint problems.[22-24] Furthermore, it has been shown that there is no relationship between marital satisfaction with sexual functioning and sexual relationship satisfaction.[25]

Mental health in terms of the GHQ questionnaire was significantly lower in patients with rheumatoid arthritis and SLE compared to people who did not have these diseases but mental health level in terms of this questionnaire showed no significant difference in the group with back pain compared to the control group. Previous studies in patients with rheumatoid arthritis and SLE have also shown that mental health in these individuals is significantly lower than in healthy individuals, which is consistent with the results of this study.[26,27] Despite the present paper, it has been shown in the study of Demyttenaere et al.,[28] which was conducted on patients with chronic back pain, that the prevalence of mental disorders in people with back pain was more than the people who do not have this disease, so that the lower intensity of this disease in the present studied people may have been the cause of no difference between the 2 groups. Factors that reduce the level of mental health in these patients may be the chronicity of the disease and its recurrence, which lead to frequent visits to the doctor and dependence on medication, which in turn results in reduced social relationships and disruption of daily life. But another reason for the decrease in the level of mental health in people can be the decrease in sexual function, which can have a negative effect on it.[26]

In this study, like the previous studies, the sexual functioning level in each of the 3 groups was significantly lower than the control group. In the Abdel-Nasser et al.[5] study which was conducted on 52 patients with rheumatoid arthritis, also similar results with the present study were obtained and the patients in that study

also had significantly low sexual functioning and 9 patients in that study had incomplete enough ability for sexual functioning that they were incapable in this respect. In a study of 106 patients with SLE, conducted by Dalboudet et al., 49% of patients believed that the disease had a negative effect on their sexual activity, so treatment of the disease could help improve their sexual activity. Furthermore, it has been shown in this study that these patients have lower sexual activity than other chronic disease patients.[9] In other studies conducted in Iran or other countries, similar results have been obtained which show that sexual function in patients with low back pain has decreased and generally has a negative effect on the quality of sexual life in these people.[29,30] It was also shown in the Shahar et al. study,[12] which was conducted on 51 Malaysian patients with rheumatoid arthritis, that almost 1/3 patients with rheumatoid arthritis has a sexual functioning disorder and there was a reverse correlation between the level of active disease and sexual functioning. But considering the present study patients, marital satisfaction showed no significant difference compared to the control group. This variable was evaluated for the first time in patients with SLE. Other studies on patients with rheumatoid arthritis have shown that marital satisfaction is lower in these patients and psychological distress and social support are the most important causes of low-quality sex in these people, which leads to poorer marital satisfaction.[25,31] According to previous studies on patients with low back pain, it has been reported that more socio-psychological disabilities and low back pain are significantly associated with marital satisfaction, but like our study, marital dissatisfaction was not associated with pain and disability in men, but this relationship has been significant in women.[10,13]

Gender and sex are a part of life. Apart from reproduction, sex can be about intimacy and pleasure and can improve a person's physical and mental well-being, while inflammatory diseases can reduce their quality of life by negatively affecting their mental health and sexual function.[32] It seems that various factors such as community culture, age, level of education, and time spent in marriage can play a role in this.[33,34] Apart from the causes of this issue, which require further research, the most important part is related to the consequences of these diseases on sexual function, mental health, and marital satisfaction. Sex is always one of the effective factors in creating deeper emotional bonds between couples and maintaining intimacy in the family.[35] Now, if a couple cannot create the necessary intimacy in the family and enjoy sufficient marital satisfaction due to these inflammatory diseases, the foundation of the family may become fragile and their relationship may end in aggression, loss of love, and eventually collapse.

Finally, as shown in this study the level of sexual functioning in patients with rheumatoid arthritis, SLE, and back pain, and also the level of mental health in all groups of patients except patients with low back pain are significantly lower than healthy people in this respect. But marital satisfaction is significantly different in none of the groups. Therefore, using the necessary and appropriate interventions, the level of mental health and sexual function in these patients should be improved to prevent the occurrence of further harmful problems that may affect the family structure.

It is suggested that another study be conducted with greater sample size and considering other causes involved in the level of marital satisfaction.

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ORIGINAL ARTICLE

Assessment of the anti-rheumatoid arthritis activity of *Gastrodia elata* (tian-ma) and *Radix aconitic lateralis preparata* (fu-zi) via network pharmacology and untargeted metabolomics analyses

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Abstract

Aim: *Gastrodia elata* and *Radix aconiti lateralis preparata* are respectively named as Tian-Ma and Fu-Zi (TF) in Chinese. We explored the active components against rheumatoid arthritis (RA) from an extensively used couplet of Chinese herbs, *Gastrodia elata* and *Radix aconiti lateralis preparata* (TF) via untargeted metabolomics and network pharmacological approaches.

Methods: Water extracts of TF were mixed at ratios 1:1, 3:2 and 2:3 (w/w). Ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) was then utilized as metabolomics screening. Human Metabolome (http://www.hmdb.ca/) and Lipidmaps (http://www.lipidmaps.org/) databases were used to annotate detected compounds. Further identification of vital genes and important pathways associated with the anti-RA properties of the TF preparations was done via network pharmacology, and verified by real-time quantitative polymerase chain reaction (RT-qPCR).

Results: Four key compounds involved in unsaturated fatty acid biosynthesis and isoflavonoid biosynthesis were identified through metabolomics analyses. Three key components of TF associated with anti-RA activity were linoleic acid, daidzein, and daidzin. Results of RT-qPCR revealed that all 3 tested TF couplets (1:1, 3:2, and 2:3) markedly suppressed the transcription of PTGS2. These results were consistent with our network pharmacological predictions.

Conclusions: The anti-RA properties of Tian-Ma and Fu-Zi are associated with the inhibition of arachidonic acid metabolism pathway.

KEYWORDS

couplet medicines, *Gastrodia elata and Radix aconitic lateralis preparata* (Tian-Ma & Fu-Zi, TF), metabolomics, network pharmacology, rheumatoid arthritis

Jie Yang and Yu Zhang have contributed equally.

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1 | INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease which influences about 1% of the global population[1] and is associated with bone degradation, destruction of the cartilage, and synovial inflammation and hyperplasia.[2] Conventionally, RA treatment has relied on the administration of disease-modifying anti-rheumatic drugs, non-steroidal anti-inflammatory drugs, inhibitors of tumor necrosis factor (TNF)-α and interleukin (IL)-6 signaling and glucocorticoids.[3] However, clinical utilities of these interventions is seriously limited by high rates of adverse events. Traditional Chinese medicine (TCM) approaches offer an alternative to these RA treatments by simultaneously suppressing multiple targets of pathological pathways in a coordinated and efficacious manner.[4]

The couplet medicinals, or Chinese medicine pair, is one of the unique features of TCM wherein the pairs of medicinal herbs are combined with a synergistic manner.[5,6] One couplet medicinal, *Gastrodia elata* and *Radix aconiti lateralis preparrata* (Tian-Ma and Fu-Zi, TF) has traditionally been used against RA.[7] There are 1159 prescriptions retrieved from ancient TCM that include TF displaying anti-RA activities.[8] TF as a useful couplet medicinal has been used as RA therapy since the Ming Dynasty (about 500 years ago).[9] Prior studies of Tian-Ma pills conducted by Chen et al. have demonstrated a promising treatment-associated cure rate of 85.30%.[10] Our former works displayed that TF could alleviate joint swelling and pain in RA rats.[11] However, the bioactive components of TF against RA are poorly understood.

Network pharmacology is an advanced approach that has been successfully employed to identify major targets and pathways associated with the therapeutic activity of TCM preparations.[12,13] Using such an approach, investigators have annotated active ingredients in a couplet medicinal, *Gastrodia elata* and *Ligusticum chuanxiong* hort, and annotated 48 molecular targets involved in headache treatment.[14,15] Another couplet medicinal, *Gastrodia elata* and *Ramulus uncariae cum uncis*, demonstrated multiple targets and pathways in the treatment of cerebral ischemia.[16] Therefore, we employed a combined platform, the untargeted metabolomics and network pharmacology to probe the bioactive compounds that attenuated the severity, or cured RA disease. The experimental flow is shown in Figure 1.

2 | MATERIALS AND METHODS

2.1 | Reagents

Bovine type II collagen and complete Freund's adjuvant were purchased from the Chondrex Reagents. BeyoFast™ SYBR Green quantitative polymerase chain reaction (qPCR) Mix (2X) was purchased from Biyuntian Biotechnology. PrimeScript™ RT reagent kit was purchased from TaKaRa Co., Ltd.

2.2 | Animals

The animal studies were approved by the Laboratory Animal Ethics Committee of Chengdu University of TCM. Forty male

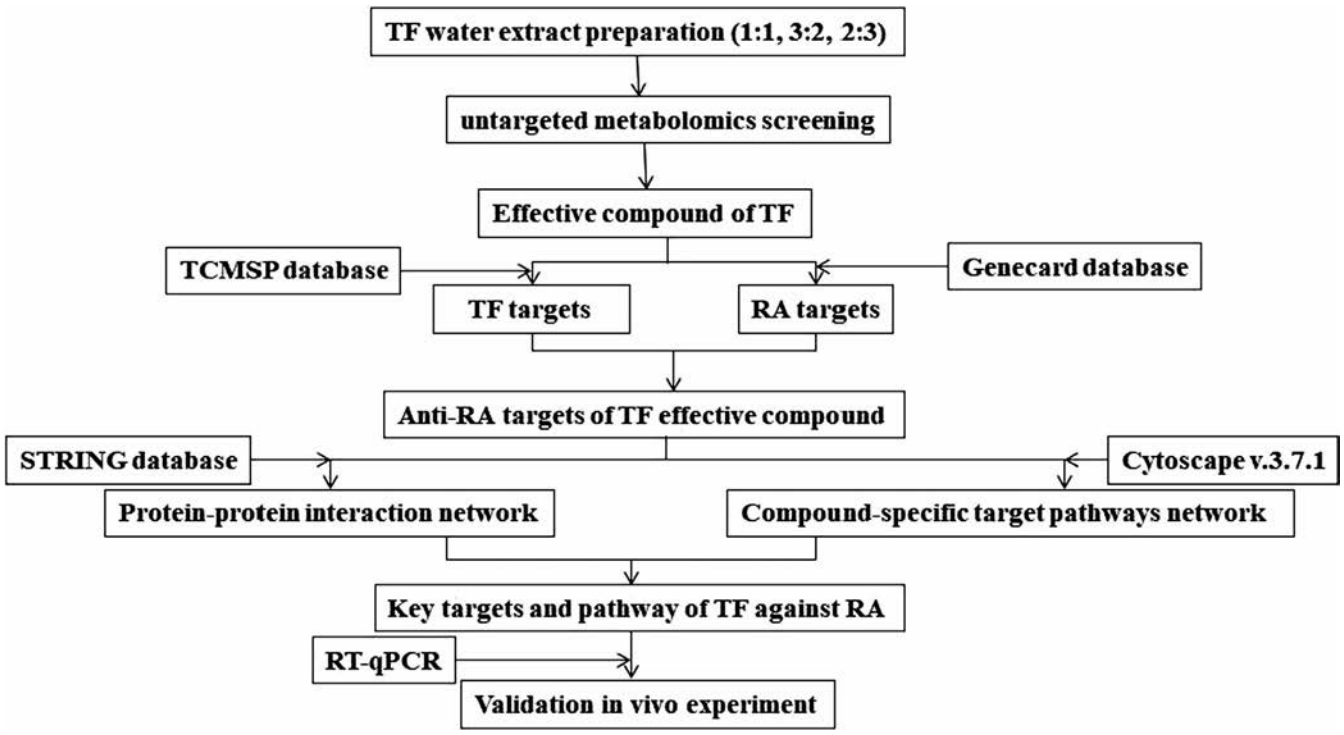

FIGURE 1 Experimental flow. TF, Tian-Ma and Fu-Zi. RA, rheumatoid arthritis. TCMSP, traditional Chinese medicine system pharmacology database and analysis platform

Sprague-Dawley rats (180 to 200 g) were purchased from Chengdu Dashuo Animal Co., Ltd., and were individually housed in a climate-controlled facility (25°C, 40% relative humidity; 12 h dark/light cycle).

2.3 | Preparation of TF solution

The Tian-Ma sample was obtained from Dafang county (N105°36' and E27°09'), Guizhou province, China. Fu-Zi was obtained at Jiangyou county (N104°76' and E31°78'), Sichuan province, China. Both genuine medicinal herbs were identified by Professor Yun Deng, Chengdu University of TCM. Tian-Ma and Fu-Zi were combined at ratios 1:1, 3:2 and 2:3 (w/w) to which were added 10-fold of distilled water and placed in an incubator for 30 minutes at room temperature. The Fu-Zi was decocted for 1 hour, followed by the decoction of TF for 1 hour under a mild flame, after which the solution was filtered. Eight-fold of distilled water was added and decocted for 1 hour prior to second filtration. The first and second filtrates were then combined and condensed to yield a solution to a relevant density of 1:1.1. The freeze-dried TF powder was prepared and stored at 4°C.

2.4 | Ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS)-based untargeted metabolomics analysis

Three TF couplets were prepared based upon the ratios of Tian-Ma and Fu-Zi powders as follows: 1:1 (TF11), 2:3 (TF23), or 3:2 (TF32). Lyophilized samples (100 g) of TF couplets were ground by liquid nitrogen, and resuspended in pre-cold solution (80% methanol, 0.1% formic acid) via vortex. The resuspended solutions were incubated on ice for 5 minutes, spun at 15 000 *g* and 4°C for 5 minutes. The final diluted sample consisted of 60% methanol and 40% H_2O, filtered by a 0.22 μm filter, transferred into a clean tube, spun at 15 000 *g* and 4°C for 10 minutes, and stored in −80°C for LC-MS analysis.

LC-MS analysis was conducted with an UHPLC system (Thermo Fisher) and an Orbitrap Q Exactive series mass spectrometer (Thermo Fisher). The samples were injected into Hyperil gold column (100 × 2.1 mm; 1.9 μm) with a flow rate of 0.2 mL/min, eluted with 0.1% formic acid (A) and methanol (B) in positive ion mode, and 5 mnol/L ammonium acetate (pH 9.0; A) and methanol (B) in negative ion mode. The elution process was set as follows: 98% A, 2% B for 1.5 minutes; 100% B for 12.0 minutes; 100% B for 14.0 minutes; 98% A, 2% B for 14.1 minutes; 98% A, 2% B for 16 minutes. The MS instrument was executed in both the negative and positive ion modes, with the electrospray ionization settings as follows: spray voltage = 3.2 kV, capillary temperature = 320°C, sheath gas flow rate = 35 arb, and aux gas flow rate = 10 arb.

Compound Discoverer 3.0 (CD 3.0 by the Thermo Fisher) software was utilized to analyze the UPLC-MS/MS data. The peak alignment, selection, and quantitation for individual compounds were executed as follows: retention time tolerance = 0.2 minutes; actual mass tolerance = 5 ppm; signal intensity tolerance = 30%; signal/noise ratio = 3; and minimum intensity = 100 000. Normalization of peaks was done to total spectral intensity, after which data were used to predict the molecular formula based on the additive ions, the molecular ion peaks and the fragment ions. Peaks were matched using the mzCloud (https://www.mzcloud.org/) and ChemSpider database (http://www.chemspider.com/) to yield qualitative as well as relative quantitative results.

The Human Metabolome Database (HMDB) (http://www.hmdb.ca/) and the Lipidmaps database (http://www.Lipidmaps.org/) were applied for compound annotation. Techniques like partial least squares discriminant analysis (PLS-DA) and principal component analysis (PCA) were executed with the metaX metabolomics software. Compounds exhibiting variable importance in the projection values >1 and *P* values <.05, with a fold change value ≥2 or ≤0.5, were considered to be differentially abundant.

For heat map clustering, z-score based data normalization was applied, corresponding to the intensity areas of differentially abundant compounds, and plotted using the R "P heatmap package". The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was employed to explore functional roles of these compounds.[17] The KEGG pathway enrichment analyses were conducted when the x/n > y/N ratio condition was met, with pathways being considered to be significantly enriched when $P < .05$.

2.5 | Network pharmacological analysis

A TCM system pharmacology database and analysis platform (TCMSP) was used to identify target compounds associated with significantly enriched KEGG pathways. Identification of TF targets and RA-related targets was processed using a human gene database (https://www.genecards.org). In order to determine critical genes associated with the anti-RA activity of TF, the STRING database was employed to construct a protein-protein interaction network. Cytoscape software v.3.7.1 (https://cytoscape.org/) was used to construct a compound-specific target from target metabolic pathway networks. Finally, the DAVID database (https://david.abcc.ncifcrf.gov) was employed for functional enrichment analyses of mutual targets.

2.6 | Assessment of the in vivo anti-RA activities of TF formulas

2.6.1 | Experimental design

After adaptive feed for 7 days, 8 randomly selected rats as healthy controls and other animals were used for the construction of a collagen-induced arthritis model.[18] Rats with arthritis index more than 4 scores were identified as a successful RA model, which were randomly divided into 4 groups: RA group, TF11 group, TF32 and TF23 group, with 8 rats in each group. Three weeks after the model was made, 3 TF couplets were respectively administered via oral gavage, one time a day and lasting for 21 days. Rats in healthy normal

and model control animals were instead gavaged with normal saline (10 mL/kg). Six weeks after model was made, all animals were fasted overnight, anesthetized using 10% chloral hydrate (350 mg/kg), and 3 mL cardiac blood samples were collected for downstream analysis.

2.6.2 | Real-time (RT)-qPCR tests for PTGS1 and PTGS2 messenger RNA (mRNA) expression

About 2 mL of blood sample from each rat was combined with 3 times (v/v) of erythrocyte lysis buffer in an ethylenediaminetetraacetic acid containing tube, after which samples were spun down for 10 minutes at 3000 *g*. Supernatants were then discarded, and isolated cell washing was done twice using 2 mL of phosphate-buffered saline prior to storage at −80°C. A Multisource Total RNA Miniprep Kit (AXYGEN, AxyPrepTM, 07418KD1) was utilized to isolate total RNA from each sample, with 1 μg of RNA per sample used to generate complementary DNA (cDNA) with a PrimeScript™ RT Reagent Kit (Takara). Reaction conditions were as follows: 95°C for 30 seconds; 40 cycles of 5 seconds at 95°C, 35 seconds at 60°C and 30 seconds at 72°C. All reactions were done in a 20 μL mixture containing SYBR Green qPCR Mix (10 μL), primers (2 μL; forward + reverse), cDNA (2 μL), dH_2O (6 μL). β-actin was utilized as a normalization control, and relative gene expression was assessed via the $2^{-\triangle\triangle Ct}$ approach. Primers used herein are compiled in Table 1.

TABLE 1 Primer information

Primer name	5'→3' sequence	TM
Rats β-actin-F	CACCCGCGAGTACAACCTTC	60
Rats β-actin-R	CCCATACCCACCATCACACC	60
Rats COX-1-F	GTCTGCCTCAACACCAAGAC	60
Rats COX-1-R	ATGGCTGGCCTAGAACTCAC	60
Rats COX-2-F	GCTTCTCCCTGAAACCTTACACAT	60
Rats COX-2-R	GCTTTCAACTCTGCAGCCATT	60

2.7 | Statistical analysis

R (v3.4.3), Python (v2.7.6), and CentOS (release 6.6) were used for all statistical analyses. In case of lack of normal distribution of data, regular transformations were conducted via an area normalization approach. Univariate analyses were employed to assess the significance of statistics.

3 | RESULTS

3.1 | Compound classification of the TF extracts

936 and 1476 compounds within TF were identified in the positive and negative ion modes, respectively, of which 200 negative and 314 positive compounds ($n = 200^-$, $n = 314^+$) were categorized into 11 classes: lipids and lipid-like molecules ($n = 63^-$, $n = 55^+$), benzenoids ($n = 20^-$, $n = 46^+$), organic acids and derivatives ($n = 31^-$, $n = 74^+$), and phenylpropanoids and polyketides ($n = 31^-$, $n = 29^+$) were the majorly detected compounds. According to the Lipidmaps database, 56 positive and 68 negative compounds within these TF extracts were classified into 19 clusters. Isoprenoids ($n = 13^-$, $n = 7^+$), fatty acids and their conjugates ($n = 12^-$, $n = 10^+$) and flavonoids ($n = 11^-$, $n = 11^+$) were the most prevalent compounds identified through this analysis. Overall, these 2 databases provided significant insight into the composition of TF extracts.

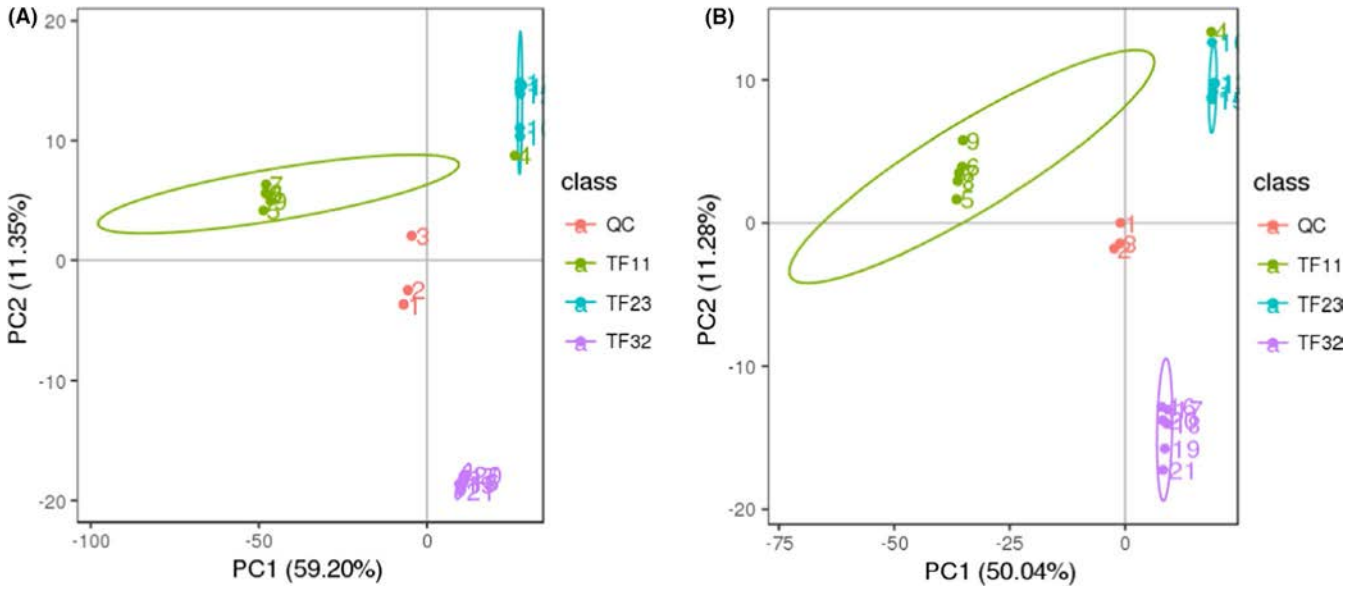

FIGURE 2 Principal component analysis score plots comparing 3 TF couplets. Three TF couplets were composed of Tian-Ma (T) and Fu-Zi (F) powders at 1:1 (TF11), 3:2 (TF32), or 2:3 (TF23) (w/w) ratios. The X-axis corresponds to principal component (PC) 1 scores, while the Y-axis corresponds to PC2 scores. A, +ive ion mode. B,-ive ion mode

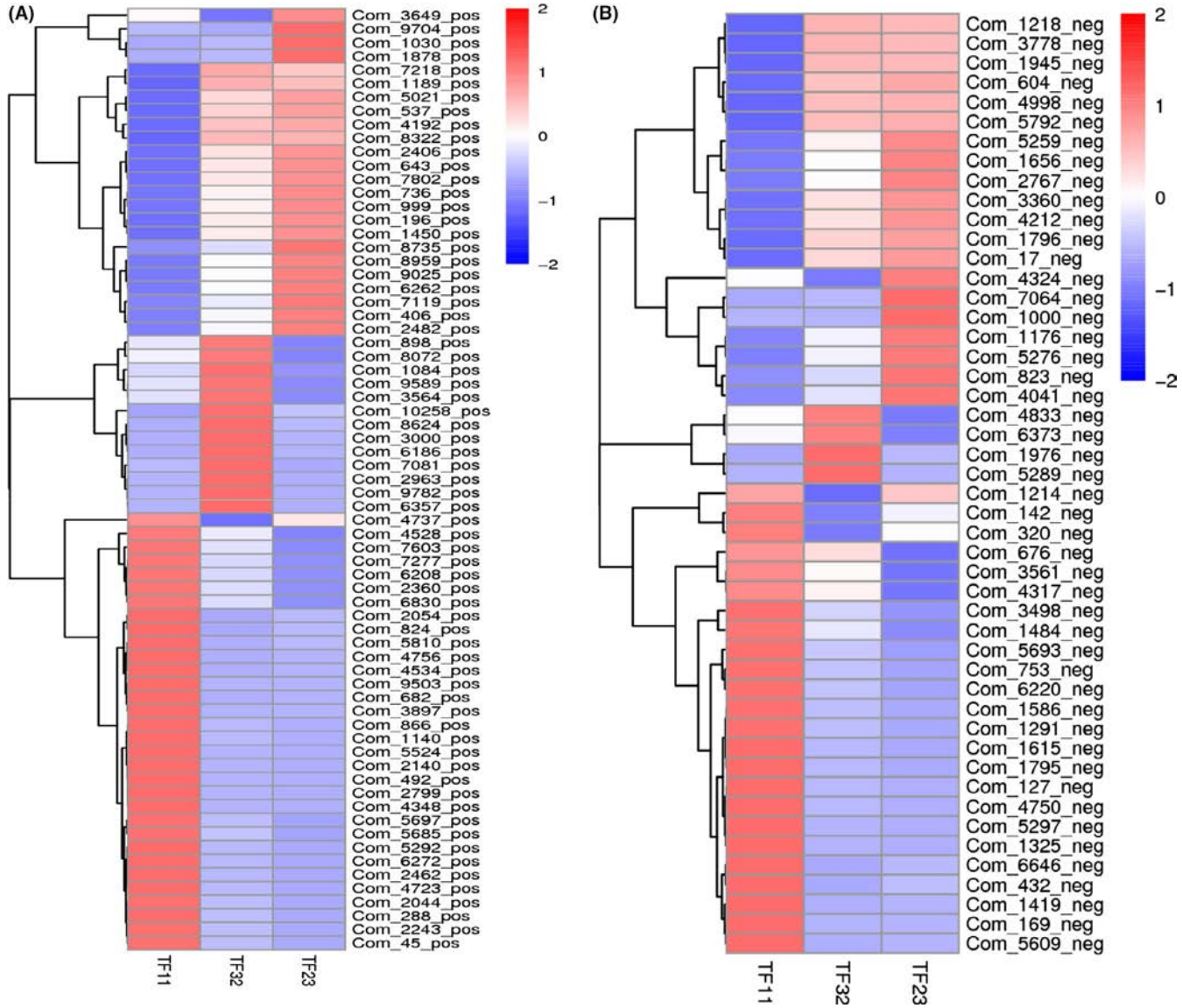

FIGURE 3 Differentially isolated compounds within TF preparations as annotated by HMDB. Three TF preparations composed of relative Tian-Ma and Fu-Zi powder weights of 1:1 (TF11), 3:2 (TF32), or 2:3 (TF23) are analyzed. Upregulated and downregulated compounds are shown in red and blue, respectively, whereas white is used to represent compounds that are not significantly differentially abundant. A, +ive ion mode. B, -ive ion mode. HMDB, the Human Metabolome Database. Differentially isolated compound descriptions are shown in the Data S1

3.2 | Analysis of the composition of TF extracts

Results of PCA analysis based on 3 TF couplets displayed differential compound profiles (Figure 2), which were consistent with our former hypothesis that distinct ratios of Tian-Ma and Fu-Zi in special TF couplets might demonstrate discrepant compound profiles.

There were 39^{-} and 48^{+} compounds identified as differentially abundant ingredients between TF11 and TF23 samples. Compared between TF11 and TF32, 31^{-} and 45^{+} compounds were selected as differentially abundant ingredients. As for TF23 and TF32, relatively few compounds, that is 13^{-} and 24^{+}, were identified in discrepant abundance according to HMDB database annotation (Figure 3). Using the Lipidmaps database, we identified 15^{-} and 13^{+} (TF11 vs TF23), 11^{-} and 12^{+} (TF11 vs TF32), and 9 and 6^{+} (TF23 vs TF32) compounds detected as differently abundant ingredients (Figure 4). We subsequently merged these differentially abundant compounds, and conducted a KEGG pathway enrichment analysis, four compounds were found particularly enriched in isoflavonoid biosynthesis and unsaturated fatty acid synthesis ($P < .05$) (Table 2).

3.3 | Network pharmacological results of key TF compounds

Fifteen targets retrieved from TCMCP database were associated with linoleic acid, one of the key compounds derived from TF couplets,

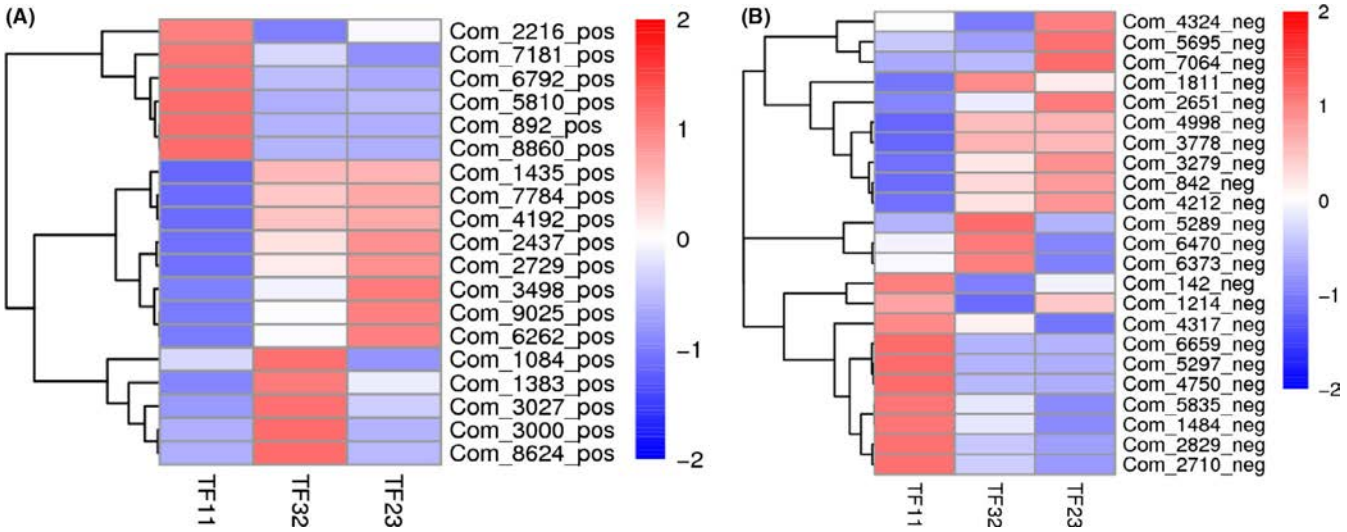

FIGURE 4 Heatmaps of differential compounds as annotated by Lipidmaps. Three TF preparations composed of relative Tian-Ma and Fu-Zi powder weights of 1:1 (TF11), 3:2 (TF32), or 2:3 (TF23) are analyzed. Upregulated and downregulated compounds are shown in red and blue, respectively, whereas white is used to represent compounds that are not significantly differentially abundant. A, +ive ion mode. B, -ive ion mode. Differentially isolated compound descriptions are shown in the Data S2

TABLE 2 Differential compounds involved in Kyoto Encyclopedia of Genes and Genomes pathways ($P < .05$)

Name	Formula	Molecular weight	The number of corresponding target	Fold change		
				TF11 vs TF23	TF23 vs TF32	TF11 vs TF32
Behenic acid	$C_{22}H_{44}O_2$	340.33	-	0.28	3.13	-
Linoleic acid	$C_{18}H_{32}O_2$	280.24	15	-	-	2.88
Daidzein	$C_{15}H_{10}O_4$	254.06	70	0.37	-	-
Daidzin	$C_{21}H_{20}O_9$	416.11	14	-	0.47	0.44

while daidzein and daidzin were associated with 70 and 14 targets, respectively. There were 3797 RA-related targets identified from the human gene database (https://www.genecards.org). An interaction network among overlapping targets of these key compounds and RA was constructed by STRING database (Figure 5), including 6 highly connected targets that were extremely connected to over 30 other targets (Figure 6). These core targets were therefore believed to be essential RA targets that were linked to the anti-RA properties of TF. These targets include caspase-3 (CASP3), cellular tumor antigen p53 (TP53), IL-6, TNF, prostaglandin G and H synthase 2 (PTGS2) and vascular endothelial growth factor A (VEGFA).

Consistent with the multi-targets and multi-pathways characteristics of TF against RA, compounds-targets and targets-pathways network were plotted by Cytoscape software v.3.7.1. This network composed 57 nodes, including 1 couplet medicine, 1 disease, 3 pharmaceutical ingredients and 52 targets. It has 120 edges, of which daidzein, daidzin and linoleic acid were associated with 26, 6, and 2 targets, respectively (Figure 7). This network highlighted the relationships between TF compounds and RA targets. Three identified TF components targeted PTGS2 which has a crucial role in inflammatory modulation.

30 targets and 20 pathways were found by targets-pathways network analysis. An average of 6.67 pathways were associated with each target, and 10.00 targets with each pathway (Figure 8). The Kaposi sarcoma-associated herpes virus infection pathway (hsa05167) displayed the maximum crosstalk with anti-RA targets. The Epstein-Barr virus infection (hsa05169), hepatitis B infection (hsa05161), and human cytomegalovirus infection (hsa05163) pathways also exhibited significant overlap with the anti-RA targets. Given that RA is categorized as a conventional autoimmune inflammatory disorder, metabolic pathways like the IL-17 signaling pathway (hsa04657) and TNF signaling pathway (hsa04668) are critically linked to the anti-RA processes. Enriched pathways including the TNF signaling, Jak/STAT signaling pathways, human cytomegalovirus infection, and arachidonic acid metabolism are all associated with excessive proinflammatory cytokine production. Therefore TF might act as inflammatory modulation and potential antivirus factors in vivo.

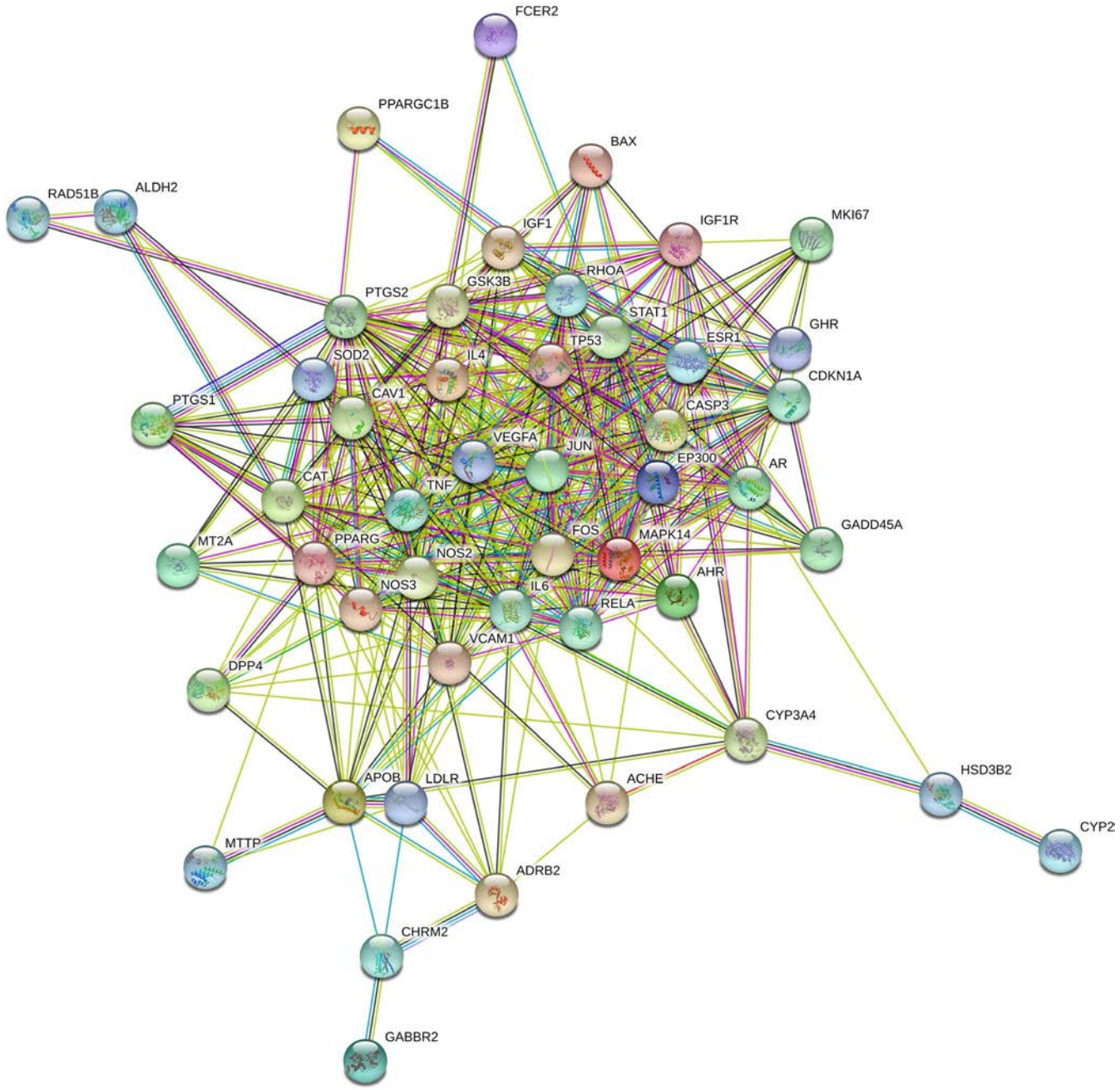

FIGURE 5 The interaction network of overlapping targets associated with Tian-Ma and Fu-Zi powder (TF) against rheumatoid arthritis (RA)

3.4 | Validation of the PTGS2 mRNA expression levels in RA rats

The RA model and therapeutic effect of TF were assessed by arthritis index. Results indicated that all TF couplets could markedly relieve the arthritis symptoms of [11] RA rats. Untargeted metabolomics and network pharmacological analysis displayed 3 key ingredients (linoleic acid, daidzein, and daidzin) had a significant impact on the expression of genes associated with the arachidonic acid metabolism pathway, including PTGS2. Results of RT-qPCR confirmed that TF water solutions could inhibit the transcription of PTGS2 (Table 3), and indicated the anti-RA activity of TF associated with the inhibition of the arachidonic acid metabolism pathway.

4 | DISCUSSION

Herein, we identified key bioactive compounds that showed different concentrations of 3 TF couplets. These compounds were significantly associated with isoflavonoid and unsaturated fatty acid biosynthesis via a combined approach of UPLC-MS/MS-mediated untargeted metabolomics[19,20] and network pharmacology. Both

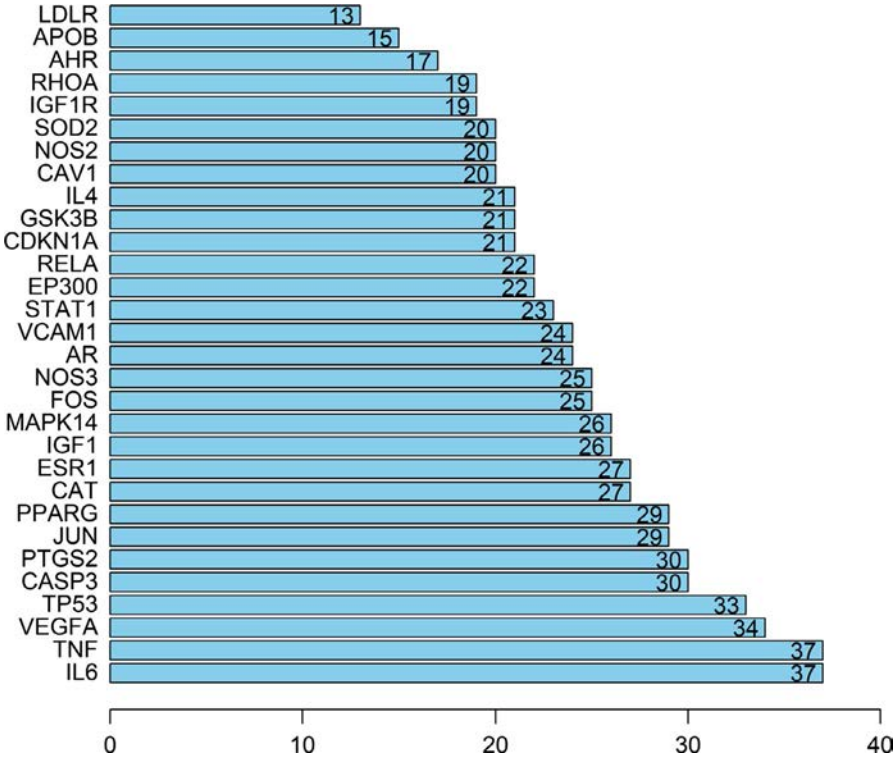

FIGURE 6 Histogram of genes or proteins associated with the anti-rheumatoid arthritis (RA) activity of Tian-Ma and Fu-Zi powder (TF). Key genes associated with the TF anti-RA activities, included *TNF-α*, *IL-6*, *VEGFA*, *CASP3*, *TP53*, and *PTGS2* (cyclo-oxygenase-2). The X-axis is the number of conjunctive nodes between genes or proteins, while the Y-axis includes gene symbols

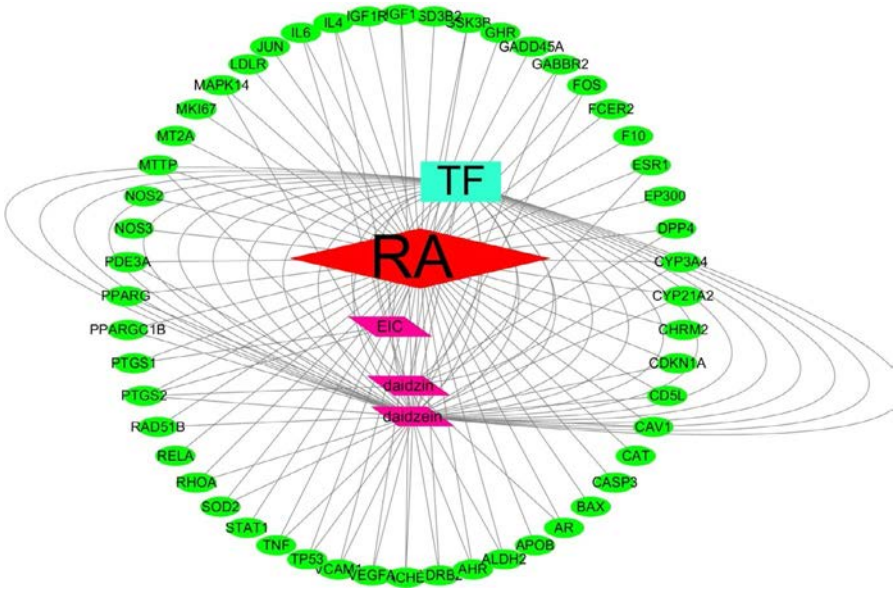

FIGURE 7 An interactive network diagram of active Tian-Ma and Fu-Zi powder (TF) components and potential rheumatoid arthritis (RA)-related disease targets. Targets of active TF components relevant in the context of RA are shown in green, while differential compounds in the 3 tested TF couplets are shown in purple. The TF couplet medicine and RA are indicated in blue and red, respectively. EIC, linoleic acid

isoflavonoids and unsaturated fatty acids have accumulated significant and multiple anti-RA activities. For instance, linoleic acid is categorized a major n-6 polyunsaturated fatty acid (PUFA), and its elongation or desaturation can give rise to gamma-linolenic acid along with arachidonic acid and as an antecedent of prostaglandin E_2.[21,22] The intake of linoleic acid can thereby induce anti-RA activity.[23-25] Daidzein and daidzin have been confirmed to suppress RA symptoms and decrease RA incidence.[26-29] Hence, these compounds might play an important role in RA treatment.

Recently, several clinical studies displayed the anti-RA properties of TF.[30,31] However, the pharmacological targets of TF remain to be clarified. Network pharmacology is an integrated approach employed in TCM to understand the interactions between herbs, ingredients, diseases, and their targets.[32] Our work and relevant publications[33-35] identified PTGS2 as a target of key bioactive ingredients of TF couplet. PTGS2 is also a regulator of arachidonic acid metabolism involved in the proinflammatory pathway. Therefore PTGS2 could be considered as a potential anti-RA target derived from TF couplet.

RA is a common autoimmune disease which might be induced by virus infection, such as Kaposi sarcoma-associated herpesvirus,[36,37] Epstein-Barr virus [38,39] and human cytomegalovirus.[40] Our results of targets-pathways analysis (Figure 8) indicated that the

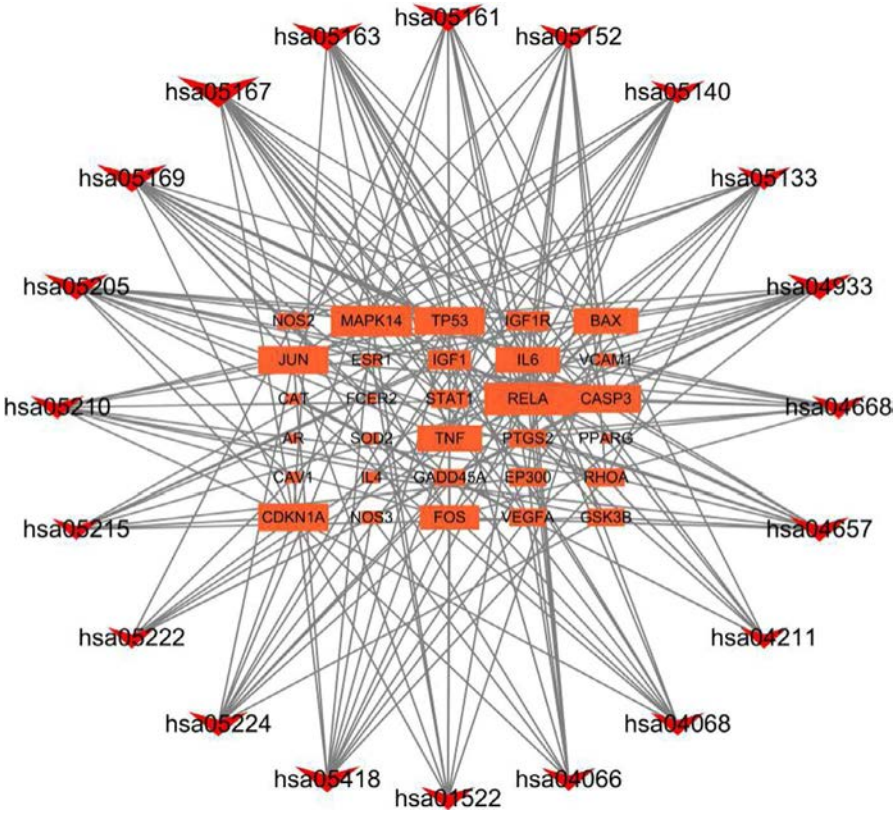

FIGURE 8 A target-pathway interactive network. Pathway IDs and genes are shown in red and brown, respectively

TABLE 3 Rats' whole blood PTGS1 and PTGS2 messenger RNA (mRNA) expression validated by real-time quantitative polymerase chain reaction ($\bar{x} \pm s$, n = 8)

Group	Dose (g/kg)	PTGS1 mRNA relative expression level	PTGS2 mRNA relative expression level
Normal	-	1.5 ± 0.6	0.9 ± 0.4
Model	-	0.4 ± 0.2*	5.2 ± 3.4*
TF11 group	2.7	0.3 ± 0.0	0.6 ± 0.2**
TF32 group	3.4	0.3 ± 0.0	0.4 ± 0.2**
TF23 group	3.4	0.4 ± 0.2	0.4 ± 0.1***

Note: Compared with normal, *$P < .05$.
Compared with model, **$P < .05$, ***$P < .01$.

identified compounds from TF couplet were involved in control of inflammation,[41] apoptosis, vascular endothelial function[42] and other RA-related processes.[43-45] Signaling pathways such as PI3K/AKT/mammalian target of rapamycin/nuclear factor-κB is involved in the modulation of autophagy, chondrocytes proliferation and apoptosis of the synovial fibroblasts, all of which are closely linked to RA.[46-50] Pathways that reduce reactive oxygen production are also valuable for inflammatory regulation in the context of RA.[51] Overall, these enriched pathways highlight a characteristic regulatory network associated with TF that display integrated activities against RA.

5 | CONCLUSION

The present work identified linoleic acid, daidzein and daidzin as key compounds of TF couplet against RA. In future studies, the pharmacological properties of these ingredients should be evaluated, and a metagenomics platform should be used to assess the metabolic processes associated with TF treatment.

CONFLICT OF INTERESTS

None.

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SUPPORTING INFORMATION

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

Supplementary Materials

Data S1: Description of negative and positive compounds of TF couplet retrieved by HMDB.

Data S2: Description of negative and positive compounds of TF couplet retrieved by lipidmaps database.

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ORIGINAL ARTICLE

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Clinical profile, treatment and outcome of Kawasaki disease: A single-center experience from a tertiary care referral center of Assam, north-east India

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Abstract

Aim: This is a retrospective study to report our experience with a cohort of 73 patients with Kawasaki disease (KD) over 2.5 years.

Method: The study was conducted in the Department of Pediatrics. Data were retrieved from medical records of Pediatric Rheumatology and Immunodeficiency Clinic collected from April 2017 to October 2019 and analyzed.

Results: Male-to-female ratio in our cohort was 2:1. The median age at diagnosis of KD was 3 years (IQR, 4.25). Fever was present in all patients. Oral mucosal changes are the second most common symptom (N = 64, 87%) followed by extremity changes (N = 58, 79%), and rash (N = 56, 76%). Nineteen (26%) children had cardiovascular complications like coronary artery abnormalities (N = 15, 20%), cardiac tamponade (N = 2, 2%), and shock (N = 1, 1%). The effusion in the patients with cardiac tamponade contained inflammatory cells and plenty of red blood cells. Sixty-eight (93%) patients with KD had received treatment with IVIg. Patients in our cohort had completed a mean follow-up of 13.6 ± 9.4 months. No fatality or any long term adverse effects were observed on follow-up.

Conclusion: Kawasaki disease is a common rheumatological disorder in children at our center with diverse clinical presentations. The disease needs to be considered as a differential diagnosis in an acute febrile illness in children persisting up to 5 days.

KEYWORDS

coronary artery aneurysm, Kawasaki disease, North-East India

1 | INTRODUCTION

Kawasaki disease (KD) is an acute vasculitis of childhood with a special predilection to involve coronary arteries. If untreated, it can lead to coronary artery aneurysms in 25% of cases.[1] In countries like Japan, the United States, and certain European countries, KD is now considered as the most common acquired heart disease in children.[2] The disease is most common in children under 5 years of age; however, it can also occur in older children, adolescents, and young adults.[1,2] The incidence of KD is highest in Japan (322 per 100 000 children under 5 per year)[3] followed by South Korea (194.7 per 100 000 children under 5 per year)[4] and Taiwan (82.8 per 100 000 children under 5 per year).[5] KD has been reported from almost all parts of India over the last 2 decades; however, nation-wide epidemiological data

are lacking. An epidemiological study carried out in Chandigarh reported an incidence of 5.35 per 100 000 children under 5 per year during 2009-2014.[6] No studies have been published on KD in Assam or other north-eastern Indian states. In this retrospective study, we report our experience in a cohort of 73 patients with KD registered at the Pediatric Rheumatology and Immunodeficiency Clinic (PRIC) of our institute over a period of 2.5 years.

2 | PATIENTS AND METHODS

This study was conducted at the Department of Pediatrics, Gauhati Medical College and Hospital (GMCH), Guwahati, Assam. The PRIC of GMCH was established in April 2017. It is the only center in north-east India that is equipped with facilities for the management of children with KD. We reviewed the data of all 73 cases of KD in the medical records of PRIC from April 2017 to October 2019. Cases were categorized as either complete KD or incomplete KD. Complete KD was defined according to criteria outlined in the 2017 American Heart Association guidelines (AHA 2017, Table 1), whereas incomplete KD was defined as fever of ≥5 days with 2 or 3 compatible clinical criteria or infants with fever for ≥7 days without other explanation. We defined intravenous immunoglobulin (IVIg) resistant KD as the persistence or recrudescence of fever after at least 36 hours and less than 7 days after completion of IVIg infusion.[1]

Demographic characteristics, clinical and laboratory parameters of each patient in our cohort were recorded in a scoring sheet (Supplementary material) before analyzing the data. During the period of study, our institution had a practice of following up all patients with KD at PRIC along with 2D echocardiography performed at the Department of Cardiology after 2 weeks and 6 weeks of initial treatment. Subsequent follow-up visits were advised after 3 months, 6 months, 1 year, and then once in every 1-2 years in patients with normal coronary artery diameters or in patients whose coronary diameters were regressed to normal size. The patients on prophylactic warfarin (patients with giant coronary aneurysms) were followed up at more frequent intervals. The serial *Z* scores of the internal diameters of coronary arteries (calculated by using new equations developed by Dallaire and Dahdah 2010[7]) were recorded in the score sheet. The study was approved by the ethics committee of our institute.

TABLE 1 Diagnostic criteria of KD[1]

A complete case of KD is diagnosed in a child with fever for at least 5 d (the day of fever onset is taken as d 1 of illness) in presence of at least 4 out of 5 following principal features. In the presence of ≥ 4 principal clinical features, particularly when redness and swelling of the hands and feet are present, the diagnosis of KD can be made with 4 d of fever. Experienced clinicians who have treated many patients with KD may establish the diagnosis with 3 d of fever in rare cases.
1. Erythema and cracking of lips, strawberry tongue, and/or erythema of oral and pharyngeal mucosa
2. Bilateral bulbar conjunctival injection without exudate
3. Rash: maculopapular, diffuse erythroderma, or erythema multiforme-like
4. Erythema and edema of the hands and feet, in acute phase and/or periungual desquamation in subacute phase
5. Cervical lymphadenopathy (≥1.5 cm), usually unilateral

Abbreviation: KD, Kawasaki disease.

2.1 | Statistical analysis

Categorical variables were analyzed as frequencies (percentages) and continuous variables were analyzed using median with interquartile range (IQR) and mean with standard deviation (mean ± SD).

3 | RESULTS

During the study period there were 73 cases of KD recorded at PRIC. Of these, 53/73 (73%) patients were complete KD, and 20/73 (27%) were incomplete KD. The median age at the time of the diagnosis of KD was 3 years (IQR, 4.25). The majority of children (52/73, 71%) were less than 5 years of age. Forty-nine (67%) patients were male and 24 (33%) were female with a male-to-female ratio of 2:1 (Table 2). In 53/73 (73%) cases, the diagnosis of KD was established within 10 days of onset of symptoms. The mean duration of fever at the presentation in our cohort was 8.5 ± 4 days. In 48 (65%) children, a diagnosis of KD was recorded in the referral letter by referring physicians. Other provisional diagnoses with which patients were referred to us included acute gastroenteritis (6 patients), viral exanthema (4 patients), pyrexia of unknown origin (4 patients), arthritis (3 patients), acute viral hepatitis (3 patients), cellulitis (1 patient), acute intestinal obstruction (1 patient), and febrile convulsion (1 patient). The frequencies of the classical features of KD in our patients are

TABLE 2 Demographic and clinical characteristics of patients with Kawasaki disease at the time of diagnosis

Characteristics	n (%)
Age in y (median): 3	
0-4 y	52 (71)
<1 y	7 (9)
1-4 y(s)	45 (61)
≥5 y	21 (28%)
Gender	
Male	49 (67)
Female	24 (33)
Changes in extremities (erythema of palms and soles, extremity edema and periungual peeling)	58 (79)
Oral mucosal changes	64 (87)
Polymorphous rash	56 (76)
Conjunctivitis	54 (74)
Cervical lymphadenopathy	44 (60)

TABLE 3 Laboratory parameters of the patients in our cohort

Parameters	Median value (interquartile range)
Hemoglobin, g/dL	10 (2.7-12.6)
WCC, $\times 10^9$/L	14.6 (4.5-29)
Platelet count, $\times 10^9$/L	403 (40-1100)
ESR, Westergren, mm/h	80 (18-140)
CRP, mg/L	97 (63-486)

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WCC, white cell count.

shown in Table 2. Other notable features in our patients were diarrhea (9 patients), asymmetric oligoarthritis involving hip, knee, and ankle joints (5 patients), perianal peeling (30 patients), and Beau's line recorded at 6 weeks follow-up (44 patients). Reactivation of bacille Calmette (BCG) site was not documented in any patient in our cohort despite BCG vaccination being near-universal in this age group in Assam. Results of laboratory investigations at presentation are shown in Table 3.

Nineteen (26%) patients had cardiovascular complications. Coronary artery aneurysm (CAA) was detected in 15 (20%) patients, pericardial effusion was documented in 11 patients (mild effusion in 9 and large pericardial effusion with cardiac tamponade in 2 patients), myocarditis was suspected in 1 patient and 1 patient had shock at presentation requiring inotropic support. The 2 patients with cardiac tamponade had prolonged fever, thrombocytosis and hypoalbuminemia and required emergency pericardiocentesis to remove 150-200 mL of blood-stained serous fluid (Table 4). One patient required repeat pericardiocentesis. The pericardial fluid showed an inflammatory cellular effusion with lymphocytic predominance. These 2 patients also had associated other serous effusions as shown in Table 4.

Sixty-eight (93%) patients in our cohort received IVIg as per the standard protocol (dose: 2 g/kg single infusion over 12-15 hours). Of those treated with IVIg, 66/68 patients received aspirin; the aspirin protocol at PRIC was 50-70 mg/kg/d in 6 hourly divided doses until 48 hours of defervescence followed by reduction of dose to 3-5 mg/kg/d. Parents of 4 patients with KD in our cohort refused treatment for their children because of unwillingness to accept the diagnosis of KD. Post-IVIg, 57/68 patients had shown a prompt response. However, 10/68 patients (14%) were IVIg-resistant. Of these, 8/10 patients were managed with infliximab (IFX), and 2/10 patients were managed with pulse injections of methylprednisolone (pulse-MP). In patients treated with IVIg, minor infusion reactions (fever, chill, and rigor) were observed in 5 patients, transient headache was observed in 11 patients, and flu-like symptoms were observed in 3 patients. No serious treatment-related adverse effects were observed in our patients.

Sixty-eight (93%) patients in our cohort had completed a mean follow-up of 13.6 ± 9.4 months and 5/73 patients were lost to follow-up. Among patients with CAA, 14 out of 15 (93.3%) patients had completed a median follow-up of 18 months (IQR 13.5). Of these, 12 patients had shown reduced coronary artery diameters on follow-up, and 2 patients had GCA who continued prophylactic warfarin (dose was titrated according to the International Normalized Ratio) and low-dose aspirin. No bleeding manifestations were observed in these 2 patients. Also, no serious infection like tuberculosis was documented in patients treated with IFX. No fatal outcome was observed in our cohort.

Two (2%) patients in our cohort had a recurrence of KD. However, no CAA was documented in these patients.

TABLE 4 Clinical and laboratory parameters of the patients with cardiac tamponade

Parameters	Patient 1	Patient 2
Age/gender	5 years/male	8 years/male
Duration of fever at admission	15	10
Associated other effusion (s)	Left sided pleural effusion	Bilateral pleural effusions and ascites
Hemoglobin (g/dL)	10.2	9.8
WCC ($\times 10^9$/L)	23.7	9.8
Platelet count ($\times 10^9$/L)	965	420
Serum albumin (g/L) (reference range: 35-52 g/L)	26	28
Pericardial fluid		
WCC ($\times 10^9$/L)	0.12	0.07
DLC	$N_{40\%}$, $L_{60\%}$	$N_{20\%}$, $L_{80\%}$
RBC	Plenty	Plenty
Protein (g/L)	43	23
LDH (IU/L)	1988	112
CB-NAAT	Negative	Negative
Culture	No growth	No growth
Pleural fluid		
WCC ($\times 10^9$/L)	0.25	0.03
DLC	$N_{30\%}$, $L_{70\%}$	$N_{20\%}$, $L_{80\%}$
RBC	Plenty	Plenty
Protein (g/L)	52 (serum protein: 68)	23 (serum protein: 70)
LDH (IU/L)	1240 (serum LDH: 680)	Not assayed
CB-NAAT	Negative	Negative
Culture	No growth	No growth
Treatment	IVIg 2g/kg (single dose), pulse-MP 30 mg/kg OD × 3 doses	IVIg 2 g/kg (single dose), pulse-MP 30 mg/kg OD × 3 doses

Abbreviations: CB-NAAT, cartridge-based nucleic acid amplification test (performed to rule out tuberculosis); CRP, C-reactive protein; DLC, differential leucocyte count; ESR, erythrocyte sedimentation rate; IVIg, intravenous immunoglobulin; LDH, lactate dehydrogenase; OD, once daily; pulse-MP, pulse methylprednisolone; RBC, red blood cell; WCC, white cell count.

TABLE 5 Comparison of demographic and clinical parameters with those observed in other studies

Parameters	Index study	Chandigarh[8]	China[9]	Switzerland[13]	Algeria[14]	Australia[15]
Total no. of patients	73	69	1016	207	133	353
Age in y	Median: 3 (range 0.25 - 11)	Mean ± SD: 4.9 ± 3	Median: 1.4 (range 0.17-10.75)	Median: 2.6 (range, 0.1-15)	Median: 3 (range 0.4 - 11)	Mean ± SD: 3.9 ± 3
Male : female	2:01	2.5:1	1.8:1	1.43:1	1.6:1	1.7:1
Duration of fever in d, mean ± SD	8.5 ± 4	10.3 ± 5.3	-	-	13 ± 6	-
Fever lasting for 5 d, n (%)	73 (100)	69 (100)	-	-	133 (100)	-
Oral mucosal lesions, n (%)	64 (87)	36 (52.2)	846 (83.3)	161 (77.8)	130 (98)	334/346 (96.5)
Conjunctival injection, n (%)	54 (74)	42 (60.9)	855 (84.2)	159(76.8)	121 (91)	306/344 (89)
Extremity changes, n (%)	58 (79)	25 (36.2)	618 (60.8)	144 (69.9)	128 (97)	257/340 (75.6)
Cervical lymphadenopathy, n (%)	44 (60)	47 (68.1)	624 (61.4)	136 (65.7)	38 (28.5)	210/335 (62.7)
Rash, n (%)	56 (76)	43 (62.3)	780 (76.8)	176 (85)	130 (98)	339 (96)
Complete KD, n (%)	53 (72)	-	716 (70.4)	146 (70.5)	131 (98.4)	314 (88.9)
Incomplete KD, n (%)	20 (27)	-	300 (29.5)	61 (29.5)	2 (0.015)	34 (9.6)
Recurrent KD, n (%)	2 (2)	-	3 (0.3)	5 (2.4)	0	0
Cardiovascular complications, n (%)	19 (26)	-	-	-	32 (24)	-
Coronary artery aneurysm, n (%)	15 (20)	8 (11.6)	240 (23.9)	96 (46.4)	30 (22.5)	19/282 (6.7)
Suspected myocarditis	1	-	-	15 (7.2)	2	-
Pericardial effusion	9	-	PE: 1.85%	54 (26)	PE: 3	-
Cardiac tamponade	2	-	-	2 (1)	-	-
Shock	1		-	-	-	-

Abbreviations: KD, Kawasaki disease; PE, pericardial effusion.

4 | DISCUSSION

This study represents the first case series of KD from the north-east region of India, and includes the records of 73 patients over a period of 2.5 years.

We report a male-to-female ratio in our cohort of 2:1; this is similar to that reported from Chandigarh[8] and China.[9] The proportion of children in our cohort who are below the age of 5 years is similar to studies conducted by other countries.[10-13] The comparisons of the principal clinical features of our cohort from other studies are shown in Table 5.[8,9,13-15]

Cardiovascular complications that are more prominent during the acute phase of KD include myocardial, pericardial, and endocardial involvements which manifest with myocarditis including KD shock syndrome (KDSS), pericarditis (including cardiac tamponade in rare cases), and valvular regurgitations.[1] CAA was observed in 20% of patients in our cohort. Effusions of the pleura or pericardium are uncommon complications of acute KD.[16,17] Two of the patients in our cohort had pericardial effusion with cardiac tamponade and pleural effusion. One of these patients also had associated ascites. The absence of any organism on the culture of the aspirated fluid from the pleura and pericardium, no response to broad-spectrum antimicrobials, and response to IVIg plus pulse-MP were significant pointers to consider KD as the etiology. To the best of our knowledge, a total of 6 cases of cardiac tamponade as a complication of KD have previously been described in the literature (Table 6).[13,18-21] Another rare presentation in our cohort was shock which was observed in 1 patient. KDSS is a shock-like syndrome that can occur in children with KD.[2] This syndrome is defined as sustained systolic hypotension (shock is defined according to published guidelines used in the intensive care setting) in a child with KD or clinical signs of poor perfusion.[22-24]

IVIg is the gold standard of treatment for KD. Ninety-three percent of patients in our cohort received treatment with IVIg which is comparable with rates reported at other Indian centers.[8] For IVIg-resistant cases, several therapeutic agents are recommended, like second dose of IVIg, IFX, and glucocorticoid.[1] During the last decade, several published case series have suggested a role for IFX in severe KD (especially in resistant KD), including some that have shown a reduction in the size of CAAs.[20,25,26] At our center, there

TABLE 6 Comparison of the patients with cardiac tamponade in our cohort with already published reports

Author	Number of cases	Age /gender	Category of KD and associated CAA	Associated other serous effusion(s)	Treatment	Outcome
Dahlem et al.[18] (1999)	1 (impending cardiac tamponade)	8 y/male	IVIg-resistant KD, no CAA	Pleural effusion, ascites	Pulse methyl prednisolone, pericardiocentesis	Prompt response within 48 h, no recurrence and sequelae
Ozdogu et al.[19](2005)	1 (cardiac tamponade)	18 y/male	Complete KD. No CAA	Pleural effusion, ascites	IVIg, pulse-MP, pericardiocentesis	No response and the patient died
Singh et al.[20] (2015)	1 (cardiac tamponade)	12 y/male	IVIg- resistant GCA	-	IFX, pericardiocentesis	Had associated GCA No recurrence of effusion
*de La Harpe et al.[13] (2019)	2 (cardiac tamponade)	One patient was 2.75 y/male	-	-	-	The patient of 2.75 y had developed pulmonary hypertension, vena cava thrombosis and died
Kim et al.[21] (2020)	1 (impending cardiac tamponade)	10 mo	Incomplete KD, mild dilatation of RCA (Z score 2.7)	Pleural effusion	IVIg (twice), 3 pulse-MP injections followed by OP, IFX, pericardiocentesis	Responded only after IFX. No recurrence and no signs of any other CTD on follow-up
Index study	2 (cardiac tamponade)	5 y and 8 y/male	Incomplete KD. No CAA	Pleural effusion in both patients and ascites in 1 patient	IVIg, 3 pulse-MP injections given 2 d after IVIg, pericardiocentesis	Responded after pulse-MP. No sequelae and recurrence; also, no signs of any other CTD on follow-up

Abbreviations: CAA, coronary artery aneurysm; CTD, connective tissue disorder; GCA, giant coronary aneurysm; IFX, infliximab; IVIg, intravenous immunoglobulin; KD, Kawasaki disease; OP, oral prednisolone; pulse-MP, pulse methylprednisolone; RBCs, red blood cells.

* Details of these patients are not reported.

is free supply of IVIg and IFX can be arranged for patients with economic constraints through hospital administration. IFX is also commercially available in Guwahati, Assam. We have been using IFX as an adjunct in the treatment of KD since the beginning of PRIC. We observed that IFX is a very useful agent in the treatment of KD, especially IVIg-resistant cases and this was similar to the studies conducted on IFX in KD.[20,25,26]

Due to the rarity of the disorder many parents are unaware about KD and do not accept the diagnosis. In our cohort, parents of 4 children had refused the diagnosis and its treatment even after proper explanation about the disease and related issues. This indicates the need for some special measures to enhance the knowledge about the disease among physicians and also which can address people of all parts of Assam.

There are several limitations to our study. This is a single-center retrospective cohort and the diagnosis of KD in many patients is likely to be underreported (especially in patients with incomplete KD). Therefore, our cohort is not necessarily a precise representative sample of the nature of KD in Assam. We were unable to address risk factors associated with the development of CAAs (such as age at onset of symptoms, gender), assessment of cardiovascular function, and dyslipidemia on follow-up. Also, pro-inflammatory cytokine levels were not assayed in patients who had received IFX. We need to have a long-term prospective study to address these issues in the future.

5 | CONCLUSION

We report the first case series of KD from north-east India. Patients in our cohort had diverse presentations including cardiac tamponade, KDSS, and recurrence of KD. Our children had tolerated IVIg and IFX well without any serious adverse events at the time of treatment or on follow-up. More work is needed to understand the different presentations of KD.

AUTHOR CONTRIBUTIONS

Dhrubajyoti Sharma (DS) - patient management, data collection, study design and writing of final manuscript; Farhin Iqbal (FI) - echocardiography and patient management; Chiranjeet Narayan Dev (CD), Shivangi Borah (SB), Ruhul Amin Hoque (RH) and Leivon Bellamy Kom (LK) - patient management, data collection, review of the manuscript.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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The readiness of pediatric rheumatology patients and their parents to transition to adult-oriented treatment

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Abstract

Introduction: Transition is a planned process of pediatric patients from child-centered to adult-oriented treatment. Transitional care for patients with chronic diseases is essential. The present study aimed to evaluate the readiness of patients with rheumatic diseases and their parents for transition process.

Method: This is a cross-sectional, single-center study. All patients and their parents were questioned about their awareness of and willingness to undergo transitional care. Transition Readiness Assessment Questionnaire (TRAQ) was applied to all the participants. TRAQ is a tool for measuring readiness for transitional care in adolescents with chronic diseases. TRAQ includes 20 items that are divided into 2 domains: self-management and self-advocacy.

Results: A total of 157 (87 girls/70 boys) patients and their parents were enrolled. Of them 64 were diagnosed with familial Mediterranean fever, 52 with juvenile idiopathic arthritis, 21 with systemic lupus erythematosus, and 20 with Behçet's disease. The median age of the patients was 16 years (15-18). However, all patients and parents accepted that transition to adult-oriented care is necessary; only one-third of them were aware about transitional care. Eighty (50.9%) patients and 147 (93.6%) of the parents stated that they were wishing to continue pediatric rheumatology treatment. The mean TRAQ self-management domain and self-advocacy domain total scores in the patients were 1.76 ± 0.51 and 1.72 ± 0.49, respectively ($P = .48$). The mean TRAQ total score was not different between patients and parents. When we assessed the factors affecting transition process, the TRAQ score was lower among patients with active disease, and requiring hospitalization during the previous year.

Conclusion: Assessment od the readiness of patients with chronic rheumatic diseases for transition care will increase the awareness of patients and their parents as well, and provide determination of the optimal time for transition.

KEYWORDS

pediatrics, rheumatology, transition care, Transition Readiness Assessment Questionnaire

GSM: 00 905 322 765 238.

1 | INTRODUCTION

Enhanced knowledge of rheumatic diseases and advancements in novel targeted treatments are improving long-term survival outcomes, increasing the number of adolescents reaching adulthood with rheumatological diseases. Consequently, transitional care has come out as a new field in pediatric rheumatology. Transitional care, as defined by the Society for Adolescent Medicine,[1] is the purposeful and planned movement of pediatric patients from child-centered to adult-oriented treatment. Cooperation between pediatric and adult rheumatologists will facilitate treatment compliance and reduce the risk of unfavorable outcomes. Transitional care for adolescents and young adults (AYA) with rheumatic diseases is fundamental due to the chronic course of such diseases, and the persistence of disease activity and morbidity during adulthood. According to the European League Against Rheumatism (EULAR)/ Paediatric Rheumatology European Society (PRES) recommendations, high-quality and coordinated transitional care is required for all AYA and should be initiated as early as possible.[2]

Although some clinics have started to establish transitional care units, the number of relevant studies is inadequate. According to a recent European survey, the majority (99%) of pediatric rheumatologists reported that a formalized process of transitional care is essential, whereas 25% of the participants reported that their centers did not provide transitional care.[3] Furthermore, a national survey from the USA reported that the transitional care success rate among adolescents with special healthcare needs was only 14%.[4] Undoubtedly, transitional care must be structured in synergy at a suitable time and it is critically important to prepare patients and determine their readiness for transitional care. Furthermore, the move to transitional care may be challenging not only for patients, but also for their parents. As such, the present study aimed to determine the readiness of AYA with rheumatic diseases and their parents for transition to adult-oriented treatment. Furthermore, we also aimed to evaluate the factors influencing the readiness for transitional care.

2 | MATERIALS AND METHODS

This cross-sectional study was performed at Kanuni Sultan Süleyman Training and Research Hospital, between January and April 2020. Patients older than 12 years of age who were admitted to the pediatric rheumatology outpatient clinic between January and April 2020 and were followed up with a diagnosis of chronic rheumatic disease for at least 6 months were included in the study. The Transition Readiness Assessment Questionnaire (TRAQ) was separately administered by a clinical nurse specialist (CNS) to AYA patients with a rheumatic disease and their parents. TRAQ is a 20-item questionnaire which measures readiness for transitional care in AYA with chronic diseases. The 20 items are divided into 2 domains: self-management and self-advocacy. The questionnaire items are answered using a 5-point Likert-type scale based on the Stages of Change Model, ranging from "I do not need to do this" to "I always do this when I need to". The responses to each item in each domain were calculated.[5] Higher scores indicate greater readiness for transitional care, but there is no cut-off value indicating whether the patient is ready for transition or not. The Turkish version of TRAQ used in this study has been validated.[6] Due to lack of a validated tool for assessing the readiness for transition of the parents having children with chronic diseases, the version of TRAQ administered to the parents was modified, so that all references to "you" in the items was changed to "your child". Furthermore, all the participants were questioned about their awareness of transitional care and their willingness to use it.

Demographic data, and clinical and laboratory findings were obtained from the patients' medical records by 2 pediatric rheumatologists. Patients were classified as having a rheumatic disease according to previously accepted criteria, including International League of Associations for Rheumatology classification criteria for juvenile idiopathic arthritis (JIA),[7] pediatric familial Mediterranean fever (FMF),[8] consensus classification criteria (PEDBD) for pediatric Behçet's disease (BD) [9] and Systemic Lupus International Collaborating Clinics criteria for juvenile systemic lupus erythematosus (SLE).[10] Furthermore, all parents were questioned in terms of demographic findings including age and education status before applying TRAQ.

Disease activity was evaluated according to the SLE Disease Activity Index (SLEDAI) for SLE,[11] BD Current Activity Form (BDCAF) [12] for BD, Juvenile Arthritis Disease Activity Score (JADAS) [13] for JIA, and Auto-Inflammatory Diseases Activity Index (AIDAI) for FMF.[14] Written informed consent was obtained from all the participants included in the study and the study protocol was approved by the local ethics committee.

2.1 | Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows v.21 (IBM Corp., Armonk, NY, USA). Descriptive analyses are presented as percentage, mean ± SD, median, and range, as appropriate. Categorical variables were compared using the Chi-square test or Fisher's exact test, as appropriate. Differences in continuous data between 2 groups were evaluated using Student's *t* test or the Mann-Whitney *U* test, as appropriate. The level of statistical significance was set at $P < .05$.

3 | RESULTS

3.1 | Baseline characteristics of the patients and parents

The study included 157 patients and their parents. Among the patients, 64 were diagnosed with FMF, 52 with JIA, 21 with SLE, and 20 with BD. The median age of the patients was 16 years (range: 15-18 years) and 87 (55.4%) of the patients were female. The

hospitalization rate during the previous year was 10.8% (n = 17). Among the patients who were hospitalized, 5 had FMF, 8 had JIA, 2 had BD, and 2 had SLE. All of them were hospitalized due to the flare of their diseases. Of the 64 patients with FMF, 21 were colchicine-resistant and all were inactive when TRAQ was administered. The 21 patients who were colchicine-resistant were treated with monthly canakinumab.

Among the 52 patients with JIA, the distribution of JIA subtypes was as follows: oligo-articular JIA, n = 19; enthesitis-related arthritis (ERA), n = 23; polyarticular JIA, n = 10. In all, 8 of the 52 JIA patients had uveitis. None of them had a concomitant disease. At the time TRAQ was administered none of the JIA patients had active arthritis, enthesitis, or morning stiffness, and all were receiving biologic drugs. In total, 41 were treated with anti-tumor necrosis factor (adalimumab n = 19; etanercept n = 22) and 9 received anti-interleukin (IL)-6 (tocilizumab). The median JADAS score was 1.1 (range: 1-2.1).

Among 21 patients with SLE, all had skin involvement, 6 had renal involvement, 6 had hematologic involvement, 1 had pulmonary involvement, and 1 had neurologic involvement concomitantly. All 21 SLE patients were using hydroxychloroquine and prednisolone, with a median dose of 7.5 mg (range: 5-10 mg). Furthermore, 8 patients were receiving mycophenolate mofetil and 3 were treated with acetylsalicylic acid. At the time TRAQ was administered the median SLEDAI score was 4 (range: 2-10) and 3 of the patients had active disease.

Of the 20 patients with BD, all had oral aphthous and genital ulcers, 12 had pseudofolliculitis, 6 had erythema nodosum, 2 had thrombophlebitis, and 2 had uveitis, and the pathergy test was positive in 6 patients. All 20 patients were receiving colchicine, 4 were treated with azathioprine, and 2 were treated with adalimumab concomitantly. All but 1 of the BD patients had inactive disease and 1 patient had active uveitis at the time TRAQ was administered.

The median age of the patients' parents was 42 years (range: 36-48 years). Among the parents, 29.9% graduated primary school, 20.4% graduated middle school, 20.4% graduated high school, and 6.4% graduated university. The remaining 22.9% of patients were literate.

3.2 | Survey responses

In total, 31.8% (n = 50) of the patients and 39.4% (n = 62) of the parents reported they knew about transitional care. In addition, 100% of the patients and their parents accepted the fact that the patients had to transition to adult-oriented care when they reached adult age; however, 50.9% (n = 80) of the patients and 93.6% (n = 147) of the parents reported they were wishing to continue pediatric rheumatology treatment because they were concerned that new doctors would not be familiar with their diseases and medical history. Furthermore, both patients who were receiving biologic drugs and their parents reported they were worried about the administration and supply of their drugs.

Mean TRAQ total score was 3.31 ± 0.82 in the patients and 3.41 ± 0.78 in the parents ($P = .54$). Mean TRAQ self-management domain and self-advocacy domain total scores in the patients were 1.76 ± 0.51 and 1.72 ± 0.49, respectively ($P = .48$); female patients had a higher mean self-management domain total score than the males (1.98 ± 0.52 vs. 1.54 ± 0.48, $P = .01$). TRAQ scores were not significantly different between the patients with FMF, JIA, SLE, and BD, whereas the patients who were receiving canakinumab and the patients with active disease had lower TRAQ scores. When we assessed the factors affecting the transition process, we could not find any association between age, gender and TRAQ total score; however, the female patients had higher self-advocacy scores. Having active disease, and hospitalization during the previous year decreased the TRAQ score and delayed readiness for transitional care among the patients.

4 | DISCUSSION

The present study evaluated readiness for transitional care in 157 AYA patients with a rheumatic disease and their parents. Readiness for transitional care depends on the specific decision capacity of the patients about their health condition. Unfortunately, an inadequate transition process can result in a lack of continuity of healthcare, and an increase in morbidity and mortality. Assessment of patient readiness for transitional care and their awareness of and willingness to transition can facilitate a more efficient transition process. Most recently, Matsumoto et al.[15] evaluated the awareness of transitional care in JIA patients and their parents, reporting that 57.1% of patients and 35.9% of their parents did not know about transitional care, and 61.2% of patients and 78.6% of their parents were worried about transition due to difficulty trusting new doctors. Nonetheless, data on attitudes of patients with other rheumatic diseases toward transitional care are lacking; therefore, the present study also investigated patients with FMF, SLE, and BD, as well as those with JIA, and their parents, and noted that although all the patients and their parents thought transition was necessary, 50% of the patients and most of the parents were concerned about whether new doctors would be familiar with their disease and medical history.

Almost all published studies on transitional care in rheumatology patients were performed in developed countries; relevant data from developing countries are limited. One survey of pediatric rheumatologists from a developing country (Brazil) reported that the rate of a well-established transitional care program was only 13%. Although 43% of the pediatric rheumatologists concluded that 18 years is an ideal age for initiating transitional care, only 33% reported that they started the transition progress at that age. Furthermore, most pediatric rheumatologists reported that they did not use a specific tool to evaluate transition readiness.[16] Turkey is a developing country and, unfortunately, despite increasing awareness of the need for transitional care in Turkey, there isn't a standard model. The Turkish health insurance system advocates transfer of patients aged > 18 years to adult healthcare departments. However, whether or not patients are

ready for such a transfer is frequently overlooked. To the best of our knowledge, the present study is the first from a developing country to evaluate awareness of and readiness for transitional care among AYA patients with rheumatic diseases and their parents.

According to American Academy of Pediatrics guidelines, assessing transition readiness using an objective tool is recommended.[17] Assessment of patient readiness for transition using a reliable and validated tool is important. There are several transition readiness assessment tools,[18] but only TRAQ was validated for use in Turkey.[6] The number of studies on rheumatic disease patient transition readiness are insufficient and mainly included patients with JIA irrespective of their biological therapy use. Jensen et al.[19] evaluated 89 patients with chronic conditions, of which 50 (56%) had a rheumatic disease. They also reported that the TRAQ self-management domain score was not significantly associated with age, gender, socioeconomic status, or diseases group. In contrast, Lazaroff et al.[20] observed that female gender, older age, and high patient activation significantly predicted higher TRAQ scores among patients with JIA. Bingham et al.[21] evaluated the variables affecting transition readiness in 76 patients with rheumatic diseases, of which 60 were diagnosed with JIA, 6 with lupus, and 12 with fibromyalgia. They reported that having a younger parent, a family member with a similar disease, longer disease duration, and a co-morbid non-rheumatic diagnosis, and having had a summer job were associated with high self-reported independence. Haro et al.[22] reported that patients with SLE had a lower healthcare utilization rate, lower medication usage, and lack of rheumatology follow-up after transition. Xie et al.[23] noted that transitional care improved self-care and quality of life, and decreased the hospital readmission rate. In the present study, we could not find any association between age, gender and TRAQ total score; while all female patients had higher TRAQ self-management domain scores, and FMF patients who were colchicine-resistant and treated with monthly canakinumab, and patients with active disease (3 SLE and 1 BD patients) at the time TRAQ was administered had lower total TRAQ scores. Administering TRAQ regularly to such patients might facilitate the progression to readiness for transitional care via increasing their awareness of the process.

The parents of children with chronic illness tend to be overprotective of their children, which can delay their development of self-care. Suris et al.[24] evaluated compelling factors associated with the transfer process of parents/caregivers of patients with chronic illness and reported that parent/caregiver transition readiness is associated with an easy transfer transition process. According to EULAR/PRES recommendations, the transition process should be structured around direct communication, not only with patients, but also parents/carers.[2] Furthermore, the transition process should meet patients' and parents' expectations; therefore, it is important to evaluate the awareness and readiness of patients and families before the transition process begins. In the present study parents and patients had similar TRAQ scores. The Spanish Society of Pediatric Rheumatology (SERPE) developed a recommendation set for transition management in rheumatic diseases by using Delphi technique. They confirmed that health professionals should communicate to patients and parents, as well. They suggested to promote specialized nursing care during transition. They also recommended to consider socioeconomic and/or cultural factors and to manage the transition process according to patients' needs and expectations.[25] In our department, all questionnaires were applied by a CNS and all parents were questioned in terms of education status. We also evaluated the awareness and willingness of the patients and parents about transition care. This preliminary study may help to design an appropriate transition process in our country.

The present study's limitations are its single-center design and the fact that evaluation of the progress in patients' readiness for transitional care was not performed during the follow-up due to the cross-sectional design. Furthermore, there is not a validated tool in Turkish for assessing the readiness for transition of the parents who give care to children with chronic diseases. This study had a cross-sectional design, so the findings cannot reflect the real distribution of rheumatic diseases in a pediatric rheumatology center. In the present study, all JIA patients were receiving biologic drugs. Evaluating only JIA patients treated with biologic drugs is another limitation of the study. However, previous studies have shown that more than one-third of JIA patients had active disease and required treatment after the adolescence period[26] and the patients with JIA requiring biologic treatment at the age of 17 years probably will have to continue this treatment when they are adults.[27] Therefore, it is important to evaluate the readiness of these patients. However, it is clear that repeating the study in a more homogeneous JIA group irrespective of their usage of biological therapy will give more accurate data on this subject. Nonetheless, the study's large cohort of patients with chronic rheumatic diseases strengthens the present findings.

5 | CONCLUSION

The present study shows that having active disease, and hospitalization during the previous year decreases the TRAQ score and delays patient readiness for transitional care. TRAQ focuses primarily on medication skills and does not evaluate the awareness of and willingness for transitional care in patients and their parents. In addition, regular administration of TRAQ during follow-up can be used to track the progression of self-management and self-advocacy; therefore, all patients and their parents should regularly be administered TRAQ to help objectively monitor their readiness for transitional care, so as to determine the optimal transition cut-off age.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

AUTHOR CONTRIBUTIONS

HES conceptualized and designed the study, drafted the initial manuscript, and had full access to all the data in the study; SGK and RK designed the study, conducted the data analyses, drafted the initial manuscript, and had full access to all the data in the study; NAA

drafted the initial manuscript. All authors reviewed and revised the manuscript and approved the final version of the manuscript.

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ORIGINAL ARTICLE

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MicroRNA-320c inhibits articular chondrocytes proliferation and induces apoptosis by targeting mitogen-activated protein kinase 1 (MAPK1)

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Abstract

Aim: To clarify the interaction of microRNA-320c (miR-320c) and mitogen-activated protein kinase 1 (MAPK1), and to investigate the effects of miR-320c on articular chondroctye proliferation and apoptosis.

Methods: Lentiviral expression vectors were constructed and dual luciferase assays containing MAPK1 3′-untranslated regions (3'-UTRs) were performed. Small hairpin RNA (shRNA) was utilized to modulate MAPK1 expression. The messenger RNA and protein expression levels were determined by quantitative real-time polymerase chain reaction (qRT-PCR) and western blotting respectively. Cell Counting Kit-8 and flow cytometry were conducted to detect the proliferation and apoptosis of Human Chondrocyte-articular (HC-a) cells. Besides that, the influences of miR-320c and MAPK1 on MAPK pathway activation were also evaluated.

Results: Our data identified MAPK1 as a direct target gene of miR-320c, and miR-320c can negatively regulate MAPK1 expression by directly binding to MAPK1 3′-UTR in HC-a cells. Further functional study displayed that miR-320c overexpression and MAPK1 shRNA significantly suppressed the proliferation of HC-a cells and promoted cell apoptosis. Meanwhile, MAPK1 shRNA could attenuate miR-320c inhibitor promotive effects on HC-a cell proliferation and reverse its inhibitory effect on cell apoptosis. MAPK1 overexpression could rescue the inhibitory effect of miR-320c on HC-a cell proliferation, and weaken the accelerating effect of miR-320c on cell apoptosis. However, neither miR-320c or MAPK1 shRNA regulate the expression of c-JUN, JNK and c-Fos.

Conclusion: miR-320c inhibits articular chondrocyte proliferation and induces apoptosis by targeting MAPK1, suggesting that miR-320c perhaps participates in the pathogenesis of osteoarthritis and acts as a potential target for the therapeutic treatment of osteoarthritis.

KEYWORDS

apoptosis, cell proliferation, chondrocytes, MAPK1, MiR-320c

1 | INTRODUCTION

Osteoarthritis (OA) is associated with progressive destruction of cartilage, inflammation, subchondral bone remodeling and synovium inflammation,[1] and seriously impairs the quality of life of patients. It has become the main cause of disability in the elderly and has brought heavy economic burden to families and society.[2] At present, treatment options are pain management and symptom control, which cannot reverse the loss of articular cartilage.[3] Therefore, it is necessary to explore new therapy targets to slow down or even prevent disease progression.

In recent years, microRNAs (miRNAs) have emerged as an important target for many diseases. miRNAs are a group of small-molecule, single-stranded, endogenous and non-coding RNAs,[4] which participate in the regulation of gene expression. They can serve critical functions in disease biological process, including cell proliferation, differentiation and apoptosis.[5,6] miRNAs usually negatively regulate the expression of the target gene through targeting 3′-untranslated regions (3'-UTRs) of messenger RNA (mRNA) in a base-pairing manner, which could promote mRNA degradation or translation inhibition.[7] The establishment of interrelationship of miRNA and its target genes can provide a better understanding of the molecular mechanism of the diseases. Accumulated evidence has supported that miRNAs play a critical role in OA.[8,9] A lot of aberrant miRNAs have been identified in patients with OA, revealing the potential involvement in the progression and development of OA.[10-12] Therefore, targeting miRNAs may be used to identify a potential therapeutic target for the clinical treatment of OA.

Our study has reported that the expression level of plasma miR-320c was upregulated in OA patients,[13] indicating miR-320c may be involved in the development of OA. But the functions of miR-320c in OA pathogenesis are still unclear. Bioinformatics software analysis revealed that mitogen-activated protein kinase 1 (MAPK1) was a candidate target of miR-320c, of which there was miR-320c binding site at the 3′-UTR of MAPK1. MAPK1, also known as extracellular signal-regulated kinase (ERK)2 is encoded by the MAPK1 gene. It belongs to the MAP kinase family and previous research has demonstrated that the MAPK signaling participated in OA[14,15] by regulating the proliferation and differentiation of chondrocytes.[16]

Accordingly, in this study we focused on the interaction between miR-320c and MAPK1 in chondrocytes to explore the underlying molecular mechanism of miR-320c in OA. First, we utilized dual luciferase reporter assays to confirm that miR-320c can negatively regulate MAPK1 expression by targeting 3′-UTR of MAPK1. Next, we utilized small hairpin RNA (shRNA) to regulate the expression level of MAPK1 and evaluated the influences of miR-320c and MAPK1 on MAPK pathway activation. Last, we performed functional experiments to assay the effects of miR-320c and MAPK1 on Human Chondrocyte-articular (HC-a) cell proliferation and apoptosis.

2 | MATERIALS AND METHODS

2.1 | Cell culture

The HC-a cells were provided by the Shanghai Chinese Academy of Sciences Health Science Research Center and cultured in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin at 37°C with 5% CO_2. The third passages of chondrocytes were used in the following experiments and all the experiments were repeated in triplicate independently in this study.

2.2 | Construction of Lentiviral vectors

Lentiviral vector pLVX-ZsGreen-miRNA-Puro, pLVX-EGFP-IRES-Neo, pLVX-puro, pLVX-Control, pLVX-Control-shRNA, pGIPZ and pLenti-shRNA-GFP-Puro were purchased from Asia-Vector Biotechnology Company (Shanghai, China). miRNA-320c (5′-AAAAGCTGGGTTGAGAGGGT-3′) sequence was amplified and cloned into EcoRI and Bam HI sites of the pLVX-ZsGreen-miRNA-Puro vector, to generate the miRNA-320c overexpression lentivector. MAPK1 sequence was amplified and cloned into pLVX-puro vector, to generate the MAPK1 overexpression lentivector. MiRNA-320c inhibitor (5′-TGGGAGAGTCAAATCGAAAATTATTGGGAGAGTCAAATCGAAAATTATTGGGAGAGTCAAATCGAAAATTATTGGGAGAGTCAAATCGAAAATTATTGGGAGAGTCAAATCGAAAATTATTGGGAGAGTCAAATCGAAAA-3′) sequence was amplified and cloned into XhoI and Bam HI sites of the pLVX-EGFP-IRES-Neo vector. shRNA targeting MAPK1 was synthesized and inserted into pLenti-shRNA-GFP-Puro vector. The target sequences for silencing MAPK1 were listed as follows: MAPK1-sh1: 5′-TGCTGTTGACAGTGAGCGATTCGAGTAGCTATCAAGAAAATAGTGAAGCCACAGATGTATTTTCTTGATAGCTACTCGAACTGCCTACTGCCTCGGA-3′; MAPK1-sh2: 5′-TGCTGTTGACAGTGAGCGCGGACATTTGGTTCTTATCAATTAGTGAAGCCACAGATGTAATTGATAAGAACCAAATGTCCATGCCTACTGCCTCGGA-3′. MAPK1-sh3: 5′-TGCTGTTGACAGTGAGCGACCACACAAGAGGATTGAAGTATAGTGAAGCCACAGATGTATACTTCAATCCTCTTGTGTGGGTGCCTACTGCCTCGGA-3′.

2.3 | Generation of stable cell lines

All lentivectors were packaged in HEK293T cells by SpeedyLenti (Asia-Vector) according to the manufacturer's instructions. Lentivirus with the best multiplicity of infection was used to infect HC-a cells. Forty-eight hours later, stable infected cells were obtained by 5 μg/mL puromycin (Sigma-Aldrich) screening and confirmed by quantitative polymerase chain reaction (qPCR) and western blotting.

2.4 | Dual luciferase reporter assay

The binding site of miR-320c in the 3'-UTR of MAPK1 was predicted by using bioinformatics tools (http://www.targetscan.org) and synthesized by PCR. The wild type (Wt) and mutant (Mut) 3'-UTR sequence of MAPK1 were cloned into the pmir-GLO reporter luciferase vector (Promega). The primer sequences were as follows: MAPK1 3'-UTR Wt (F: 5′-CTCGAGGCTAGCGAGATTTGTCAGGACAAGGGCTC-3′; R: 5′-TGCCTGCAGGTCGACCTGCACAGAAAGCTGTAACA-3′); MAPK1 3′-UTR Mut (F: 5′-CTCGAGGCTAGCGAGCTGCACAGAAAGCTGTAACA-3′; R: 5′-TGCCTGCAGGTCGACCAGAGGACTGGACGTGCTCA-3′). The recombinant plasmids named pmir-GLO MAPK1-3'-UTR Wt and MAPK1-3'-UTR Mut. Stable infected HC-a cells were planted 24-well plates for 24 hours and subsequently transfected with different vectors by Lipofectamine 2000 (Invitrogen). There were 6 groups: (a) miRNA negative control (NC) + MAPK1 3'-UTR Wt; (b) miR-320c + MAPK1 3'-UTR Wt; (c) miR-320c inhibitor + MAPK1 3'-UTR Wt; (d) miRNA NC + MAPK1 3'-UTR Mut; (e) miR-320c + MAPK1 3'-UTR Mut; (f) miR-320c inhibitor + MAPK1 3'-UTR Mut. After culturing for another 48 hours at 37°C, cells were collected and the luciferase activities were measured by the Dual Luciferase Reporter Assay System (Promega) according to the manufacturer's protocol. Renilla luciferase activities were used as normalization for each transfected well.

2.5 | Real-time PCR analysis

Total RNA was extracted from HC-a cells using Trizol reagent (Invitrogen), according to the protocols of the manufacturer. The yield of RNA was determined using a NanoDrop 2000 spectrophotometer (Thermo), and the integrity was evaluated using agarose gel electrophoresis stained with ethidium bromide. Complementary DNA (cDNA) was synthesized using high-capacity cDNA Reverse Transcription Kits (Applied Biosystems) according to the recommendations of the manufacturer. miRNA cDNA was synthesized according to miRNA First-Strand cDNA Kit (TransGen) instructions. Real-time PCR was performed using SybrGreen qPCR Master Mix (Sangon). The relative abundance of miR-320c was normalized to U6, and MAPK1, c-JUN, JNK and c-FOS were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Reactions were incubated at 95°C for 3 minutes, followed by 45 cycles of 95°C for 7 seconds, 57°C for 10 seconds, 72°C for 15 seconds. Each sample was run in triplicate for analysis. The expression levels were computed using ABI Prism7700 SDS Software (Thermo Fisher) and calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences for real-time PCT were shown in Table 1.

2.6 | Western blot analysis

Western blot analysis was used to evaluate the protein expression level. A total of 48 hours following transfection, the cells were collected and total protein were extracted by RIPA Lysis Buffer (Pierce) according to the manufacturer's instructions. The concentration of total protein was examined by using a Bicinchoninic Acid Protein Assay Kit (Pierce). Equal amounts of protein (20 μg) were subjected to sodium dodecyl sulfate (SDS) - 10% polyacrylamide gel electrophoresis (PAGE), followed by transferring to polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat milk for 1 hour and incubated overnight at 4°C with the following primary antibodies against MAPK1 (1:5000, Abcam), c-FOS (1:5000, Abcam), JNK (1:2000, Santa Cruz), c-JUN (1:1000, Santa Cruz) and GAPDH (1:1000, Santa Cruz) at 4°C overnight, followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 hour at room temperature (1:10 000; Santa Cruz). The blots were visualized by using the enhanced chemiluminescence western blotting kit (Thermo) according to the manufacturer's instructions. Immunoreactive bands were quantified using ImageQuant (Bio-Rad).

TABLE 1 Primer sequences for real-time polymerase chain reaction

Gene	Primer sequence
miR-320c	F : AAAAGCTGGGTTGAGAGGGT
	R :ACCCTCTCAACCCAGCTTTT
MAPK1	F : TCACACAGGGTTCCTGACAGA
	R : ATGCAGCCTACAGACCAAATATC
c-JUN	F: TCCAAGTGCCGAAAAAGGAAG
	R: CGAGTTCTGAGCTTTCAAGGT
JNK	F: TCTGGTATGATCCTTCTGAAGCA
	R: TCCTCCAAGTCCATAACTTCCTT
c-FOS	F: GGGGCAAGGTGGAACAGTTAT
	R: CCGCTTGGAGTGTATCAGTCA
U6	F:CTCGCTTCGGCAGCACA
	R:AACGCTTCACGAATTTGCGT
GAPDH	F: GGAGCGAGATCCCTCCAAAAT
	R: GGCTGTTGTCATACTTCTCATGG

Abbreviations: F, forward; MAPK1, mitogen-activated protein kinase 1; R, reverse.

2.7 | Cell proliferation and apoptosis assay

Cell Counting Kit-8 (CCK-8) (BBI Life Sciences) was used to assess the cell proliferation of HC-a cells and flow cytometry was performed to measure the cell apoptosis rate. There were 7 groups: (a) miRNA NC; (b) miR-320c; (c) MAPK1 overexpression (OE); (d) miR-320c + MAPK1 OE; (e) miR-320c inhibitor; (f) MAPK1 shRNA; (g) miR-320c inhibitor + MAPK1 shRNA.

The cell proliferation assay was performed after transfection for 12, 24, 48 and 72 hours. CCK-8 reagent (10 μL) was added to the culture medium. After incubating at 37°C for 2 hours, the absorbance was measured at 450 nm. The cell apoptosis assay was conducted at

48 hours post-transfection. The cells were fixed and washed twice with phosphate-buffered saline, then were incubated with Annexin PE and 7-AAD (Yeasen) at room temperature for 15 minutes in the dark. Then the samples were analyzed by Beckman Coulter FC500 flow cytometer (Beckman Coulter) according to the manufacturer's instructions.

2.8 | Statistical analysis

Statistical analysis was conducted by SPSS 20.0 software. Data are presented as the mean ± standard deviation (SD). The differences among groups were analyzed using Student's *t* test, and one-way analysis of variance. The statistically significant differences were identified as $P < .05$.

3 | RESULTS

3.1 | miR-320c negatively regulated MAPK1 expression

To assess the effects of miR-320 on MAPK1 expression in chondrocytes, miR-320c, miR-320c inhibitor, and the miRNA NC were transfected into HC-a cells. Furthermore, quantitative real-time PCR and western blot were carried out to detect the mRNA and protein expression levels. The result showed that compared with miRNA NC group, the mRNA expression level of MAPK1 was downregulated in the miR-320c group and significantly upregulated in the miR-320c inhibitor group (Figure 1A $P < .05$). Western blot results showed that the expression levels of MAPK1 have no differences among these 3 groups, while the protein expression level of phosphorylated MAPK1 (p-MAPK1) was decreased in miR-320c group and increased dramatically in miR-320c inhibitor group (Figure 1B), which was consistent with the mRNA levels. All these results revealed MAPK1 expression was suppressed by miR-320c and elevated by miR-320c inhibitor in HC-a cells, indicating that MAPK1 was a direct target gene of miR-320c.

3.2 | miR-320c regulates MAPK1 expression by directly targeting MAPK1 3'-UTR

To validate the predicted binding of miR-320c to the MAPK1 3'-UTR, we transfected the reporter vectors containing MAPK1 3'-UTR Wt/Mut into stable infected HC-a cells. The MAPK1 3'-UTR Wt/Mut sequences binding to miR-320c are shown in Figure 2A. The results demonstrated that the luciferase activities were almost equal in the miRNA NC + MAPK1 3'-UTR Mut group and miRNA NC + MAPK1 3'-UTR Wt group. The relative luciferase activities of MAPK1 were markedly reduced in the miR-320c + MAPK1 3'-UTR Wt group compared with the miRNA NC + MAPK1 3'-UTR Wt group (Figure 2B $P < .05$). Transfection with MAPK1 3'-UTR Mut abolished this suppression (Figure 2B $P < .05$). Furthermore, the luciferase activity of MAPK1 was significantly increased in the miR-320c inhibitor + MAPK1 3'-UTR Wt group compared with the miRNA NC + MAPK1 3'-UTR Wt group (Figure 2B $P < .05$), whereas the luciferase activity was attenuated in the miR-320c inhibitor + MAPK1 3'-UTR Mut group compared with the miR-320c inhibitor + MAPK1 3'-UTR Wt group (Figure 2B $P < .05$). All the results indicated miR-320c directly binds to MAPK1 3'-UTR.

3.3 | Overexpressions of miR-320c and MAPK1 shRNA inhibit MAPK1 expression

To further address whether miR-320c functions through targeting MAPK1, MAPK1 shRNA was used to silence MAPK1. Compared with the miRNA NC group, miR-320c reduced the MAPK1 mRNA expression level (Figure 3A $P < .05$), while MAPK1 OE increased the MAPK1 mRNA expression (Figure 3A $P < .001$). The relative mRNA level of MAPK1 in the miR-320c + MAPK1 OE group was significantly lower than in the MAPK1 OE group (Figure 3A $P < .05$). Moreover, transfection with shMAPK1 resulted in decreased MAPK1 protein expression in HC-a cells compared with the miRNA NC group. The expression of MAPK1 was significantly upregulated in HC-a cells in the miR-320c inhibitor group, but this upregulation was dramatically attenuated by shMAPK1 (Figure 3A $P < .05$). Western blot analysis

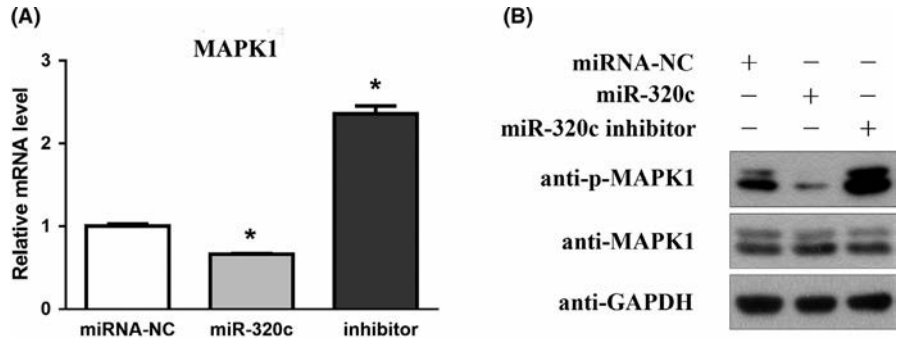

FIGURE 1 MicroRNA-320c (miR-320c) regulates mitogen-activated protein kinase 1 (MAPK1) expression in Human Chondrocyte-articular (HC-a) cells. (A) MAPK1 messenger RNA expression level after transfecting with miRNA negative control (NC), miR-320c or miR-320c inhibitor; (B) MAPK1 protein expression level after transfecting with miRNA NC, miR-320c or miR-320c inhibitor. *$P < .05$ compared with miRNA NC group. GAPDH, glyceraldehyde 3-phosphate dehydrogenase

showed the protein level of p-MAPK1 was consistent with the real-time PCR results (Figure 3B). Therefore, our data confirmed that MAPK1 was a directly targeted gene of miR-320c in HC-a cells. MiR-320c plays the same role as MAPK1 shRNA in cells and served as a negative regulator of the MAPK1 expression.

3.4 | mRNA and protein expression levels of c-JUN, JNK and c-FOS

To evaluate the influence of miR-320c and MAPK1 on the MAPK pathway activation, the mRNA and protein expression levels of c-JUN, JNK and c-FOS were determined. Real-time PCR results showed that neither miR-320c nor MAPK1 regulate the expression of c-JUN, JNK and c-FOS compared with the miRNA NC group, while no significant difference was showed among all these groups (Figure 3C-E $P > .05$). Moreover, western blot results showed that miR-320c overexpression and miR-320c inhibitor cannot regulate the protein levels of c-JUN/p-c-JUN, JNK/p-JNK and c-FOS/p-c-FOS either (Figure 3F). Indirectly, the results indicated that miR-320c regulated MAPK1 expression may not be via MAPK signaling pathway.

3.5 | miR-320c and MAPK1 shRNA suppressed chondrocytes proliferation

To explore the biological function of miR-320c and MAPK1 in HC-a cells, the cell proliferation was detected at 12, 24, 48 and 72 hours after transfection (Figure 4). The results showed HC-a cell proliferations both in the miR-320c overexpression group and in the MAPK1 shRNA group were significantly suppressed at 24, 48 and 72 hours compared with that in the miRNA NC group ($P < .05$). But the cell proliferation was significantly increased in the MAPK1 OE group from 24 hours till 72 hours ($P < .05$). MAPK1 overexpression could rescue the inhibitory effect of miR-320c on HC-a cell proliferation at 48 and 72 hours compared with that in miR-320c overexpression group ($P < .05$). In contrast, the cell proliferation was significantly enhanced in the miR-320c inhibitor group at 72 hours compared with the miRNA NC group ($P < .05$). And MAPK1 shRNA partially attenuated this promotive effect at 72 hours compared with that in the miR-320c inhibitor group ($P < .05$). These results demonstrated that both miR-320c and MAPK1 shRNA could suppress HC-a cell proliferation, indicating miR-320c inhibit the proliferation of HC-a cells by MAPK1.

3.6 | miR-320c and MAPK1 shRNA promoted cell apoptosis

Cell apoptosis was performed at 48 hours after transfection to identify the effects of miR-320c and MAPK1 in the HC-a cells (Figure 5A). Flow cytometry data showed that the miR-320c group and the MAPK1 shRNA group notably promoted cell apoptosis rate in HC-a cells compared with the miRNA NC group (Figure 5B $P < .05$). The cell apoptosis rate dramatically reduced in the MAPK1 OE group, and MAPK1 can attenuate the accelerating effect of miR-320c on cell apoptosis compared with the miR-320c group (Figure 5B $P < .05$), whereas the apoptosis rate in the miR-320c inhibitor group was clearly lower than that in the miRNA NC group ($P < .05$). MAPK1 shRNA could reverse the inhibitory effect of miR-320c inhibitor on cell apoptosis compared with the miR-320c inhibitor group (Figure 5B $P < .05$). Taken together, these results suggested that miR-320c overexpression could promote cell apoptosis by targeting MAPK1.

4 | DISCUSSION

This study explored the effects of miR-320c and the exact mechanism underlying its role in modulating cell proliferation and apoptosis.

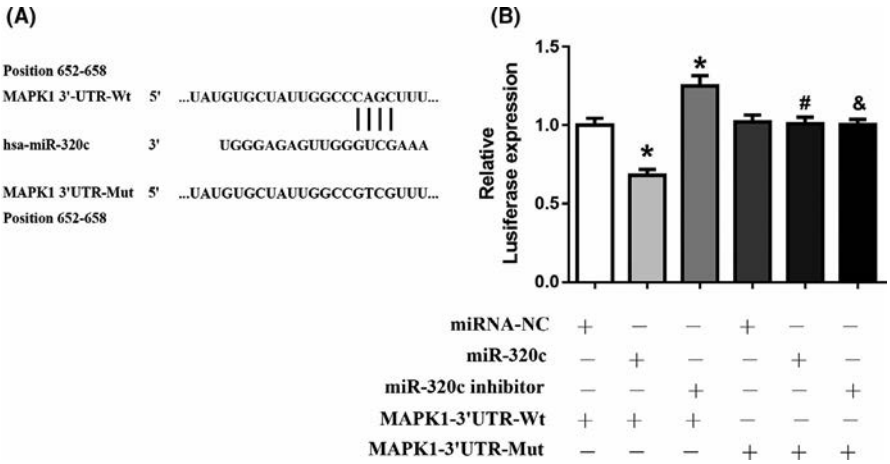

FIGURE 2 MicroRNA-320c (miR-320c) target mitogen-activated protein kinase 1 (MAPK1) 3'-untranslated regions (UTR) in Human Chondrocyte-articular (HC-a) cells. (A) Binding sites between MAPK1 3′-UTR and miR-320c; (B) relative luciferase activities of miR-320c, miR-320c inhibitor, MAPK1 3'-UTR wild type (Wt) and MAPK1 3'-UTR mutant (Mut). $^{*}P < .05$ compared with miRNA negative control (NC) group; $^{\#}P < .05$ compared with miR-320c + MAPK1 3'-UTR Wt group; $^{\&}P < .05$ compared with miR-320c inhibitor + MAPK1 3'-UTR Wt group

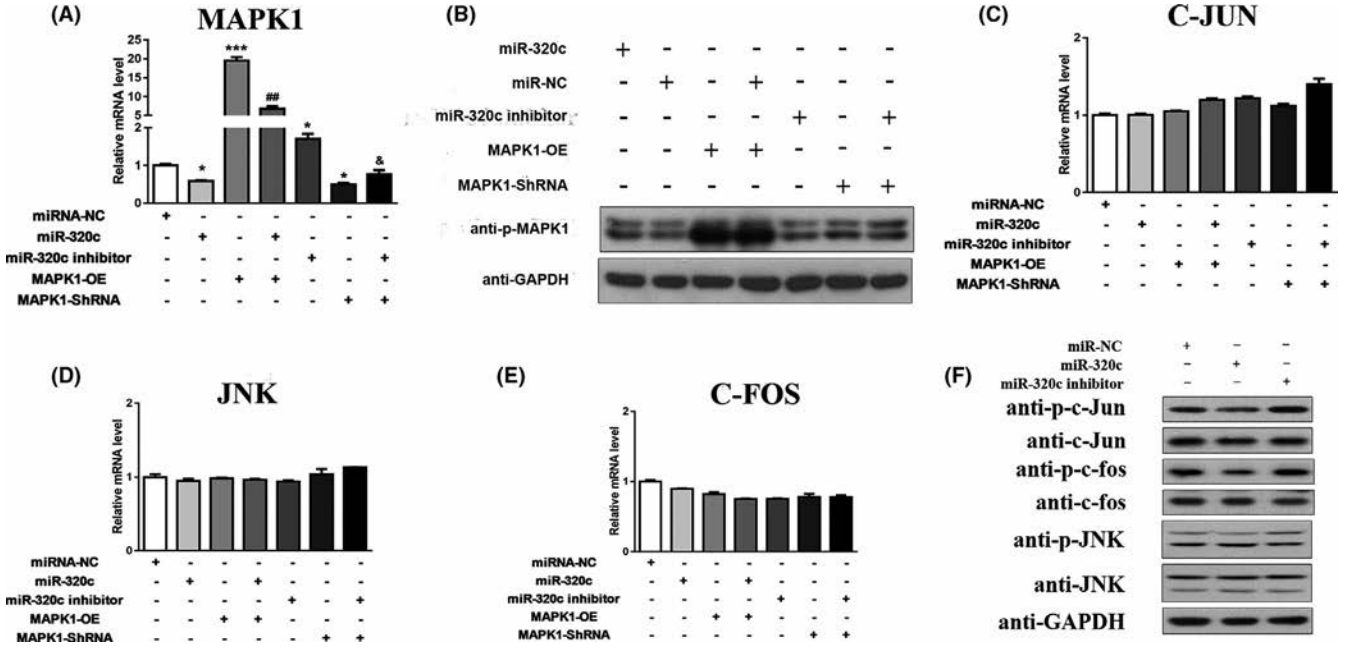

FIGURE 3 Interaction of microRNA-320c (miR-320c) and mitogen-activated protein kinase 1 (MAPK1) and the effects on activation of MAPK pathway in Human Chondrocyte-articular (HC-a) cells. (A) MAPK1 messenger RNA (mRNA) expression levels and (B) MAPK1 protein expression levels after transfection 48 h in HC-a cells. (C, D, E) The mRNA expression levels of c-JUN, c-FOS, JNK after transfection 48 h in HC-a cells. (F) The protein expression levels of JNK/p-JNK, c-FOS/p-c-FOS, c-JUN/p-c-JUN after transfection 48 h in HC-a cells. $^{*}P < .05$ compared with miRNA negative control (NC) group; $^{***}P < .001$ compared with miRNA negative control (NC) group; $^{\#}P < .05$ compared with miR-320c group; $^{\&}P < .05$ compared with miRNA-320c inhibitor group

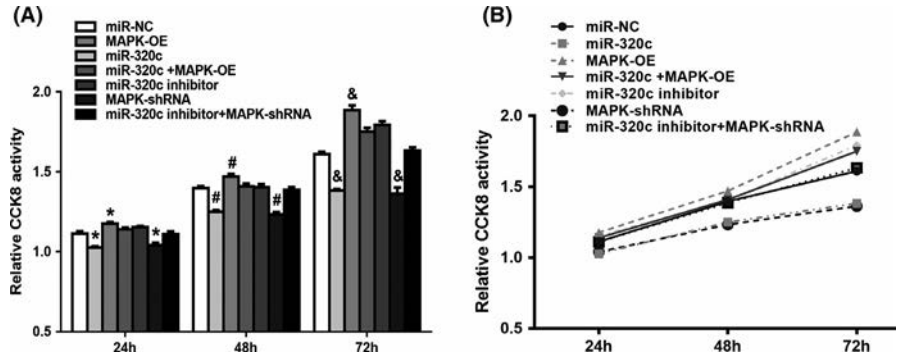

FIGURE 4 Influence of microRNA-320c (miR-320c) and mitogen-activated protein kinase 1 (MAPK1) short hairpin RNA (shRNA) on cell proliferation of Human Chondrocyte-articular (HC-a) cells. (A) The relative Cell Counting Kit-8 (CCK-8) activities of HC-a cells in each group are shown at different time points after transfection. (B) The CCK-8 activities curve of HC-a cells in each group. $^{*}P < .05$ compared with miRNA negative control (miR-NC) group; $^{\#}P < .05$ compared with miR-320c group; $^{\&}P < .05$ compared with miRNA-320c inhibitor group

The data identified MAPK1 as a direct target gene of miR-320c, and MAPK1 expression was negatively regulated by miR-320c by binding to MAPK1 3′-UTR in HC-a cells. Functional studies indicated that miR-320c could suppress proliferation and promote apoptosis of HC-a cells through targeting MAPK1.

Chondrocytes are the dominant cells in the articular cartilage and play a major role in OA development.[17,18] The proliferation and apoptosis of chondrocytes are in a dynamic balance, maintaining the number and function of articular chondrocytes.[19,20] miRNAs have been reported that can modulate plenty of physiological and pathological processes.[21] They can promote chondrocytes apoptosis and inhibit cell proliferation by regulating its downstream target molecule.[22,23] So, investigating functions of miRNAs may provide unique insights into the molecular mechanisms of diseases. Previous studies have demonstrated that miRNAs were changed significantly in OA and involved in regulating chondrocytes function.[24,25]

We have reported that plasma miR-320c expression level was upregulated in patients with OA.[13] Till now, studies regarding miR-320 were focused on cancers. It was either upregulated or downregulated in different cancers and was associated with cell proliferation, apoptosis in various pathological processes.[26-30] Little research has addressed the relation between miR-320 and OA pathogenesis. Two studies displayed miR-320/320c was decreased in OA

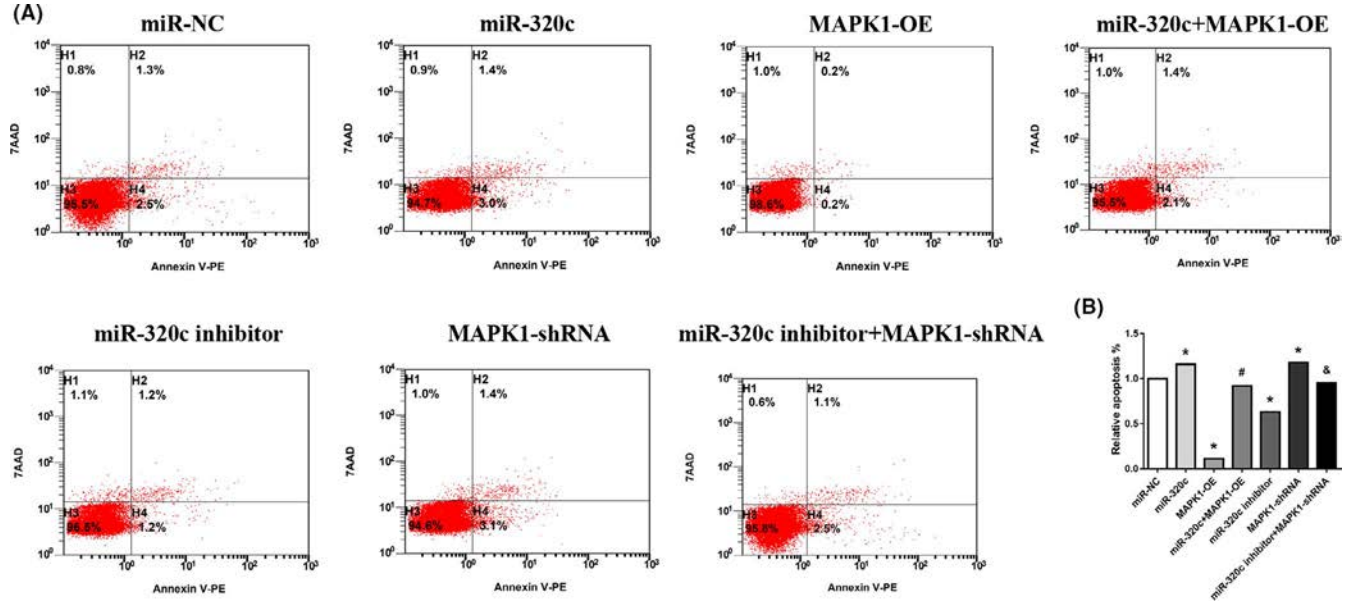

FIGURE 5 Influence of microRNA-320c (miR-320c) and mitogen-activated protein kinase 1 (MAPK1) short hairpin RNA (shRNA) on apoptosis. (A) Human Chondrocyte-articular (HC-a) cell apoptosis rates in each group by flow cytometry. (B) Analysis of cell apoptosis rates of HC-a cells in each group. $^{*}P < .05$ compared with miRNA negative control (miR-NC) group; $^{\#}P < .05$ compared with miR-320c group; $^{\&}P < .05$ compared with miRNA-320c inhibitor group

chondrocytes.[31,32] The other research found miR-320 expression was elevated in chondrogenic and hypertrophic ATDC5.[33] Hence, the roles of miR-320c involved in OA are still unclear and need more research. Our current study proved miR-320c played an important role in chondrocytes proliferation and cell apoptosis, maintaining the dynamic balance of chondrocytes.

miRNAs regulate numerous genes by binding to a complementary sequence in 3′-UTR of the target genes.[34,35] They can inhibit gene transcription or induce gene degradation[36] and exert different roles in diseases. Previous research has found miR-320 can target multiple genes, such as *SP1*,[26] *PBX3*,[27] *ELF3*,[28] *E2F1*[29] and *AKIP1*.[30] Although bioinformatic analysis revealed that MAPK1 contains a complementary site for miR-320c, no study has focused on the interaction between MAPK1 and miR-320c. Our results confirmed MAPK1 as a target of miR-320c. The upregulation of miR-320c decreased MAPK1 3'-UTR luciferase activity and the inhibitory effect was abolished by MAPK1 3'-UTR Mut, validating that the MAPK1 3'-UTR contains a direct binding site for miR-320c. Moreover, miR-320c overexpression significantly weakens the transcriptional activity of MAPK1 in HC-a cells. All these results suggest miR-320c can negatively regulate MAPK1 expression by directly targeting the 3'-UTR of MAPK1 in HC-a cells. Interestingly, we found that MAPK1 stayed unchanged despite the dramatic change in phosphorylation status of MAPK1 in the miR-320c and miR-320c inhibitor groups. It has been known that the activation of MAPK requires its phosphorylation. Upon activation, phosphorylated MAPK1/ERK2 translocates to the nucleus of the stimulated cells, where it phosphorylates nuclear targets. We speculated that the unchanged MAPK1 protein level might be affected by other regulatory mechanisms. The specific reasons need to be further explored.

Additionally, MAPK pathway activation was also be assessed in this experiment. MAPK kinases have proven to be involved in numerous biochemical signals[37,38] and cell proliferation.[39] MAPK1 could be targeted by various miRNAs (miR-127-5p,[40] miR-140-3p,[41] miR-143,[42] miR-342,[43] miR-378,[44] miR-585,[45] et al) and manipulate cell biological function through regulating the downstream signaling pathway.[46] Hence, we investigated whether miR320c targets MAPK1 through activating the MAPK pathway. Nevertheless, the results in this study showed that neither miR-320c nor MAPK1 shRNA regulate the expression levels of c-JUN/p-c-JUN, JNK/p-JNK and c-FOS/p-c-FOS in HC-a cells. The findings suggested that the interaction of miR-320c and MAPK1 may not activate the MAPK signaling pathway.

Since the function and mechanism of miR-320c in OA remain undefined, cell proliferation and apoptosis were performed to further investigate the biological function of miR-320c and MAPK1 in HC-a cells. Our data identified that miR-320c overexpression inhibited the proliferation and induced apoptosis of HC-a cells, whereas MAPK1 overexpression resulted in the opposite phenomenon. The proliferation rate in the miR-320c inhibitor group was improved dramatically and the apoptosis was suppressed, suggesting that miR-320c may act as a suppressor in the progression of OA. Furthermore, MAPK1 shRNA displayed similar functions to those induced by miR-320c, which suppress cell proliferation and promote apoptosis. Besides that, MAPK1 shRNA could attenuate miR-320c inhibitor promotive effects on HC-a cell proliferation and reverse its inhibitory effect on cell apoptosis. On the basis of these results, we considered that MAPK1 is a direct functional mediator of miR-320c in HC-a cells, and the functions of miR-320c may be mediated by MAPK1.

However, there are still some limitations in our study. The present study only investigated the relationship among miRNA-320c, MAPK1 and the MAPK signaling pathway. We could detect more signaling pathways to exploring the mechanism of miRNA-320c, and the possibility of therapeutically targeting miRNA-320c in OA should also be investigated in the further study.

5 | CONCLUSION

In conclusion, our study demonstrated that miR-320c negatively regulated MAPK1 expression directly binding with its 3′-UTR in HC-a cells. Furthermore, overexpression of miR-320c could suppress cell proliferation and induce apoptosis of chondrocytes via suppression of MAPK1. These results revealed the suppressive role of miR-320c in OA, making miR-320c a novel cellular therapeutic target for the treatment of patients with OA. It not only lays foundation for in-depth study on the molecular mechanism of miR-320c, but also raises the prospect of new treatments for OA in the future.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interests.

AUTHOR CONTRIBUTIONS

Like Zhao and Rongwei Zhou contributed to study design, data analysis and interpretation; Rongwei Zhou, Qian Wang and Yongjing Cheng carried out the experiment. Ming Gao and Cibo Huang conceived of the study, coordinated the study, and analyzed data. All authors were involved in writing the paper and gave final approval of the submitted and published versions.

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*HLA-B*27:04* associated with enthesitis and younger age of onset, and HLA-B allele profile in patients with ankylosing spondylitis in Thailand: A cross-sectional study

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Abstract

Aim: The aims of this study were to estimate human leukocyte antigen (*HLA)-B* allele frequency, to identify alleles associated with ankylosing spondylitis (AS), and to explore manifestations in various *HLA-B*27* in Thai AS patients.

Methods: This was a cross-sectional study. Thai patients older than 18 years with diagnosed AS according to modified New York criteria who visited Siriraj Hospital (Bangkok, Thailand) were consecutively enrolled. *HLA-B* alleles were determined by reverse sequence-specific oligonucleotide assays, and were assigned at a 4-digit resolution. *HLA-B* alleles of 334 unrelated healthy Thai donors who participated in a previous phase 2b dengue vaccine clinical trial were included as controls. Odds ratio (OR) and Fisher's exact test were used to estimate association between allele and AS. The *P* value significance threshold was calculated according to Bonferroni.

Results: Among the 88 patients who were recruited, 34 *HLA-B* alleles were identified, and all patients were heterozygous. The prevalence of *HLA-B*27* was 89.8%, and 4 alleles of *HLA-B*27* were identified. *HLA-B*27:04* (OR: 39.4, $P < .0001$) and *HLA-B*27:05* (OR: 13.8, $P = .0011$) were associated with AS. In contrast, *HLA-B*27:06* was not found to be associated with AS (OR: 0.4, $P = .241$). AS patients carrying *HLA-B*27:04* were more likely to have enthesitis and younger age at onset than those carrying *HLA-B*27:05*.

Conclusions: *HLA-B*27:04* and *HLA-B*27:05* were both found to be strongly associated with Thai AS. *HLA-B*27:06* showed a neutral allele for Thai AS. AS patients with *HLA-B*27:04* had more enthesitis and younger age at onset than those with *HLA-B*27:05*.

KEYWORDS

ankylosing spondylitis, HLA-B subtype, *HLA-B*27:04*, HLA-B27, Thai

1 | INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory disease. The majority of patients have inflammatory back pain, and other common manifestations include enthesitis, arthritis, and uveitis. Onset of symptoms occur most often in people aged less than 40 years, and diagnosis is made based on modified New York criteria.[1] AS is highly heritable.[2] *Human Leukocyte Antigen-B*27 (HLA-B*27)* is a gene that is well-known to be associated with AS; however, not all AS patients carry *HLA-B*27*, and not all individuals who carry *HLA-B*27* have AS. The *HLA-B*27* gene is a highly polymorphic gene. At least 213 alleles of *HLA-B*27* are known.[3] However, only some of those HLA-B*27 subtypes are associated with AS, and the prevalence of HLA-B*27 subtypes and their association with AS varies among ethnicities. The common alleles are *HLA-B*27:05* in Western populations, *HLA-B*27:02* in Mediterranean populations, and *HLA-B*27:04* in Chinese and other Asian populations.[3] In addition, AS patients with various HLA-B*27 subtypes may present with different manifestations.[4] *HLA-B*27:04* was shown to associated with peripheral arthritis.[4] Other genes may also associate with AS. Other alleles in major histocompatibility complex (MHC) class I (*HLA-B*40* and *HLA-C*15*) are associated with AS,[5] and an allele in non-MHC (ERAP 1) was reported to be associated with HLA-B27-positive AS in both Europeans and East Asians.[5]

In Thailand, the prevalence of positive HLA-B27 in AS patients was 91%,[6] and the prevalence in healthy populations was estimated to be 4%.[6,7] Lopez-Larrea et al. studied 45 Thai AS patients with positive HLA-B27 who were diagnosed as having AS according to the European Spondyloarthropathy Study Group (ESSG), and the *HLA-B*27:04*, *-B*27:05*, and *-B*27:07* subtypes were identified in those patients.[8] Patients who meet the ESSG criteria should be diagnosed as having spondyloarthritis, and those patients may also fulfill modified New York criteria for AS if a patient has radiography of at least bilateral grade 2 or unilateral grade 3 sacroiliitis, and at least 1 associated clinical criteria.[1] The genetic background may differ among spondyloarthritic diseases.[9] There is no study of *HLA-B** allele profile in Thai AS patients who may be negative or positive HLA-B27. Our study aimed to estimate *HLA-B* allele frequency, to identify alleles associated with AS, and to explore manifestations in various *HLA-B*27* alleles in Thai AS patients.

2 | METHODS

2.1 | Patients

This cross-sectional study enrolled Thai patients from the outpatient clinics of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand from 15 August 2018 to 27 December 2019. Siriraj Hospital is a 2300-bed national tertiary center, and Thailand's largest teaching hospital.

All included patients were aged older than 18 years, had pelvic and spinal radiographs, and were evaluated and diagnosed with AS by 1 rheumatologist (PC) according to modified New York criteria.[1] Patients unwilling to undergo genetic study and related patients were excluded. Written informed consent was obtained from all participants. The protocol for this study was approved by the Siriraj Institutional Review Board (SIRB) (COA no. Si 247/2012), and complied with the 1964 Declaration of Helsinki and all of its later amendments.

2.2 | HLA-B genotyping

Genomic DNA was extracted from the buffy coat obtained from citrate-phosphate-dextrose blood using the guanidine-HCl extraction method.[10] The purity of the genomic DNA for each sample was determined by measuring the absorbance at 260 and 280 nm. If the A260/A280 values were within the range of 1.7-1.9, the concentration of the DNA was adjusted to 20 ng/μL. The DNA samples were determined for *HLA-B* alleles by reverse sequence-specific oligonucleotide assay (rSSO; LABType™ XR Class I B Locus Typing Kit, One Lambda Inc, Canoga Park, CA, USA) according to the manufacturer's instructions, and using a LABScan3D™ (Luminex FLEXMAP 3D) flow analyzer for data acquisition.[11] The rSSO method was tested in 100% concordance with other methods (polymerase chain reaction–sequence-specific amplification and next-generation sequencing) in an annual HLA proficiency testing program in Thailand. The HLA alleles were assigned at a 4-digit resolution using HLA Fusion version 4.2 software.

2.3 | Data collection

Characteristics, including age, gender, ethnicity, and spondyloarthritis features according to the Assessment of Spondyloarthritis International Society criteria (inflammatory back pain, arthritis, dactylitis, enthesitis, anterior uveitis, inflammatory bowel disease, psoriasis, good response to non-steroidal anti-inflammatory drugs [NSAIDs], family history),[12] were collected during a face-to-face interview and from medical records. Presence/absence and age at onset of spondyloarthritis features were also collected.

2.4 | Statistical analysis

Using the previously reported ~80% prevalence of *HLA-B*27:04* in AS patients with positive HLA-B27[13] and a 5% error, a sample of 62 AS patients with positive HLA-B27 was calculated. Since 80% of AS patients had positive HLA-B27 in Siriraj Hospital (article in preparation), 88 AS patients were needed. Regarding our analysis for association between *HLA-B*27* alleles and AS, data from full-length sequencing of HLA class I in a previously studied healthy Thai cohort was used as control in the present study.[14] The control group consisted of 334 unrelated healthy donors from the Muang District of Ratchaburi Province, Thailand who had previously participated in a phase 2b dengue vaccine clinical trial.[14]

To estimate association between allele and AS magnitude, the odds ratio (OR) and its 95% confidence interval (95% CI) were calculated for each allele. Test for association was performed for each allele using Fisher's exact test. Due to multiple testing of 34 alleles, the type I error was set at 0.0015 according to Bonferroni's correction.

Comparison of continuous variables was determined by Student's *t* test or Mann-Whitney *U* test according to the pattern of data distribution. Pearson's χ^2 test or Fisher's exact test was used to compare categorical data, as appropriate. A $P < .05$ was considered significant. Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

3 | RESULTS

Among 114 AS patients, 94 patients provided signed consent for further genetic blood testing. Of those, 88 unrelated AS patients were consecutively included. The mean (standard deviation, SD) age of patients was 42.0 (12.1) years, and 57 (64.7%) patients were male. Most patients live in central Thailand (73.9%), followed by western (10.2%), eastern (6.8%), southern (5.7%), and northeastern (3.3%) Thailand. No patients had psoriasis, and the characteristics of patients are shown in Table 1.

There were 34 *HLA-B* alleles identified in this study, and all patients were heterozygous. The most common alleles were *HLA-B*27* (89.8%) (Table 2). Four alleles of *HLA-B*27* (*HLA-B*27:04*, *-B*27:05*, *-B*27:06*, and *-B*27:15*) were observed in AS patients, while only 3 alleles (*HLA-B*27:04*, *-B*27:05*, *-B*27:06*) were reported in the control group.[14] A higher allele frequency of *HLA-B*27:04* was found in AS patients compared to controls (39.8% vs 1.6%; OR: 39.4, $P < .0001$). The allele frequency of *HLA-B*27:05* was quite low in AS patients; however, it was significantly higher in study patients than in controls (4.0% vs 0.3%; OR: 13.8, $P = .0011$). Conversely, the frequency of the *HLA-B*27:06* allele (1.1%) was lower in AS patients than in controls (2.7%); however, the association did not reach statistical significance (OR: 0.4, $P = .241$). Only one *HLA-B*27:15* allele was observed in AS patients, while none were observed in the control group. Considering only cases and controls carrying *HLA-B*27*, *HLA-B*27:04* was positively and *HLA-B*27:06* was negatively associated with AS when compared to the healthy controls from the dengue vaccine clinical trials[14] and the donors[15] (Table 3). The frequencies of *HLA-B*44:03* (1.7% vs 6.0%) and *HLA-B*46:01* (5.7% vs 11.2%) were lower in AS patients than in controls ($P = .02$ and $P = .025$, respectively).

TABLE 1 Characteristics of patients with ankylosing spondylitis

Characteristics	All (N = 88)	*HLA-B*27:04* (n = 70)	*HLA-B*27:05* (n = 7)	Negative HLA-B27 (n = 9)
Age, mean (SD), y	42.0 (12.1)	42.2 (12.7)	39.8 (6.9)	40.7 (11.4)
Male, n (%)	57 (64.7)	48 (68.6)	4 (57.1)	4(44.4)
Age at first symptom, mean (SD), y	28.8 (11.9)	27.3 (12.2)	32.4 (4.0)	35.7 (10.3)
First axial symptoms, n (%)	69 (78.4)	53 (75.7)	5 (71.4)	9 (100.0)
Disease duration, median (IQR), y	11.7 (4.6-19.7)	14.4 (5.7-20.7)	4.4 (2.4-10.1)	2.6 (1.9, 9.9)
Inflammatory back pain, n (%)	75 (85.2)	60 (85.7)	4 (57.1)	9 (100.0)
Arthritis, n (%)	66 (75.0)	53 (75.7)	5 (71.4)	6 (66.7)
Enthesitis, n (%)	50 (56.8)	42 (60.0)	1 (14.3)	6 (66.7)
Uveitis, n (%)	25 (28.4)	23 (32.9)	2 (28.6)	0 (0.0)
Dactylitis, n (%)	10 (11.4)	9 (12.9)	1 (14.3)	0 (0.0)
Inflammatory bowel disease, n (%)	1 (1.1)	1 (1.4)	0 (0.0)	0 (0.0)
Good response to NSAIDs, n (%)	49 (55.7)	38 (54.3)	4 (57.1)	5 (55.6)
Family history, n (%)	28 (31.8)	25 (35.7)	0 (0.0)	2 (22.2)

Abbreviations: Family history, presence in first-degree or second-degree relatives of any of the following: ankylosing spondylitis, psoriasis, uveitis, reactive arthritis, inflammatory bowel disease; First axial symptom, the first presentation with inflammatory back pain and/or buttock pain; Age at first symptom, age at onset of the first musculoskeletal symptoms; IQR, interquartile range; NSAIDs, non-steroidal anti-inflammatory drugs; SD, standard deviation.

TABLE 2 *HLA-B** allele distribution in ankylosing spondylitis patients and controls[#]

	Ankylosing spondylitis (N = 176 alleles)		Controls (N = 668 alleles)				
*HLA-B** allele	Number	Frequency	Number	Frequency	OR	95% CI	*P*
B*08:01	1	0.006	1	0.001	3.8	0.2-61.2	.345
B*13:01	5	0.028	39	0.058	0.5	0.2-1.2	.120
B*13:02	2	0.011	8	0.012	1.0	0.2-4.5	.947
B*15:02	9	0.051	61	0.091	0.5	0.3-1.1	.090
B*15:11	2	0.011	4	0.006	1.9	0.4-10.5	.458
B*15:12	1	0.006	1	0.001	3.8	0.2-61.2	.345
B*15:13	1	0.006	9	0.013	0.4	0.1-3.3	.410
B*15:21	1	0.006	4	0.006	0.9	0.1-8.4	1
B*15:25	7	0.040	16	0.024	1.8	0.7-4.4	.206
B*18:01	2	0.011	20	0.030	0.4	0.1-1.6	.186
B*18:02	2	0.011	17	0.025	0.4	0.1-1.9	.275
B*27:04	70	0.398	11	0.016	39.4	20.2-76.9	***<.0001***
B*27:05	7	0.040	2	0.003	13.8	2.8-67.0	***.0011***
B*27:06	2	0.011	18	0.027	0.4	0.1-1.8	.241
B*27:15	1	0.006	0	0.000	11.4	0.5-281.7	.136
B*35:01	1	0.006	10	0.015	0.4	0.05-3.1	.368
B*35:03	1	0.006	11	0.016	0.4	0.05-2.7	.319
B*35:05	2	0.011	14	0.021	0.5	0.1-2.4	.414
B*38:02	2	0.011	14	0.021	0.5	0.1-2.4	.414
B*39:01	1	0.006	8	0.012	0.5	0.1-3.8	.480
B*39:09	3	0.017	11	0.016	1.0	0.3-3.8	.957
B*39:15	1	0.006	2	0.003	1.9	0.2-21.1	.600
B*40:01	12	0.068	57	0.085	0.8	0.4-1.5	.461
B*40:06	3	0.017	9	0.013	1.3	0.3-4.7	.722
B*44:03	3	0.017	40	0.060	0.3	0.1-0.9	.031
B*46:01	10	0.057	75	0.112	0.5	0.2-0.9	.033
B*51:01	3	0.017	34	0.051	0.3	0.1-1.1	.064
B*51:02	2	0.011	10	0.015	0.8	0.2-3.5	.72
B*52:01	2	0.011	17	0.025	0.4	0.1-1.9	.275
B*55:02	2	0.011	12	0.018	0.6	0.1-2.8	.545
B*56:01	2	0.011	4	0.006	1.9	0.3-10.5	.458
B*57:01	1	0.006	9	0.013	0.4	0.1-3.3	.410
B*58:01	11	0.063	42	0.063	1.0	0.5-2.0	.986
B*67:01	1	0.006	0	0.000	11.4	0.5-281.7	.136

Note: The 78 alleles in #controls were not shown in the table, as follows: 9 *HLA-B*07:06*; 6 *HLA-B*07:02*; 3 *HLA-B*15:01*; 5 *HLA-B*15:17*; 3 *HLA-B*15:27*; 7 *HLA-B*15:32*; 4 *HLA-B*15:35; 4 HLA-B*15:450*; 2 *HLA-B*35:02*; 3 *HLA-B*37:01*; 2 *HLA-B*38:01*; 6 *HLA-B*40:02*; 5 *HLA-B*44:02*; 2 *HLA-B*48:01*; 4 *HLA-B*54:01*; 5 *HLA-B*56:04*; and, 1 each for *HLA-B*15:04*, *-B*15:08*, *-B*15:18*, *-B*48:04*, *-B*51:07*, *-B*52:04*, *-B*55:01*, and *-B*73:01* because they are not found in the ankylosing spondylitis patients. There were no statistically significant differences when comparing with the ankylosing spondylitis patients ($P > .05$).

Abbreviations: CI, confidence interval; NA, not applicable; OR, odds ratio.

[#]Controls were unrelated healthy donors who participated in a phase 2b dengue vaccine clinical trial in Thailand.[14]

$P < 0.0015$ was considered significant according to Bonferroni's correction.

The 20 total alleles identified in patients with negative HLA-B27 are described, as follows: 3 (16.6%) *HLA-B*40:01*; 3 (16.6%) *HLA-B*15:25*; 2 (11.1%) *HLA-B*13:01*; and 1 each (5.6%) for *HLA-B*13:02*, *B*15:02*, *-B*39:09*, *-B*39:15*, *-B*44:03*, *-B*46:01*, *-B*51:02*, *-B*52:01*, *-B*55:02*, and *-B*58:01*.

The 78 alleles (9 *HLA-B*07:06*; 6 *HLA-B*07:02*; 3 *HLA-B*15:01*; 5 *HLA-B*15:17*; 3 *HLA-B*15:27*; 7 *HLA-B*15:32*; 4 *HLA-B*15:35*; 4 *HLA-B*15:450*; 2 *HLA-B*35:02*; 3 *HLA-B*37:01*; 2 *HLA-B*38:01*; 6 *HLA-B*40:02*; 5 *HLA-B*44:02*; 2 *HLA-B*48:01*; 4 *HLA-B*54:01*; 5 *HLA-B*56:04*; and, one each for *HLA-B*15:04*, *-B*15:08*, *-B*15:18*,

TABLE 3 Relative predispositional effects analysis of *HLA-B*27* subtypes in Thai ankylosing spondylitis patients and controls from published studies

	Number (%)				OR (95% CI)		
*HLA-B** allele	AS (N = 79)	Control1 (N = 31)	Control2 NET (N = 63)	Control2 NT (N = 24)	AS vs Control1	AS vs Control2 NET	AS vs Control2 NT
B*27:04	70 (88.6)	11 (35.5)	14 (22.2)	7 (29.2)	14.1 (5.1-38.9)*	27.2 (10.9-67.9)*	18.9(6.2-57.8)*
B*27:05	7 (8.9)	2 (6.5)	2 (3.2)	1 (4.2)	1.4 (0.3-7.2)	3.0 (0.6-14.8)	2.2(0.3-19.1)
B*27:06	2 (2.5)	18 (58.1)	46 (73.0)	14 (58.3)	0.02 (0.004-0.09)*	0.01 (0.002-0.04)*	0.02(0.004-0.09)*
B*27:07	0	0	1 (1.6)	2 (8.3)	NA	NA	NA
B*27:15	1 (1.3)	0	0	0	NA	NA	NA

Note: Control1 were unrelated healthy donors who participated in a phase 2b dengue vaccine clinical trial in Thailand and were assumed to carry heterozygous *HLA-B*27*.[14]

Control2 were unrelated healthy donors who participated in a validation of high-resolution polymerase chain reaction–sequence-specific amplification technique to define *HLA-B*27* subtypes in Thailand.[15]

Results reported as number and percentage of persons carrying the alleles.

Abbreviations: AS, ankylosing spondylitis patients; CI, confidence interval; N, number of persons; NA, not applicable; NET, northeastern Thailand; NT, northern Thailand; OR, odds ratio.

*P value < .0001.

*-B*48:04, -B*51:07, -B*52:04, -B*55:01, and -B*73:01)* were observed only in controls.[14] There was no statistically significant difference when compared to AS patients ($P > .05$; data not shown).

3.1 | Characteristic of patients with various HLA-B*27 subtypes

AS patients carrying *HLA-B*27:04* were significantly more likely to have younger age at onset of their first presenting musculoskeletal symptoms (mean age: 27.3 ± 12.2 years) compared with those carrying *HLA-B*27:05* (mean age: 32.4 ± 4.0 years) ($P = .023$) (Table 1). Patients carrying *HLA-B*27:05* were significantly less likely to have enthesitis (1/7, 14.3%) than those carrying *HLA-B*27:04* (42/70, 60.0%) ($P = .040$).

Two females carried *HLA-B*27:06*. One carried *HLA-B*27:04/HLA-B*27:06* with the first presentation of severe inflammatory back pain at 21 years of age, and having peripheral arthritis and history of her father's diagnosis of AS. The other women carried *HLA-B*27:06/HLA-B*44:03* with the first presentation of inflammatory back pain at 44.9 years of age, and having peripheral arthritis with no family history of spondyloarthritis.

Regarding extra-articular manifestation, uveitis was most commonly observed (25 patients, 28.4%). All of those patients were positive *HLA-B*27*. No patients with negative HLA-B27 had uveitis. One patient with inflammatory bowel disease carried *HLA-B*27:04*. The proportions of male gender, first presentation with axial symptoms, inflammatory back pain, history of arthritis, dactylitis, good response to NSAIDs, and family history of spondyloarthritis were comparable between *HLA-B*27:04* and *HLA-B*27:05*.

4 | DISCUSSION

Among the *HLA-B* alleles identified in this study, *HLA-B*27:04* (79.5%) was the most commonly observed allele in Thai AS patients, and it was found to be significantly associated with AS when compared with healthy controls[14] (OR: 39.4). This finding is consistent with that from a meta-analysis study in AS patients (relative risk 1.14),[4] and from a previous study in Thai spondyloarthritis patients.[16] *HLA-B*27:04* is the most common allele in east Asian populations,[17] and is the most common allele associated with AS in East Asia, except Korea.[4] *HLA-B*27:05* is the most widely distributed *HLA-B*27* allele, with a reported presence in nearly all populations of the world.[3,17]

*HLA-B*27:05* was the second most common allele of *HLA-B*27* in Thai AS patients (8.0%) in this study, and it was also associated with AS when compared with controls.[14] This association was not reported in a previous study of 45 Thai spondyloarthritis patients.[16] This difference in findings may be due to the small sample size in that study, the low prevalence of that allele (allele frequency of 0.3%) in a Thai population,[14] or the possibility that genetic association with AS may be different from genetic association with spondyloarthritis. A previous meta-analysis that included a majority of AS patients from Asia showed *HLA-B*27:05* to have slightly increased susceptibility to AS (relative risk 1.5) with high heterogeneity.[4] Considering only cases and controls carrying *HLA-B*27*, Liu et al. reported[18] *HLA-B*27:05* was less strongly associated with AS than *HLA-B*27:04*. Our study showed *HLA-B*27:05* was not associated with AS while *HLA-B*27:04* was associated with AS when considering only those carrying *HLA-B*27*.

A relatively high prevalence of *HLA-B*27:06* in southeast Asian populations (Thai, Indonesian, and Malaysian) as compared to other regions was reported.[19] *HLA-B*27:06* is a common allele of

*HLA-B*27* in Thai populations, as follows: 58% in both the central[14] and northern regions,[15] and 73% in the northeastern region.[15] *HLA-B*27:06* showed low frequency in AS patients, and it demonstrated marginally negative association with AS in the present study compared to controls.[14] *HLA-B*27:06* was reported to be a protective allele against spondyloarthritis in 1995.[16] However, a different study[20] found *HLA-B*27:06* not to be protective. Alternatively, it should be a disease neutral subtype[3] if it has co-inherited other disease-predisposing genes, which is consistent with our study since 2 AS patients were found to carry heterozygous *HLA-B*27:06*.

The association of *HLA-B*27:15* and AS was unclear in this study since only 1 AS patient carried this allele, while it was not observed in the healthy controls.[14] This finding was consistent with the previous reports from Asian cohorts.[13,21] *HLA-B*27:07* was an uncommon allele associated with AS.[22] *HLA-B*27:07* was identified in 2 Thai spondyloarthritis patients;[16] however, it was not observed in AS patients in this study.

Regarding alleles other than *HLA-B*27*, a previous study in 1637 east Asian AS patients[5] reported that *HLA-B*40* was associated with AS at a suggestive level (OR: 1.65, $P = 2.54 \times 10^{-4}$) after controlling for *HLA-B*27*. *HLA-B*40* was a quite common allele in both Thai AS and the healthy,[14] and it did not show association with AS. Moreover, *HLA-B*38*,[23] *-B*39*,[23] *-B*52*,[23] and *HLA-B*60*[24] were positively associated with AS in previous reports. Our study did not show these associations may be due to the small sample size and it could not further be analyzed for controlling the effect of *HLA-B*27*.

Conversely, *HLA-B*44:03* and *-B*46:01* both demonstrated marginal negative association with AS in our study. *HLA-B*46*[21] and *HLA-B*07:02*[23] were reported to be protective alleles; however, we did not observe this finding in our study. The sample size of the current study may be too small to detect all negative associations with AS, so a future larger study is needed to identify these associations.

Regarding disease characteristics, the patients who carried *HLA-B*27:04* were more likely to have a younger age at onset than those carrying *HLA-B*27:05*. This is consistent with the findings of a previous study in Chinese AS.[25] A previous meta-analysis reported association between *HLA-B*27:04* and peripheral joint involvement, and association between *HLA-B*27:05* and uveitis;[4] however, neither of these associations were found in our study. This may be due to the fact that most of our AS patients carried *HLA-B*27:04*. Moreover, a large proportion of our AS patients had arthritis, which is consistent with a previous report from Thailand.[26] Manifestations may differ among ethnicities and genetics (both MHC and non-MHC). No AS patients with negative HLA-B27 had uveitis, which is consistent with a report by Lin et al.[4]

This study has some limitations. First, our study had a cross-sectional design, which means that data were collected at 1 point in time. However, it is possible that manifestation of disease could emerge later in the course of the disease. Longitudinal study is, therefore, needed. Second, the included study patients came from a single tertiary care hospital where the patients might be more severe than a primary hospital. Third, the high proportion of *HLA-B*27* AS patients could make other alleles appear under-represented in AS patients. Finally, the participants' ethnicities were self-reported. The potential strength in this study is that clinical data were collected by reviewing medical records and from a face-to-face interview with patients.

5 | CONCLUSION

This study profiles the *HLA-B* alleles in Thai AS patients. *HLA-B*27:04* and *HLA-B*27:05* were the first and the second most prevalent susceptibility alleles in our Thai AS population. Heterozygous *HLA-B*27:06* can also be observed in Thai AS patients. AS patients carrying *HLA-B*27:04* were more likely to have their first musculoskeletal manifestation at younger age at onset and to have enthesitis than those carrying *HLA-B*27:05*. However, a larger sample study is needed to identify low-frequency alleles that are associated AS, and a long-term study is needed to characterize the manifestations in various HLA-B* subtypes.

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CONFLICTS OF INTEREST

PC received a research grant from the Siriraj Research Fund, Faculty of Medicine Siriraj Hospital, Mahidol University, and from Pfizer (Thailand), Ltd. (investigator-initiated research). MP, KL, and PP have no conflicts of interest.

AUTHOR CONTRIBUTIONS

PC contributed to conception of the work, data collection, data analysis, data interpretation, and drafting of the manuscript. MP, KL, and PP contributed to conception of the work, data interpretation, and drafting of the manuscript. All the authors read and approved the final manuscript.

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ORIGINAL ARTICLE

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Fatigue in psoriatic arthritis: Is it related to disease activity?

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Abstract

Aim: Fatigue is commonly associated with psoriatic arthritis (PsA). However, information about its prevalence and associated factors is sparse. The primary objective here was to find the prevalence and magnitude of PsA fatigue. The secondary objective was to explore its associated risk factors, particularly emphasis on the effect of disease activity control.

Methods: PsA patients who fulfilled Classification Criteria For Psoriatic Arthritis were consecutively recruited from local rheumatology clinics. Fatigue was assessed by a 13-item self-administered questionnaire (Functional Assessment of Chronic Illness Therapy – Fatigue [FACIT-F]) (0-52). Data collected and analyzed included: demographic data, disease activity data, comorbidities and medications use.

Results: There were 231 eligible PsA patients recruited. The mean FACIT-F score was 37.5 ± 9.1. Severe fatigue, defined as FACIT-F score < 30, was found in 49 (22.1%) of them. The univariate model identified these associated factors of fatigue: tender and swollen joint count, dactylitis count, Psoriasis Area and Severity Index (PASI) score, pain and general health perception, Disease Activity in Psoriatic Arthritis (DAPSA) score, Health Assessment Questionnaire, the use of cyclosporine, sulphasalazine and biologic agents. The final regression model identified DAPSA and PASI were closely associated with severe fatigue ($P = .003$ and $P = .04$ respectively). No associations with fatigue were found between age, gender, disease duration, comorbidities and medication use. However, there were weak correlations between the magnitude of FACIT-F score, DAPSA and PASI with $r = -.3$ and $r = -.26$ respectively.

Conclusion: Severe fatigue was common in PsA patients, and its magnitude was closely correlated with DAPSA and PASI score, indicating its multifactorial nature. Achieving DAPSA and PASI remission could significantly alleviate the fatigue intensity to a certain extent. However, treatment for PsA-related fatigue should adopt a multidisciplinary approach in addition to disease activity control.

KEYWORDS

disease activity, fatigue, psoriatic arthritis, skin psoriasis

1 | INTRODUCTION

Fatigue is described as a feeling of "extreme weakness, substantial tiredness or persistent loss of vitality" and is one of the most frequent encountered complaints in patients with chronic rheumatic diseases.[1,2] Possible explanations are controversial, with some investigators suggesting it as an unpleasant component of active rheumatic disorders, while others explaining it as a solely personal perception.[2,3]

Psoriatic arthritis (PsA), which is characterized by arthritis and skin psoriasis, is one of the most prevalent rheumatic diseases in the world.[4] Fatigue is an important symptom in addition to joint pain and skin itchiness.[5] A sense of tiredness, loss of energy and exhaustion not only decrease quality of life, but also affect academic or work performance, daily activity, exercise level and social interaction.[2,3] Fatigue may even contribute to depression.[5]

Most PsA patients accepted that fatigue as a constant companion of psoriatic conditions, or as a side-effect of medication, that is conventional or biologic disease-modifying antirheumatic drugs (DMARDs).[2,3] Rheumatologists believe that fatigue results from chronic inflammation of the skin and joints via cytokine release.[2,3] However, fatigue may also develop from the psychological stress of embarrassing cutaneous and nail conditions, joint deformities and functional incapacities.[2,3,5]

Fatigue is difficult to manage as it often persists even after disease activity is well under control.[1,2] The exact underlying mechanism of fatigue is still poorly understood, and the relevant research is scarce, especially in Asia.[5] The majority of trials have been confined to Western populations and limited to skin psoriasis (PsO), regardless of arthritis and its comorbidities such as diabetes mellitus and ischemic heart disease.[2] Moreover, variations in ethnicity, culture and living style might contribute to the wide range of fatigue severity in psoriatic disease.[6-8] Thus, the primary aim of this study was to determine the magnitude and the prevalence of severe fatigue in Hong Kong Chinese PsA patients. The secondary aim was to explore its associated factors, with particular emphasis on the effect of PsA disease activity in the hopes of improving management.[9,10]

2 | METHODOLOGY

This was a multi-center, cross-sectional observational study. The protocol was reviewed and approved by the Clinical Research Ethics Committee in our locality. The primary objective was to find the magnitude and the prevalence of fatigue in Hong Kong Chinese patients with PsA. The secondary objective was to investigate the associated factors of fatigue, with particular emphasis on the effect of PsA disease activity, in terms of Disease Activity in Psoriatic Arthritis (DAPSA) and Psoriasis Area and Severity Index (PASI).[9,10]

Subjects were recruited consecutively from the rheumatology clinics of Tseung Kwan O Hospital (TKOH), United Christian Hospital (UCH) and Queen Mary Hospital (QMH) from January 2019 to March 2020. All patients fulfilled the CIASsification criteria for Psoriatic ARthritis (CASPAR), were aged 18 years or above, and could provide written consent. Patients excluded had: (a) ethnicity other than Chinese; (b) hemoglobin level less than 10; (c) history of depression or other psychiatric diseases; (d) history of carcinoma; and (e) with stage 5 kidney disease. Relevant information was retrieved from the local electronic Central Management System (CMS).

Demographic and clinical data included age, gender, body mass index (BMI) and disease duration. PsA disease activity parameters were measured: (a) tender (68) and swollen joint count (66); (b) dactylitis count; (c) Leeds Enthesitis Index (LEI); (d) PASI; (e) C-reactive protein (CRP); (f) erythrocyte sedimentation rate (ESR); (g) Health Assessment Questionnaire Disability Index (HAQ-DI); (h) pain score on a visual analog scale (VAS, 0-100 mm); and (i) general health (GH) score VAS, 0-100 mm.[4,5,9,11] Higher scores indicated more severe pain and worse general well-being. HAQ-DI assessed physical disability.[9,11]

Other data recorded were: comorbid conditions (eg, hypertension, ischemic heart disease and diabetes mellitus), and use of conventional DMARDs (eg, acitretin, methotrexate, sulphalsalazine, cyclosporine, and leflunomide), and biological agents (ie, anti-tumor necrosis factor [TNF] biologics and non-TNF biologics).[9] DAPSA score was calculated, as the sum of the following components: (a) tender and swollen joint count; (b) pain score/10; (c) GH score/10; and (d) CRP (mg/L)/10.[10] DAPSA values below or equal to 4 are classified as arthritis remission, while values between 4.01 and 14, between 14.01 and 28, and above 28, are classified as low, moderate and high activity, respectively.[10] PASI score below or equal to 1 is defined as skin remission.[12] Kidney function was expressed as estimated glomerular filtration rate (eGFR), using the Cockcroft-Gault formula.[13]

Fatigue was measured using the version 4 Traditional Chinese FACIT-F (Functional Assessment of Chronic Illness Therapy – Fatigue) questionnaire.[14,15] It comprises 13 items with 5-point scales, total scores range from 0 to 52.[14-16] Higher score indicates lesser degree of fatigue.[14-16] Severe or significant fatigue was defined as scores less than 30.[14-16] Written consent from the FACIT organization was obtained for the formal use of a validated traditional Chinese language version of the FACIT-F questionnaire.

2.1 | Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) 17.0 for Mac (Chicago, IL, USA). All clinical and related parameters were expressed as median with interquartile range or percentages and mean ± standard deviation (SD).

Severe or significant fatigue, was defined by a FACIT-F cutoff score less than 30.[14,15] In univariate analyses, demographics and clinical variables differences between the 2 patient groups (with or without severe fatigue) were compared using Student's *t* test or Mann-Whitney *U* test for variables as appropriate, and Chi-square test for categorical variables in univariate analyses. Subsequently, a logistic regression model was used to determine the associated

TABLE 1 Demographics and characteristics of PsA patients (231)

	Number (%)	Mean (± SD)
M/F ratio	151/80 (65.4/34.6)	
Age, y		52.3 ± 12.2
Body mass index, (kg/m^2)		24.9 ± 4.3
Duration of psoriasis, y, median		14 (8-22)
Duration of PsA, y		5 (2-10)
Diabetes mellitus	42 (18.2)	
Ischemic heart disease	10 (4.3)	
Disease activity		
Swollen joint count, 0-66, median		2 (0-5)
Tender joint count, 0-68, median		2 (0-4)
DAPSA score, median		13.3 (6-20.5)
Patients with DAPSA remission, ≤4	33 (14.3)	
Patients with low DAPSA activity, 4-14	86 (37.2)	
Patients with moderate DAPSA, 14-28	81 (35.1)	
Patients with high DAPSA, >28	31 (13.4)	
Patients with dactylitis, median		0 (0-2)
Leeds Enthesitis Index, 0-6, median		0
Positive skin condition	191 (82.7)	
PASI score, 0-72, n = 191, median		3.2 (1.2-7.4)
Severe skin condition, PASI > 10	29 (12.6)	
Erythrocyte sedimentation rate, mm/hr, median		20 (10-39)
C-reactive protein, mg/dL, median		3.5 (3.1-9.6)
Creatinine clearance, mL/min, median		93 (77-112)
Medication use		
Patients on single conventional DMARD	88 (38.1)	
Patients on dual conventional DMARDs	29 (12.6)	
Methotrexate	82 (35.5)	
Sulphasalazine	44 (19)	
Lefluonamide	13 (5.6)	
Cyclosporine	19 (8.2)	
Patients on biologics	45 (19.5)	
Patients on anti-tumor necrosis factor biologics	28 (12.1)	
Quality of life measurement		
FACIT-F score, 0-52		37.5 ± 9.1
Severe fatigue, FACIT-F < 30	49 (21.2)	
Health Assessment Questionnaire, 0-3, median		0.13 (0-0.63)
Pain VAS, 0-100 mm, median		20 (2-50)
General health VAS, 0-100 mm, median		50 (20-60)

DAPSA, Disease Activity in Psoriatic Arthritis; DMARD, disease-modifying antirheumatic drugs; FACIT-F, Functional Assessment of Chronic Illness Therapy – Fatigue; PASI, Psoriasis Area and Severity Index; PsA, psoriatic arthritis; VAS, visual analog scale

factors of severe fatigue. Statistical significance was defined as a *P* value of less than .05, 2-tailed.

3 | RESULTS

A total of 231 eligible participants were recruited. Table 1 summarizes the baseline characteristics of all the participants. There were 80 females and 151 males, with mean age of 51.3 ± 12.2 years. Mean BMI was 24.9 ± 4.3 kg/m^2. The median duration of skin psoriasis was 14 (8-22) years and the median duration of PsA was 5 (2-10) years. Forty-two (18.2%) patients had diabetes mellitus and 10 (4.3%) had ischemic heart disease. The median creatinine clearance was 93 (77, 112) mL/min. Thirty (13%) were active smokers, 14 (6.1%) were ex-smokers and 9 (3.9%) were chronic drinkers.

The median detected tender joint count (68) was 2 (0-4) and swollen joint count (66) was 2 (0-5). The median ESR level was 20 (10-39) mm/h and the median CRP level was 3.5 (3.1-9.6) mg/L. Both tender enthesitis and tender dactylitis were relatively uncommon in the PsA participants at the time of survey, with prevalence of 18.6% (43/231) and 32.5% (75/231) respectively. Among them, the median LEI was 2 (1-2) and the median dactylitis count was 2 (2-4). There were 82.7% (191/231) of patients who had skin psoriasis lesions. The median PASI score was 3.2 (1.2-7.4) among those with skin lesions.

The median body pain VAS was 20 (2-50) among them, while for patients' GH, the median VAS was 50 (20-60). The median HAQ-DI was 0.13 (0-0.63). The median DAPSA score was 13.3 (6-20.5). Among all PsA participants, 33 (14.3%) of them were in DAPSA remission, with the score below or equal to 4. Eighty-six (37.2%) of them were in low arthritis activity with DAPSA score above 4-14. Eight-one (35.1%) of them were in moderate activity, with DAPSA score between 14-28 and 31 (13.4%) were in high activity with DAPSA score above 28.

TABLE 2 Univariate analysis of PsA patients with and without severe fatigue

Parameter	Patients with severe fatigue, N = 49	Patients without severe fatigue, N = 182	*P* value
Gender			
Male, n (%)	29 (59.2)	122 (67)	.31
Female, n (%)	20 (40.8)	60 (33)	
Age in y, mean ± SD	50.4 ± 12.7	51.6 ± 12	.52
Body mass index (kg/m^2), mean ± SD	24.8 ± 5.5	24.9 ± 3.9	.90
Duration of PsO, median	12 (10-23)	14 (7-22)	.92
Duration of PsA, median	4 (2-10)	5 (2-10)	.75
Diabetes mellitus, n (%)	7 (14.3)	35 (19.2)	.43
Ischemic heart disease, n (%)	2 (4.1)	8 (4.4)	.92
Creatinine clearance, mL/min, median	89 (76-120)	94 (78-110)	.19
Disease activity			
Swollen joint count, 0-66, median	4 (1-8)	2 (0-5)	.03
Tender joint count, 0-68, median	3 (1-8)	2 (0-4)	.01
Dactylitis count, median	0 (0-3)	0 (0-1)	.02
Leeds Enthesitis Index , 0-6, median	0 (0-1)	0	.11
PASI score, 0-72, median	4.2 (1.2-10.7)	2.1 (0.3-5.6)	.01
PASI score ≤ 1, n (%)	10 (20.4)	65 (35.7)	.04
Erythrocyte sedimentation rate, mm/hr, median	18 (11-47)	20 (10-35)	.62
C-reactive protein, mg/L, median	4.6 (3.1-11.1)	3.5 (3.1-9.5)	.44
DAPSA score, median	18.3 (11.4-29.9)	11.3 (5.3-18)	.002
DASPA score ≤ 4, n (%)	3 (6.1)	30 (16.5)	.07
Medication use			
On single conventional DMARD, n (%)	11 (47.8)	30 (43.5)	.64
On dual conventional DMARDs, n (%)	3 (13)	10 (14.5)	
Methotrexate, n (%)	15 (30.6)	67 (36.8)	.42
Sulphasalazine, n (%)	15 (30.6)	29 (15.9)	.02
Lefluonamide, n (%)	0 (0)	13 (7.1)	.06
Cyclosporine, n (%)	9 (18.4)	10 (5.5)	.004
Acitretin, n (%)	0 (0)	4 (2.2)	.30
Patients on biologic agent, n (%)	4 (8.2)	41 (22.5)	.02
Patients on anti-tumor necrosis factor biologics, n (%)	3 (6.1)	25 (13.7)	.15
Quality of life measurement			
Health Assessment Questionnaire (0-3), median	0.75 (0.13-1.13)	0 (0-0.38)	<.001
Pain VAS, median	50 (15-70)	10 (0-43)	<.001
General health VAS, median	60 (40-80)	40 (20-60)	<.001

Abbreviation: DAPSA, Disease Activity in Psoriatic Arthritis; DMARD, disease-modifying antirheumatic drugs; PASI, Psoriasis Area and Severity Index; PsA, psoriatic arthritis; PsO, skin psoriasis; VAS, visual analog scale.

Concerning the medications use, 88 (38.1%) were on a single conventional DMARD, 29 (12.6%) received dual DAMRDs. Thirteen of them (5.6%) were on leflunomide, 44 (19%) were on sulphasalazine, 19 (8.2%) were on cyclosporine, 4 (1.7%) were on acitretin and 82 (35.5%) were on methotrexate. Forty-five (19.5%) were receiving biological agents, while 28 of them (62.2%) were receiving anti-TNF biologics.

The mean FACIT-F score was 37.5 ± 9.1 and 21.2% (49/231) of all PsA participants were noted to have severe fatigue (FACIT-F score < 30). The mean age of those with severe fatigue was 50.3 ± 12.7 years. Twenty-nine (59.2%) were men and 20 (40.8%) were women.

Univariate analysis found that (a) the use of DMARDs (leflunomide and cyclosporine), (b) self-perceived pain and GH scores, (c)

HAQ-DI, (d) PASI and skin remission, (e) disease activity – tender and swollen joint count, and dactylitis count, and (f) DAPSA score, were closely associated with severe fatigue in PsA. Table 2 summarizes the univariate analysis of associated factors for PsA-related fatigue.

Since the DAPSA score contains the components of tender and swollen joint count, self-perceived pain and GH scores, they were all excluded from the subsequent logistic regression model. The final model identified that DAPSA and PASI score were the 2 significant associated factors with severe fatigue in PsA, with *P* values of .003 and .04 respectively. In Spearman's rho correlation, the FACIT-F score showed a negative relationship with the DAPSA, with weak strength of association ($r = -.3$, $P < .001$). PASI also showed a weak strength of correlation with FACIT-F score with $r = -.26$, $P < .001$. Figure 1 shows the scatterplots of the relationship between FACIT-F, DAPSA and PASI in PsA cohorts.

No associations with severe fatigue were found with age, gender, BMI, renal function, inflammatory markers, the duration of psoriatic conditions, dactylitis count, LEI, medications use (either conventional DMARD or biologic agent), HAQ-DI and comorbid conditions in the final model.

4 | DISCUSSION

Fatigue is an important feature of chronic rheumatic diseases; however, it is often under-recognized, under-evaluated and under-treated by rheumatologists.[1,2] The collected data confirmed that severe fatigue was common among Hong Kong Chinese PsA patients, with prevalence of 21.2%. The mean FACIT-F score was 37.5 ± 9.1 among them. This distressing symptom was not only correlated with core disease domains, but also with their perceived pain and well-being.

Fatigue is an abstract symptom, which varies across individuals, cultures and ethnicities.[6] To date, there is no international consensus on its definition and use of measurement instruments. The 13-points FACIT-F scale was used because it had been validated in the 2007 Toronto PsA study, and it covered a broader range of fatigue-related manifestations.[17,18] Other assessment tools for fatigue, including the Multidimensional Fatigue Inventory (MFI-20) scale, and the Fatigue Visual Analog Scale, are not specific to psoriatic diseases, except the modified Fatigue Severity Scale (FSS).[9,11]

Data on the prevalence of severe fatigue in PsA is sparse. Using the fatigue VAS, FSS and SF-36 vitality scale, Skoie et al. found that up to 50% of patients with skin psoriasis suffered from substantial fatigue.[5] Using the modified FSS scores, Husted et al. identified that 49.5% of PsA patients had at least moderate fatigue and 28.7% had severe fatigue.[18] In 2016, Tobin et al. reported that about 40% of PsA patients had fatigue, based on the VAS.[19] Recently, Pilgaard et al. found that severe fatigue was extremely common not only in PsA (approximately 27%), but also across rheumatoid arthritis (RA) (approximately 25%) and axial spondyloarthropathy (axSpA) (approximately 28%), from the Danish National Rheumatology Registry (DANBIO).[20] Our results showed 21.2% prevalence of severe fatigue, consistent with previous studies.[20] This was also consistent across different fatigue assessment methods, cultures and ethnicities.[5,18-20] Based on the above findings, fatigue phenomenon in PsA should not be neglected and under-estimated.

Information about the associated factors for PsA-related fatigue were found to be limited and inconsistent. A prospective observational study by Behrens et al. found that a DMARD, leflunomide, could effectively reduce PsA patients' sense of fatigue, in addition to pain, skin and nail manifestations, and dactylitis.[21] On the other hand, Husted et al. identified that "ever used" methotrexate might provoke PsA-related fatigue.[18] In univariate analysis of the present study, cyclosporine and sulphasalazine were found to be associated with severe fatigue, but not in the final regression model. Since the cumulative dose and the duration of DMARDs used were not recorded in all of them, their relationship with fatigue was not conclusive.

To most rheumatologists, fatigue is intimately related to the chronic inflammation of rheumatologic diseases.[2,3] Much evidence, including randomized trials, demonstrated that fatigue in RA was closely linked with joint inflammation.[17,22] Biologic agents reduced RA-related fatigue, via cytokine inhibition.[17,22] Bluthé et al. postulated that interleukin-1 (IL-1) cytokines and TNF-alpha might play a key role in fatigue.[23] IL-1 and TNF-alpha crossed the blood-brain barrier in mice and induced the behavioral changes of immobility, loss of appetite and social exploration, which all share common symptoms with severe fatigue.[23] Studies about the effect of biologic agents on PsA fatigue are limited.[24] A meta-analysis of 7 randomized controlled trials concluded that anti-TNF biologics (adalimumab, certolizumab pegol), non-TNF biologics (secukinumab, ustekinumab) and apremilast had a small effect on fatigue at 24 weeks in PsA.[24] Consistently, our collected data did not find PsA fatigue having any correlation with the use of conventional DMARDs and biologic agents, which indicated that fatigue severity in PsA was not independently related to medications use, but more depended on the tight disease control with individual medication.

In 2016, Gudu et al. highlighted that the intensity of fatigue in PsA was largely related to disease severity, including extent of skin involvement, tender joint count and enthesitis.[18] On the other hand, a large study (n = 499) of PsA patients revealed that fatigue was more correlated to pain perception, gender, physical disability, medication use and emotional stress, than disease-related activities.[18] Our data were consistent with both in that PsA fatigue was correlated with its disease activities including tender joint counts, and also pain and GH perception, but not gender, physical disability or medication use. Two respective studies by Skoie et al. and Tobin et al. had reported there were lack of correlations between fatigue magnitude and PASI in psoriatic patients.[5,13] Although only weak correlations were found between fatigue severity and PASI score in the final model, a significant numerical improvement of FACIT-F score was found in the subgroup analysis of skin remission patients (PASI ≤ 1) versus skin non-remission patients (39.8 ± 8.7 vs. 36.5 ± 9.2; $P = .009$), indicating that cutaneous involvement did play a role in causing fatigue

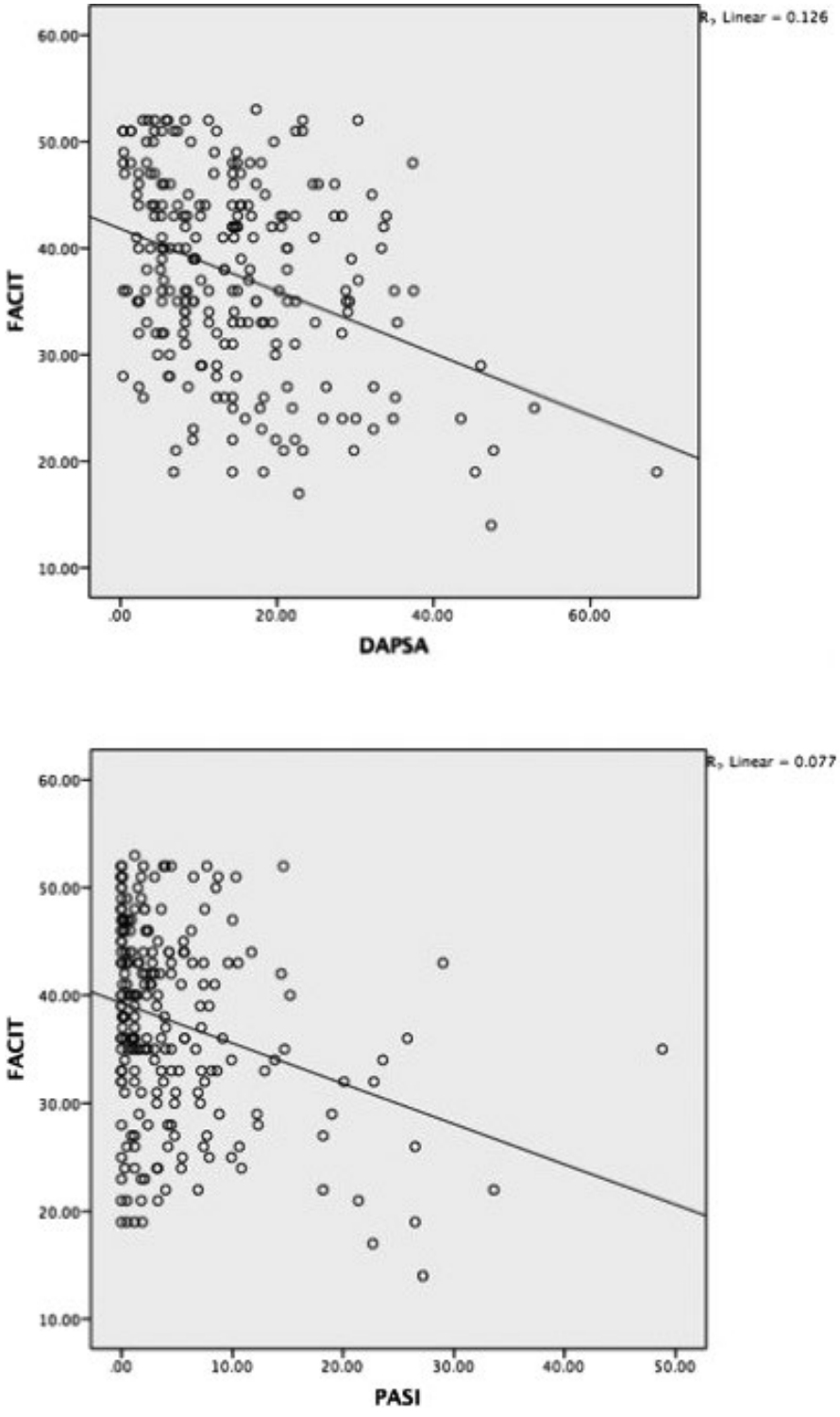

FIGURE 1 Scatterplots showing the relationship between Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-F), Disease Activity in Psoriatic Arthritis (DAPSA) and Psoriasis Area and Severity Index (PASI) score in psoriatic arthritis (PsA) patients (n = 231)

feeling in psoriatic conditions. Certainly, skin dryness and itchiness in poorly controlled PsO can induce sleep deprivation, which can cause fatigue feeling.[25,26]

Our findings showed that severe PsA fatigue was significantly associated with DAPSA as well as PASI. DAPSA score consists of 5 components: (a) tender joint count; (b) swollen joint count; (c) CRP; (d) pain perception; and (e) GH perception.[10] This showed that the underlying cause of PsA fatigue was multifaceted and that no single factor could fully explain it.[18,27] Our collected data also found that the majority of severely fatigue patients were men (59.2%) of middle age, who were the main workforce of our society and the income producers of families in a Chinese society. Understanding the way to tackle fatigue feelings among them definitely can improve not only an individual's work efficiency and quality of life, but also their families and the society. Since PsA fatigue magnitude was negatively correlated with DAPSA and PASI score, prompt disease activity control in arthritic and cutaneous conditions could definitely alleviate these unpleasant feelings to a certain extent.

However, there was not any association between DAPSA and PASI remission and the occurrence of severe fatigue among them; this finding reinforced the belief of the multifactorial nature of PsA fatigue etiology. Achieving DAPSA and PASI remission in PsA could not completely eliminate their fatigue feelings. Furthermore, although DAPSA and PASI were found to be closely associated with fatigue, their magnitude of correlation was actually weak. Their weak correlations indicated that neither DAPSA nor PASI were the perfect predictor for PsA-related fatigue. In addition to skin and joint examination, a more comprehensive assessment including psychological, emotional, physical and quality of life aspects, should be employed in predicting PsA-related fatigue. Furthermore, a multidisciplinary approach to management of fatigue is required; this may include psychological therapy and counseling which help to build and boost psychological well-being. In view of its common prevalence and multidimensional nature, fatigue assessment should be routinely made in all our PsA patients.

Our study was one of only a few conducted internationally, to explore fatigue in PsA. Our findings provide useful information in understanding the psychological aspect of PsA patients, and hope to raise rheumatologists' awareness to this troublesome problem. Another strength of this study was that multiple confounding factors were considered, particularly comorbidities, medication use and the use of DAPSA. The current data highlighted the importance of treating PsA to target alleviating fatigue magnitude.

There were a few limitations in this study. First, several potential confounding factors such as socioeconomic factors and sleep-related factors, were not considered in the present study. In previous studies, psychosocial adversity and social factors play pivotal roles in fatigue occurrence.[6,18] Data on marital status, living environment, employment, and financial status assist understanding of the psychosocial factors of fatigue. Sleep disorders or sleep quality were not taken into account.[26] There is substantial symptom overlap between fatigue and sleep disorders.[26] Verhoeven et al. had highlighted a high prevalence of pruritus (50%) among those with general skin conditions.[28] Skin itchiness and joint pain in PsA patients worsen sleeping quality, which may result in depressive symptoms, and exaggerate the fatigue feeling.[22,25,26] Sleep-related questionnaires such as the Global Sleep Assessment Questionnaire and itching measurement such as the 5-D itch scale, should be included in assessment.[27,29]

Second, fibromyalgia was not specifically excluded.[30] A 2013 pilot study reported that 55% of PsA patients had concomitant fibromyalgia syndrome, in which a core symptom was severe fatigue.[30] Third, variations in the magnitude of fatigue may be contributed by different degrees of HAQ-DI and pain level from subtypes of PsA such as predominant axial spondylitis and oligo-articular arthritis, which were not recorded in this study.

Finally, the limitation of a cross-sectional study is clear. The findings of association between fatigue magnitude, DAPSA and PASI score do not indicate the casual relationship. Longitudinal study with a validated assessment tool is definitely required to examine the link between the change in PsA disease activity and fatigue magnitude.

5 | CONCLUSION

Severe fatigue was prevalent in patients with PsA, its occurrence and magnitude was closely associated with the disease activity – DAPSA and PASI. It was not correlated with age, gender, duration of psoriatic disease, medication use and comorbid conditions. Controlling disease activities in terms of DAPSA and PASI could not completely eliminate PsA-related fatigue, but could relieve it to a certain extent.

PsA-related fatigue was multifaceted, not a single marker could predict its occurrence. In view of its complexity, more holistic and multidisciplinary approaches should be adopted to alleviate this burdensome symptom.

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CONFLICT OF INTEREST

No conflicts of interest declared.

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ORIGINAL ARTICLE

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Neutrophil extracellular traps and inflammatory response: Implications for the immunopathogenesis of ankylosing spondylitis

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Abstract

Aim: Ankylosing spondylitis (AS) pathogenesis has focused on the adaptive immune response; however, innate immune responses may also play a role in the inflammatory response of AS. Dysregulated neutrophil activation can induce tissue damage and contribute to the pathogenesis of immune-related diseases. Hence, the aim of this study was to assess the effect of immune complexes formed with the p30 of *Salmonella typhimurium* and anti-p30 antibodies present in the sera of AS patients and controls in inducing the release of neutrophil extracellular traps (NETs) and the secretion of pro-inflammatory cytokines.

Methods: We collected polymorphonuclear leukocytes (PMNs) from healthy donors. The PMNs isolated were stimulated with p30 alone or in immunocomplexes formed with antibodies presents in sera of AS patients or control subjects. Then, the NETs were analyzed by fluorescence microscopy. Concentrations of interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1β, IL-8 and IL-10, were determined using the Cytometric Bead Array kit.

Results: Significant difference was observed in the release of NETs between the neutrophils stimulated with p30 + AS (70.52 ± 16.24) those unstimulated neutrophils (9.94 ± 12.12; $P = .0095$), stimulated with phorbol 12-myristate 13-acetate (39.78 ± 14.50; $P = .0190$), stimulated with control serum (CS) (10.85 ± 5.33; $P = .0082$) and serum of AS patient (10.28 ± 6.15; $P = .0087$). The stimulation of neutrophils with p30 alone induced a relatively low production of IL-6 (64.5 pg/mL), IL-8 (2658.3 pg/mL), IL-1β (31.11 pg/mL), and TNF-α (3.8 pg/mL), compared to p30 + AS and p30 + CS groups.

Conclusion: Our results show that neutrophils release NETs and pro-inflammatory cytokines in response to p30 in immunocomplexes. These findings could improve our understanding of the role of innate immunity in the initiation and/or maintenance of inflammatory responses, and in the progression of AS.

KEYWORDS
ankylosing spondylitis, cytokines, immunopathogenesis, inflammation, neutrophil extracellular traps, p30 of *Salmonella typhimurium*

1 | INTRODUCTION

Ankylosing spondylitis (AS) is a chronic, systemic disease which represents the most common form of chronic inflammatory spondyloarthritis. The global prevalence of AS is generally estimated between 0.1% and 1.4%,[1] while reports for Mexico give a figure of 0.09%.[2] The onset of AS occurs most often during the third decade of life and is more frequent in men than women. The uncontrolled evolution of AS results in serious impairment of spinal mobility and physical function, thus adversely impacting patients' quality of life.

As is the case with other autoimmune diseases, the etiology of AS remains unknown, although it is believed to involve a combination of genetic and environmental factors that produce chronic inflammation.[3] The main genetic risk factor is attributable to the *HLA-B27* gene;[4] however, other non-major histocompatibility complex genes or genetic regions have also been associated with susceptibility to AS, including *IL-23*, *IL-27*, *FCRs*, and *ERAP1/2*.[5] Environmental factors are also thought to play an important role in the activation and exacerbation of AS. Infections by bacterial pathogens like *Yersinia*, *Shigella*, *Salmonella*, *Campylobacter*, and *Chlamydia* are considered the main environmental factors associated with the development of AS.[3] In this regard, our working group reported previously that patients with AS have high levels of antibodies (immunoglobulin [Ig]G total and IgG3) against a 30 kDa protein from *Salmonella typhimurium* (p30).[6]

Although AS is considered an autoimmune disease, and research on its pathogenesis has focused on adaptive immune responses, some spondyloarthritides (SPA) were recently re-classified as autoinflammatory diseases, in which innate immune responses could play a major role.[7-10] Interestingly, 2 factors involved in the activation of the innate immune response – bacteria and local mechanical stress – have been postulated as possible participants in the pathogenesis of AS.[11,12] The innate immune response is the first line of host defense because it provides an immediate response to foreign agents. Several types of cells participate in this system, including macrophages, dendritic cells, natural killer cells, mast cells, and neutrophils. The latter are the most abundant immune cells in circulation. They are of the phagocytic type and are known to be the main inflammatory cells.[13] In addition to their phagocytic capacity, they have other microbicidal mechanisms, such as reactive oxygen species production, degranulation, the synthesis and secretion of a large repertoire of cytokines, and the release of neutrophil extracellular traps (NETs).[14]

NETs are structures composed of cytosolic and granular proteins assembled on a scaffold of decondensed chromatin. Their function is to neutralize and kill bacteria,[15] fungi, viruses, and parasites.[16-18] However, if NETs are dysregulated, they can actually contribute to the pathogenesis of immune-related diseases. This has led to the conclusion that AS patients suffer from a chronic inflammatory process characterized by the infiltration of cells from the innate immune system.[19] In light of the foregoing, the present study was designed to determine whether the immune complexes (IC) formed with the p30 of *Salmonella typhimurium* and anti-p30 antibodies present in the serum of AS patients could induce the release of NETs and cytokine secretion, and so contribute to our understanding of the inflammatory process that characterizes AS.

2 | METHODS

2.1 | Subjects

This study is based on sera drawn from 5 patients diagnosed according to current criteria[20] at the Clinica de Reumatologia, Servicio de Medicina Interna, at the Instituto Mexicano de Seguro Social (HGZ No. 1) in Tepic, Nayarit. In addition, the sera from 5 healthy donors were used as a control group (CS). All participants signed written consent according to the Helsinki Declaration.[21]

2.2 | Cell isolation

A total of 12 mL of peripheral venous blood was collected from healthy donors in citrated tubes. Polymorphonuclear leukocytes (PMNs) were isolated by standard density gradient centrifugation using Ficoll (Sigma Aldrich), as previously reported.[22] To remove contaminating erythrocytes, the PMNs were subjected to short cycles of hypotonic lysis with deionized water.

2.3 | The 30-kDa band of *S. typhimurium* (p30)

The p30 was obtained from an *S. typhimurium* strain as previously described.[6]

2.4 | Depletion of anti-p30 antibodies by immunoadsorption

Antibodies to p30 in aliquots from the sera of AS patients and CS were removed by immunoadsorption. Briefly, p30 of *S. typhimurium* diluted in coupled buffer (0.1 mol/L $NaHCO_3$, 0.5 mol/L NaCl, pH 9.0) was coupled to 0.5 g of Sepharose CL-4B (Sigma Chem. Co.) for 2 hours at room temperature. After washing, free binding sites were blocked with 1 mol/L ethanolamine (Sigma Chem. Co.) for 16 hours at 4°C. This material was then washed twice (in coupled buffer) to ready it for use. For immunoadsorption, 50 μL of each serum from patients and controls were incubated with the immobilized p30 for

30 minutes at room temperature. Next, the sera were eluted with phosphate-buffered saline (PBS) and concentrated to the original volume using a Spin-X™ Centrifuge Tube Filter (Corning™). Depletion of the anti-p30 antibodies was confirmed by enzyme-linked immunosorbent assay (ELISA). The immunoadsorbed sera were stored at −20°C until use.

2.5 | Quantification of the anti-p30 antibodies

The relative concentration of the anti-p30 antibodies present in the complete and immunoadsorbed sera of AS patients and CS was determined by ELISA (Figure 1).

2.6 | In vitro NETs formation

The isolated neutrophils (5×10^5 cells/well) were seeded on cover slips (Knittel Glass, Germany), placed in 6-well plates in Roswell Park Memorial Institute (RPMI) 1640 medium without phenol red (Sigma Aldrich), and incubated for 1 hour at 37°C to allow adherence to the coverslips. The following stimuli were then added: (a) 100 ng/mL of phorbol 12-myristate 13-acetate (PMA) (Sigma Aldrich) to induce NETs formation; (b) 1 μg of p30 of *S. typhimurium* (p30); (c) 10 μL of serum from controls (CS); (d) 10 μL of serum from the AS patients (AS); (e) 1 μg of p30 of *S. typhimurium* combined with 10 μL of serum from the AS patients (p30 + AS); (f) 1 μg of p30 of *S. typhimurium* combined with 10 μL of serum from controls (p30 + CS); PBS was used as the negative control vehicle. The release of NETs was recorded at 37°C and 5% CO_2 at 3 hours. The NETs were subsequently fixed with 4% (v/v) paraformaldehyde (PFA) in PBS and incubated for 20 minutes at room temperature. Samples were blocked with 1% (v/v) fetal bovine serum in PBS for 30 minutes at 37°C; then the DNA was stained with a solution containing 1 μg of 4′,6-diamidino-2-phenylindole (DAPI) in PBS for 24 hours at 4°C. Neutrophil elastase (NE) was identified by immunofluorescence with a specific antibody, based on our previous work. The NETs were identified manually in the acquired images as DAPI/NE-positive structures emanating from neutrophils. They were visualized and photographed on the same day using an Axio Vert.A1 microscope (ZEISS) equipped with a Ds-fi1 camera (Nikon) adapted for phase-contrast, transmitted light, and epi-fluorescence microscopy. Each experiment was repeated at least 5 times.[22]

2.7 | In vitro cytokine release

The isolated neutrophils (1×10^6 cells) were seeded on cytometry tubes in RPMI 1640 medium without phenol red (Sigma Aldrich). Then the following stimuli were added: (a) 2 μg of lipopolysaccharide (*Escherichia coli* 0111:B4 Sigma Chem. Co.) to induce cytokine production (positive control); (b) 0.5 μg of p30 of *S. typhimurium* (p30); (c) 0.5 μg of p30 combined with 5 μL of serum from AS patients (p30 + AS); (d) 0.5 μg of p30 combined with 5 μL of serum from control subjects (p30 + CS); (e) 0.5 μg of p30 combined with 5 μL of immunoadsorbed serum from AS patients (p30 + IAS); and (f) 0.5 μg of p30 combined with 5 μL of immunoadsorbed serum from controls (p30 + ICS). RPMI 1640 medium without phenol red (Sigma Aldrich) was used as the negative control vehicle. Cytokine release was recorded at 37°C and 5% CO_2 at 24 hours. Supernatants were collected to quantify cytokine concentrations.

Concentrations of pro-inflammatory (interleukin [IL]-6, tumor necrosis factor alpha [TNF-α], IL-1β, IL-12, IL-8) and anti-inflammatory (IL-10) cytokines were determined following the manufacturer's

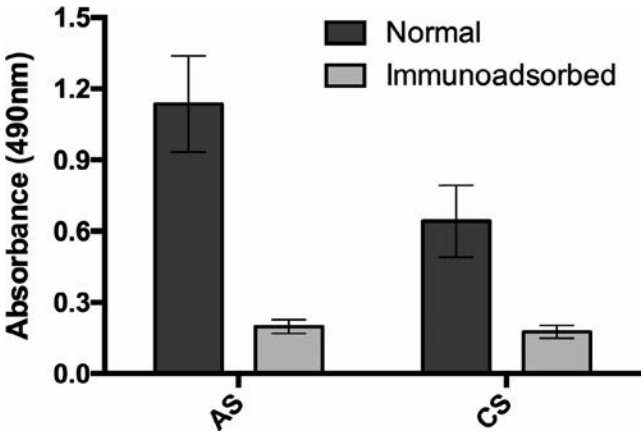

FIGURE 1 Depletion of anti-p30 antibodies by immunoadsorption. The concentration of anti-p30 antibodies in the normal and immunoadsorbed sera of patients (AS) and control subjects (CS) was determined by enzyme-linked immunosorbent assay

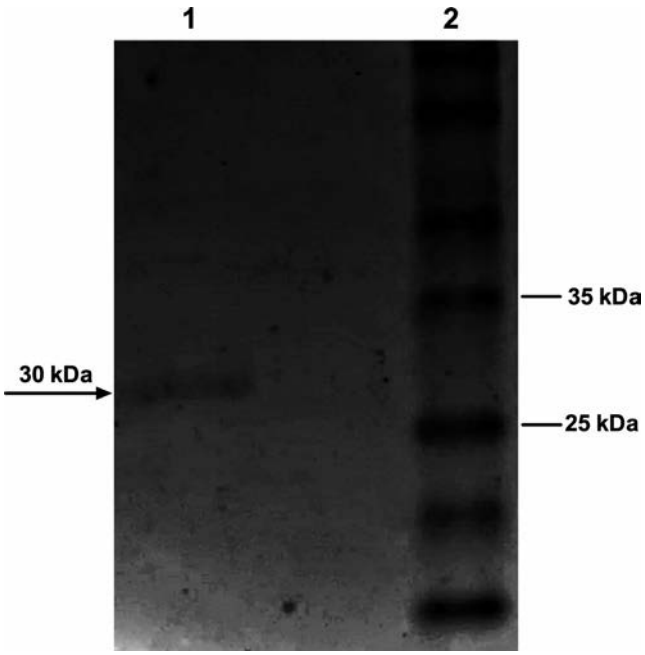

FIGURE 2 Visualization of the electroeluted p30. The purity of the electroeluted p30 was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. (1) Electroeluted p30. (2) Molecular masses of standard markers

specifications in the Human Inflammatory Cytokine kit (BD™, Cytometric Bead Array [CBA], 551811). Samples were analyzed in a BD™ Accuri™ II Flow Cytometer, and FCAP Array™ software was used to analyze the data obtained. Each experiment was repeated at least 5 times.

2.8 | Statistical analyses

All data were analyzed using GraphPad Prism 5.0 (Graph Pad Software Inc., San Diego, CA, USA). The results of all the in vitro assays represent at least 5 independent experiments. Unpaired, two-tailed Student's *t* tests (Mann-Whitney *U* tests) and analyses of variance were used to assess the statistical significance of the different groups. Significant results are defined as $P < .05$ (*) and $P < .01$ (**).

3 | RESULTS

3.1 | ICs formed with p30 and sera from AS patients induce the release of NETs

The p30 was electroeluted, purity was confirmed by , sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Figure 2), and the protein concentration was determined using the Lowry method.[23] We then determined the release of NETs by stimulating the neutrophils in vitro with p30 and IC formed with p30 + serum from AS or CS. Results show that stimulation of the neutrophils with p30 + AS induced the release of NETs, but that the neutrophils stimulated with p30 alone, sera from patients or control subjects alone, or with p30 + CS, did not induce their release (Figure 3A). Upon analyzing the images obtained by fluorescence microscopy, a significant difference was observed in the release of NETs between the neutrophils

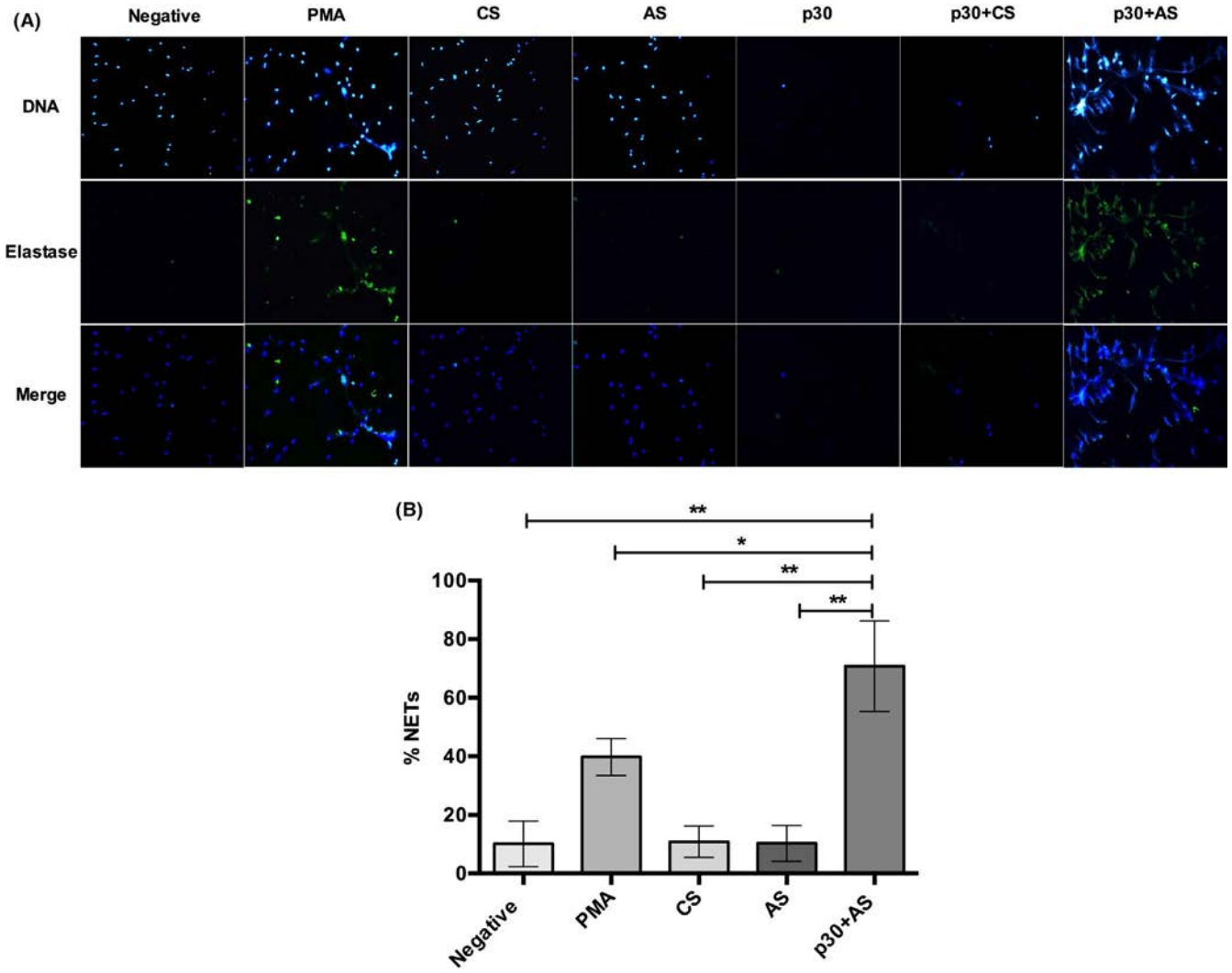

FIGURE 3 p30 in immunocomplexes induces the formation of neutrophil extracellular traps (NETs). Immunofluorescence staining for DNA (blue) or elastase (green) was performed in unstimulated neutrophils (negative), or neutrophils stimulated with phorbol 12-myristate 13-acetate (PMA), p30, control serum (CS), ankylosing spondylitis (AS), p30 + CS, and p30 + AS, respectively (A). The quantification of NETs was performed by Image J software. The percentage of NETs is shown for the unstimulated neutrophils (negative), and the neutrophils stimulated with PMA, CS, AS and p30 + AS (B). *$P < .05$, **$P < .001$

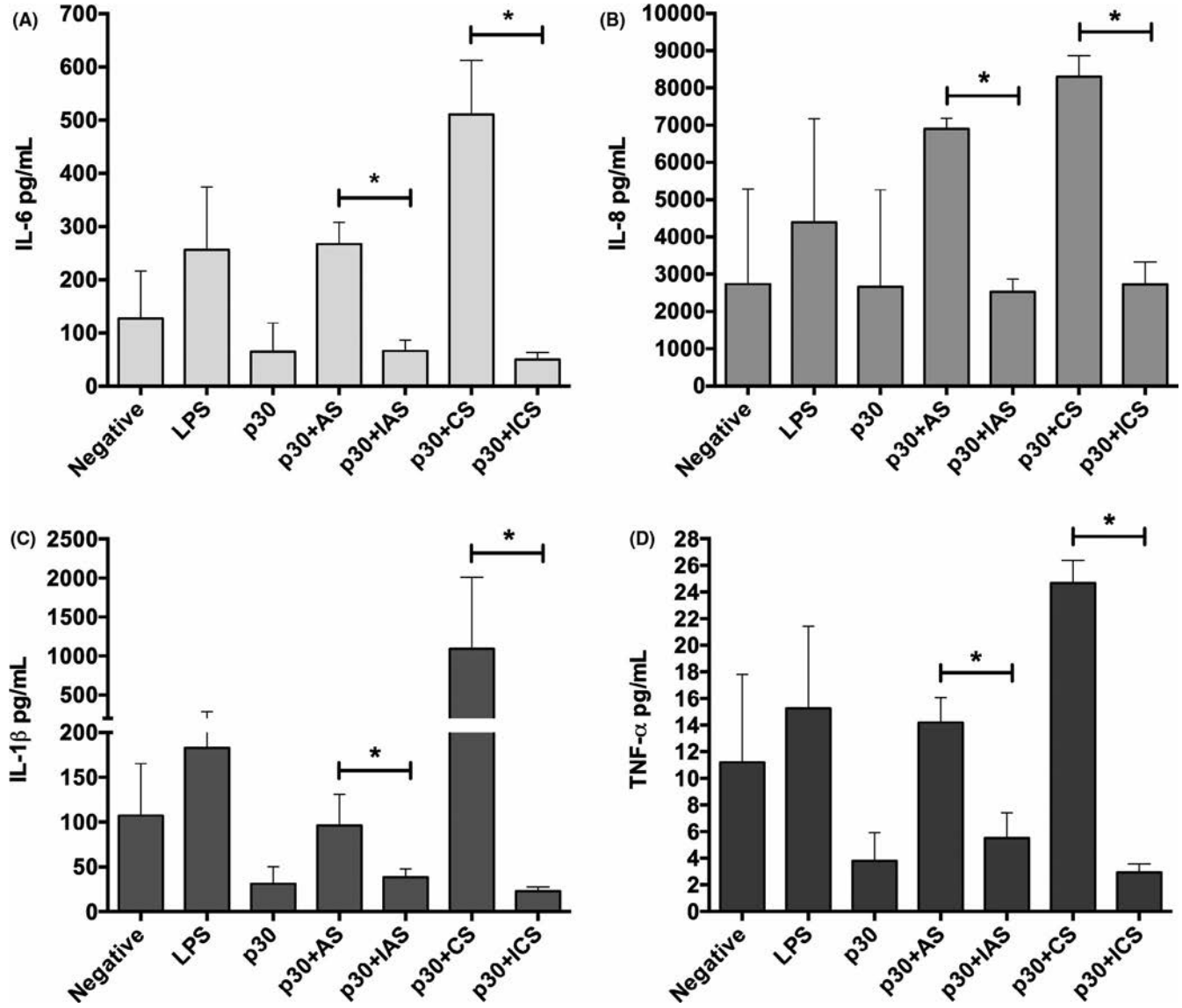

FIGURE 4 Presence of cytokines in the supernatants from the neutrophils. The concentrations of interleukin (IL)-6 (A), IL-8 (B), IL-1β, (C), and tumor necrosis factor (TNF)-α (D) are shown according to each of the stimuli used. *$P < .05$. AS, ankylosing spondylitis; CS, control serum; LPS, lipopolysaccharides

stimulated with p30 + AS (70.52 ± 16.24) those unstimulated neutrophils (9.94 ± 12.12; $P = .0095$), stimulated with PMA (39.78 ± 14.50; $P = .0190$), stimulated with CS (10.85 ± 5.33; $P = .0082$) and serum of AS patient (10.28 ± 6.15; $P = .0087$) (Figure 3B).

3.2 | Pro-inflammatory profile induced by IC formed with the p30 of *S. typhimurium*

Pro-inflammatory cytokine (IL-6, IL-8, IL-1β and TNF-α) and anti-inflammatory cytokine (IL-10) levels were determined in the supernatants taken from the neutrophils stimulated in vitro as described above. Observations showed that the stimulation of neutrophils with p30 induced a relatively low production of IL-6 (64.5 pg/mL), IL-8 (2658.3 pg/mL), IL-1β (31.11 pg/mL), and TNF-α (3.8 pg/mL), compared to p30 + AS, which produced the following results: IL-6 (267.1 ± 81.34), IL-8 (6989 ± 569), IL-1β (96.3 ± 59), and TNF-α (14.21 ± 3); and to p30 + CS: IL-6 (510.5 ± 203), IL-8 (8299 ± 1141), IL-1β (1092 ± 1833), and TNF-α (24.69 ± 3]). In addition, the neutrophils stimulated with p30 + CS showed the highest pro-inflammatory cytokine secretion. We further demonstrated that this effect is due to the IC formed with anti-p30 antibodies, since cytokine secretion was significantly depleted when anti-p30 antibodies were eliminated by immunoadsorption (Figure 4A-D).

4 | DISCUSSION

The pathogenesis of AS is not yet completely understood, although genetics and environmental factors could be involved in the chronic inflammation observed in this disease. The peri-fibrocartilaginous bone and entheses represent a primary site and tissue target where

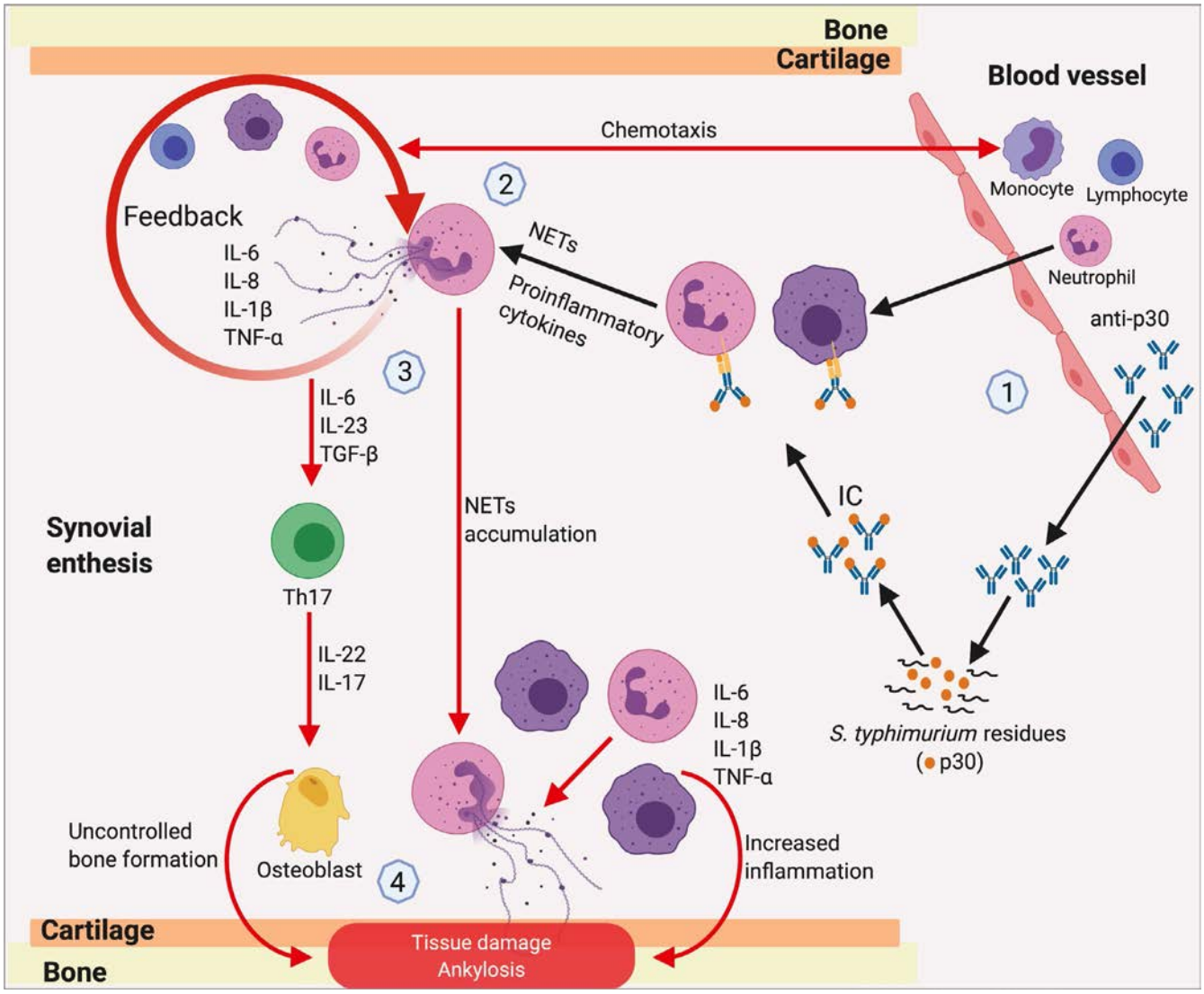

FIGURE 5 Neutrophil contribution to the immunopathogenesis of ankylosing spondylitis (AS). Hypothetical model to explain our understanding of the participation of the uncontrolled activation of neutrophils in the immunopathogenesis of AS. IL, interleukin; NETs, neutrophil extracellular traps; TGF, transforming growth factor; Th17, T-helper cell 17; TNF, tumor necrosis factor

innate and adaptive responses occur, initially as a repair process, but that in later phases could generate remodeling effects that include bone edema, osteitis, new bone formation, and a ramping up of the inflammatory response with a subsequent excessive inflammatory repair reaction that may culminate in ankylosis.[24] In this context, several studies have demonstrated that neutrophils actively participate in the tissue and organ damage associated with other inflammatory diseases, such as rheumatoid arthritis, vasculitis, and inflammatory bowel disease.[25-27]

This study describes that immunocomplexes formed with p30 and its specific antibodies present in the sera of AS patients induce high levels of NET release after stimulation of the neutrophils (Figure 3A,B). This effect could be due to the amount of anti-p30 antibodies present in the sera of AS patients, since the sera from CS contain anti-p30 antibodies (Figure 1), sera from patients or control subjects or p30 alone, were unable to induce the release of NETs. In addition, a decrease or depletion in the number of neutrophils stimulated with p30 alone was observed, likely because they died due to some mechanism distinct from NETosis (Figure 3A). However, this hypothesis requires further exploration in future studies. This is an important finding because, while the role of NETs in autoimmune diseases like rheumatoid arthritis,[28] and in such autoinflammatory diseases as Crohn's disease and ulcerative colitis[29] has been reported, ours is the first study on the release of NETs in the context of AS.

We have previously reported high levels of antibodies to *S. typhimurium* in AS patients,[6] but here we propose that the anti-p30 antibodies could be involved in the maintenance of the chronic inflammatory response observed in this disease through the uncontrolled induction of NETs, in light of the fact that around 70% of the neutrophils stimulated with p30 + AS formed NETs (Figure 3B), but no release of NETs was observed when the neutrophils were stimulated with p30 + CS (Figure 3A). According to our results, dysregulated NET formation is involved in autoimmune and autoinflammatory diseases,[30] and the release of NETs

could act as a modulator of the inflammatory response in autoimmune disorders.[31]

Additionally, research has shown that the components of NETs are involved in activating other immune and structural cells by upregulating the production of pro-inflammatory cytokines and amplifying joint inflammation.[32] There are also reports that innate cytokines play a pivotal role in AS pathogenesis;[33] for example, the increased levels of TNF-α have shown a correlation with AS activity.[34] Animal models, meanwhile, have demonstrated that IL-1β can activate bone resorbing osteoclasts and induce joint destruction,[35] while an increased expression of IL-1β has been found in the synovium of spondyloarthritis patients.[36] The present study determined that neutrophils stimulated with p30 + AS or CS release pro-inflammatory cytokines such as IL-1β, IL-6, IL-8 and TNF-α, and that these cytokines could be involved in the pathogenesis of AS. These pro-inflammatory cytokines could induce high levels of oxidative stress markers[37] that may be related to the release of NETs.[38] Therefore, the inflammatory response in AS could be promoted by both pathways; that is, the release of NETs and the secretion of pro-inflammatory cytokines by neutrophils that could exert positive feedback on NETs.

Here, we found that immunocomplex-stimulated neutrophils formed with CS serum secrete a higher concentration of inflammatory cytokines, compared to those secreted by neutrophils stimulated with immunocomplexes formed with the sera of patients with AS. As we have mentioned above, IC formed with the sera of patients with AS induce the death of neutrophils due to NETosis. Then, this condition could affect the capacity of cytokine secretion, because of the lower numbers of viable neutrophils capable of secreting these cytokines. In addition, when the sera from patients with AS and CS were immunoadsorbed to deplete the anti-p30 antibodies, no significant differences in the production of cytokines were observed in neutrophils stimulated with IC formed with those sera. All these results suggest that the cytokines production by neutrophils observed is due to the IC formed by the antibodies present in the sera of patients with AS and CS.

Finally, the release of NETs could represent an important source of material that contributes to the pathogenesis of AS by perpetuating inflammation. Taking all this information into account, a hypothetical model to explain how our results could contribute to our understanding of the participation of the uncontrolled activation of neutrophils in AS is shown in Figure 5, divided into 4 steps. (a) Anti-p30 antibodies from the bloodstream reach the synovial enthesis where some *S. typhimurium* residues, including p30, could be also found, which allows the formation of immunocomplexes (IC). (b) Neutrophils and macrophages present in synovial enthesis could be activated by ICs formed by p30 inducing the production of NETs and cytokines such as IL-6, IL-8, IL-1β and TNF-α, contributing to the increase in cellular chemotaxis. (c) The constant activation of neutrophils could establish a positive feedback of the inflammatory process, resulting in an excess in the production of NETs and pro-inflammatory cytokines. (d) The uncontrolled production of NETs in patients with AS could promote the recruitment of more inflammatory cells, that could result in the production of more NETs and then, tissue damage that could have a role in the generation of the characteristic ankylosis observed in patients with AS.

5 | CONCLUSION

Taken together, our results show that neutrophils release NETs and pro-inflammatory cytokines in response to p30 in immunocomplexes. These findings could improve our understanding of the role of innate immunity in the initiation and/or maintenance of inflammatory responses, and in the progression of AS. Although these findings open the door to broad areas of further studies, it is necessary to conduct research that will enhance our understanding of the mechanism that innate immunity employs to initiate inflammatory responses which generate subsequent excessive inflammation that may culminate in ankylosis.

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CONFLICT OF INTEREST

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

AUTHOR CONTRIBUTIONS

Zambrano-Zaragoza contributed to study design, patient collection, data analysis, and manuscript writing. Agraz-Cibrián contributed to study design, data analysis and manuscript writing. Gutiérrez-Franco, Durán-Avelar, Vibanco-Pérez, Ortiz-Martínez, Ayón-Pérez and Vázquez-Reyes contributed to study conception and data interpretation.

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ORIGINAL ARTICLE

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Evaluation of an ankylosing spondylitis education and self-management program: Beneficial effects on ankylosing spondylitis specific outcomes

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Arthritis and Osteoporosis WA

Abstract

Background: Self-management programs have demonstrated significant health benefits in people with musculoskeletal diseases.

Aim: To examine the benefits of a tailored ankylosing spondylitis self-management program (ASSMP) delivered by trained health professionals for people with ankylosing spondylitis (AS) relative to health status, quality of life and disease activity.

Methods: ASSMP was developed within a continuous quality improvement framework following a needs assessment and focus group discussions. Formal feedback from the group after each 6 week program cycle group by questionnaire helped refine the ASSMP. Patient health status, quality of life and disease activity were assessed at multiple time points up to 12 months.

Results: Fifty-five percent were female; mean age 48.5 ± 15.2 years. Median time to AS diagnosis was 4 years (interquartile range: 1-10). AS disease activity Bath Ankylosing Spondylitis Global Score scores improved at 3, 6 and 12 months ($P < .001$). Bath Ankylosing Spondylitis Disease Activity Index improved at 6 weeks and was sustained at 3, 6 and 12 months ($P < .001$). The Ankylosing Spondylitis Quality of Life improved at 3, 6 and 12 months ($P < .001$). Bath Ankylosing Spondylitis Functional Index improved by 12 months ($P < .001$). Participants reported less nocturnal back pain at 6 months and was sustained at 12 months ($P < .001$). Patients Global Disease Activity improved by 6 months ($P = .012$), Multi-Dimensional Assessment of Fatigue and a Global Fatigue Index at 6 months ($P = .003$), Hospital Anxiety and Depression Scale - Anxiety at 12 months ($P = .001$), Evaluation Ankylosing Spondylitis Quality of Life at 6 months ($P = .001$) and Pain Self-Efficacy Questionnaire at 12 months ($P = .002$).

Conclusion: This ASSMP demonstrated significant and sustained benefit in symptoms, disease activity measures and quality of life in a condition that results in significant impairment, disability and poorer quality of life. The cost effectiveness and benefit of this program should be tested.

KEYWORDS

ankylosing spondylitis, education

1 | INTRODUCTION

Ankylosing spondylitis (AS) is a progressive chronic inflammatory condition which primarily affects the sacroiliac joints and the spine but other joints and occasionally other organs can be affected.[1] The symptoms of AS are predominantly pain, fatigue, depression and physical limitations but these symptoms can vary greatly from person to person. For some the symptoms may start in a peripheral joint, causing pain, stiffness and swelling at the site of ligament or tendon attachment to a bone (enthesitis or enthesopathy) or the ribs where they attach to the thoracic spine and the sternum (costochondritis). The pain of AS tends to differ from other types of backache as it improves with exercise but not with rest or inactivity and is often accompanied by alternating buttock pain. The pain and stiffness are usually worse during the night, often severe enough to interrupt sleep and in the early morning these symptoms often last minutes to hours. The onset is often gradual and spasmodic but can be sudden and relentless. Other symptoms may include mild fever, weight loss and malaise.

AS prevalence worldwide ranges between 0.007% to 1.7% of the total population.[1,2] Incidence of AS and its genetic association, the Human Leukocyte Antigen B27 gene, varies between ethnic groups. AS is more prevalent in male adults with most symptoms presenting in the late teens to early 30s.[1,2] It is less likely to present after 45 years. Once diagnosed, AS is a manageable condition with good outcomes.

Although the etiology of AS is unknown, life events and stress, both physiological and psychological, can affect it. All inflammatory arthritis conditions have flares and remissions and sometimes these may be triggered by lifestyle events. Furthermore, limitations related to the disease or flares can impact on patients' lifestyles relating to self-care, work and recreation. Understanding these factors will allow patients to avoid precipitants, accept limitations, modify lifestyle and adhere to strategies that have been demonstrated to modify the course of the disease.

Exercise including stretching, aerobic and group exercises and anti-inflammatories form the cornerstone of disease management.[1] Furthermore, new targeted medications have improved health outcomes of AS significantly. Patients with the more severe disease can now be managed with newer pharmacological agents with remission induction as the treatment goal.[1]

Self-management (SM) programs which are patient-centered, problem-focused and action-oriented are important strategies in the optimization of patient management in chronic disease.[3] SM programs utilize educational, behavioral and cognitive strategies to enhance patient empowerment and participation in their disease management.[4-8] Evidence supports the benefit of SM programs in arthritis[3,8-10] with improvement in health status[11,12] with disease-specific[13,14] and tailor-made programs providing the best outcomes.[15,16] We have successfully developed and delivered a program in osteoarthritis (OA)[17,18] and rheumatoid arthritis (RA).[19]

Arthritis & Osteoporosis Western Australia developed and tested a novel AS education and self-management program (ASSMP) modeled on the OA and RA programs to help patients with AS better manage the non-clinical aspects of their disease. The ASSMP was developed with input from various stakeholders including rheumatologists, academics, health professionals and consumers. The ASSMP sought to improve the overall health, quality of life and disease-specific outcomes of AS participants,[20] under ethically approved research conditions.

The main purposes of this project were to:

1. develop an ASSMP
2. have trained allied health professionals deliver the ASSMP to participants
3. implement a project to evaluate the benefit to patients of this program by measuring patient disease-related outcomes in response to the ASSMP
4. if successful, develop facilitators' manuals and toolkits for use by health professionals
5. design and develop "Train the trainer workshop" to train health professionals.

We report the patient-reported outcomes from this program. The detailed methodology and development of the program will be reported separately.

2 | METHODS

2.1 | Ethical approval

The study was approved by the Human Research Ethics Committee at Curtin University, Western Australia (PT245/2013).

2.2 | ASSMP development

Following advisory group participation, consumer input, needs assessment utilizing the Arthritis Education Needs Assessment Tool (ENAT)[21] and focus group discussions, the plan, do, study, act action-oriented research model (PDSA) (Figure 1) was used. This enabled the development of a patient-centered, disease-specific interactive education program using self-management constructs. The intervention was designed to be delivered by trained health professionals.

The 6 week ASSMP was delivered in small-group settings. Delivery included didactic information sessions, interactive teaching and group discussions. The content focused on knowledge, education, self-efficacy, self-management and empowerment of participants. Although each module had a distinct knowledge-based subject, for example "medications", the modules were inter-related and each week built upon information and learning from the previous week. Each week different stretching and relaxation techniques were taught with continuous feedback and goal setting to reinforce self-management facilitated by the same leaders to promote group cohesion and bonding (Table 1).

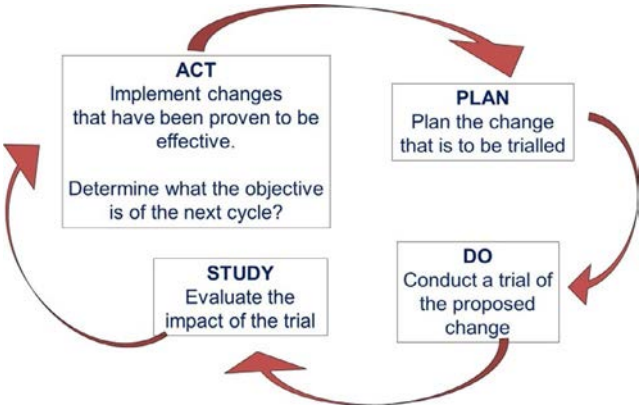


FIGURE 1 Action research model for development (plan, do, study, act [PDSA]) [Colour figure can be viewed at wileyonlinelibrary.com]

2.3 | Study design

The initial pilot cohort comprised of 59 AS patients (Cohort 1) and the second larger cohort (Cohort 2) comprised 143 patients. Additional information was collated in Cohort 2 including employment, smoking status, relevant comorbid diseases and medication use. A case cohort (within subjects) repeated measures study was used to determine change in disease-specific outcomes over time. Baseline demographics and characteristics were completed with follow up assessments at 6 weeks, 3, 6 and 12 months.

2.4 | Recruitment

Patients with AS were recruited by referral from specialist rheumatologists in Western Australia. Inclusion criteria were clinical diagnosis of AS as confirmed by their rheumatologist and aged 18 years or older. Exclusion criteria included non-English speaking, comorbid inflammatory musculoskeletal disease and/or visual, auditory or cognitive impairment.

2.5 | Intervention

Following baseline assessment, participants attended a weekly 2.5 hours self-management education session facilitated by the same 2 health professionals over 6 weeks. The scripted content incorporated multi-dimensional strategies including stretches and supervised exercise classes.

2.6 | Outcomes measures and statistical analysis

Evaluation of the ASSMP was conducted at baseline and at 6 weeks, 3, 6 and 12 months. To evaluate the outcome and effectiveness of the ASSMP, patients completed questionnaires covering the following: pain (nocturnal and total back pain), fatigue by Multi-Dimensional Assessment of Fatigue (MAF) and a Global Fatigue Index (GFI), anxiety and depression by Hospital Anxiety and Depression Scale (HADS), health distress by Health Distress Questionnaire (HDQ), Pain Self-Efficacy Questionnaire (PSEQ) and Patients Global Disease Activity (PGDA). AS-specific measures of outcomes were analyzed for Bath AS indices – activity by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and function by Bath Ankylosing Spondylitis Global Score (BAS-G) and Bath Ankylosing Spondylitis Functional Index (BASFI), Ankylosing Spondylitis Quality of Life (ASQoL) and the Evaluation Ankylosing Spondylitis Quality of Life (EASi-QoL).

Information was collected based on self-administered questionnaires that are validated.[22,23]

Intensity of nocturnal back pain and total back pain during the last week were each measured using a 1-item numerical rating scale

TABLE 1 Setting SMART goals and program overview

Setting "SMART" goals
A "SMART" goal needs to be:
Something you want to do
Small and specific
Measurable
Achievable
Realistic
Timebound
To be "SMART", you do need to be confident you can complete your goal this week.
Brief program overview (HAND-OUT)
Self-management philosophy and smart goal setting
Learning about ankylosing spondylitis and spondyloarthropathies
Learning about joints and spine
Relaxation and a good night's sleep
Stretches, exercise and fitness for ankylosing spondylitis
Managing fatigue
Ankylosing spondylitis: immune driven–what happens?
Pain management strategies
Cognitive behaviour techniques
Living and coping with ankylosing spondylitis
Medications for ankylosing spondylitis
Blood tests and radiography
Posture and balance
The costs of ankylosing spondylitis
Working with ankylosing spondylitis
Diet for ankylosing spondylitis
Cam (Complementary and Alternative Medicine) for ankylosing spondylitis
Osteoporosis
Pregnancy and ankylosing spondylitis
Sport and ankylosing spondylitis

TABLE 2 Baseline characteristics

Patient characteristic	Category	Cohort 1 N = 59	Cohort 2 N = 143	Combined N = 202
		n (%)	n (%)	n (%)
Gender	Female	32 (54.2)	80 (55.9)	112 (55.4)
	Male	27 (45.8)	63 (44.1)	90 (44.6)
Age, y, mean (SD)		57.0 (14.3)	45.3 (14.3)	48.5 (15.2)
Age at onset, y, mean (SD)		25.0 (4.2)	28.6 (12.9)	28.5 (12.8)
Age at diagnosis, y, mean (SD)		41.1 (15.8)	36.0 (13.4)	37.4 (14.2)
Onset to diagnosis, y, median (IQR)			4 (1, 10)	4 (1, 10)
Human leukocyte antigen-B27+	No		64 (31.7)	
	Yes		112 (55.4)	
	Test not done		11 (5.4)	
	Unknown		15 (7.4)	
Radiographic sacroiliitis	No	9 (15.3)	41 (28.7)	50 (24.8)
	Unilateral	9 (15.3)	10 (7.0)	19 (9.4)
	Bilateral	32 (54.2)	87 (60.8)	119 (58.9)
	Unknown	9 (15.3)	5 (3.5)	14 (6.9)
Peripheral arthritis	Yes		33 (25.0)	
Inflammatory bowel disease	Yes		30 (26.1)	
Employed			103 (72.0)	
Employment status	Unemployed		40 (30.1)	
	Part-time		40 (30.1)	
	Full-time		53 (39.8)	
Smoker	Never		82 (57.3)	
	Previous		49 (34.3)	
	Current		12 (8.4)	
Baseline medications	Analgesics		122 (85.3)	
	NSAIDs		117 (81.8)	
	Nonselective NSAIDs		89 (62.2)	
	Selective NSAIDs		46 (32.2)	
	Opioids		8 (5.6)	
	Neuropathic		7 (4.9)	
	Sulfasalazine		10 (7.0)	
	Methotrexate		17 (11.9)	
	Biological disease-modifying agents		46 (32.2)	
	Corticosteroid tablets		10 (7.0)	
	Corticosteroid injections		20 (14.0)	

Abbreviations: IQR, interquartile range; NSAIDs, nonsteroidal anti-inflammatory drugs; SD, standard deviation.

(NRS), (range, 0-10). A higher score indicates greater pain. Numerical rating scales are simple to use, reliable and validated in AS.[22,24]

The PGDA was used to evaluate how the disease activity affected the patient in the past week and is routinely used in rheumatology clinics.[23,25] It is a 1-item NRS (range, 0-10) with a higher score indicating greater disease activity. It is quick, simple to use, reliable and validated in AS.[22,23]

The BAS-G score was used to measure the patient's well-being in the last week and the last 6 months. It is a 2-item NRS with a higher score indicating a greater perceived effect of the disease on the patient's well-being (range, 0-10).[20,26] It is simple to use, reliable (r = .84 for 1 week, r = .96 for 6 months), valid and appropriate for use in the clinical setting[20,26] (https://nass.co.uk/wp-content/uploads/2018/09/Bath-Indices.pdf).

The 6-item BASDAI was used to measure patient-reported disease activity. It covers major symptoms of AS (fatigue, spinal pain, joint pain and swelling, areas of localized tenderness and morning stiffness). A higher score indicates greater disease activity (range, 0-10) with a cut-off of 4 indicating active disease.[20,27] BASDAI is quick, simple to use, the reliability is good ($r = .93$)[27] and is validated.[28]

The 10-item BASFI was used to determine the degree of functional limitation. Eight items assess functional anatomy (bending, reaching, changing position, standing, turning and climbing steps) and 2 assess the patient's ability to cope with everyday life. A score of 10 indicates maximal impairment (range, 0-10).[20,29] BASFI is quick, simple to use, reliable ($r = .87$) and validated.[20,26,29,30]

The ASQoL scale was used to measure the impact of AS on health-related quality of life. It consists of 18 items relating to the impact of AS on sleep, mood, motivation, coping, activities of daily living, independence, relationships and social life.[20,31] A higher score indicates a greater impairment of health-related quality of life (range, 0-18). The ASQoL is quick, simple to use and reliable ($r = .91$-$.92$).[20,31] It correlates moderately well with other AS-specific health outcome measures including BASFI (correlation coefficient 0.72-0.75)[31] and BASDAI (0.79).[28]

MAF was used to assess multiple dimensions of fatigue and provides for a GFI.[32-35] It consists of 16 items covering various dimensions of fatigue (degree, severity, distress, interference in activities of daily living) and frequency over the previous week. A higher score indicates greater fatigue (range, 1-50). MAF is quick, easy to use and is used in clinical practice.[36] The MAF correlated moderately with pain ($r = .39$).[35]

HADS measures cognitive and emotional aspects of anxiety and depression.[37] HADS is a 14-item questionnaire consisting of 2 subscales: anxiety (HADS-A) and depression (HADS-D) each with 7 items.[37] Higher scores indicate greater severity. The scores for HADS range 0-42 and HADS subscale range 0-21. It is reliable (Cronbach's alpha: HADS-A .68-.93 and HADS-D .67-.90) and are validated.[37,38]

The 4-item HDQ was used to evaluate the level of discouragement, fear, worry and frustration experienced in relation to the condition over the previous month.[39] Higher scores indicate more distress about health (range 0-5)[39] (https://www.selfmanagementresource.com/docs/pdfs/English_-_healthdistress.pdf).

The EASi-QoL questionnaire consists of 20 items and measures the impact of AS on 4 quality of life domains: physical function (PF), disease activity (DA), emotional well-being (EWB) and social participation (SP).[40,41] Scores for individual domains are as follows: PF (6 items, range 0-24), DA (4 items, range 0-16), EWB (5 items, range 0-20) and SP (5 items, range 0-20). A higher composite score indicates a more severe impact (range 0-80). The EASi-QoL scale is reliable (Cronbach's alpha = .90 or higher, except for DA [.88]) and a valid measure of AS-specific quality of life.[40,41]

The PSEQ was used to measure a patient's confidence in performing specific activities despite their pain.[42] It is a 10-item questionnaire and a higher score reflects a stronger self-efficacy belief (range 0-60).[42] It is reliable (Cronbach's alpha = .92) and is validated.[42,43]

Patient characteristics at baseline were summarized using frequency distributions for categorical data and means and standard deviations (SDs) or medians and interquartile range (IQR) for continuous data. Linear mixed models (LMM) with random subject effects were used to estimate change in longitudinal measures over 5 time points. LMM use maximum likelihood estimation methods to estimate parameters using all available cases, regardless of missing data points. Results were summarized as estimated marginal means and mean change from baseline with 95% confidence intervals (CI). Longitudinal categorical data were summarized using contingency tables and time by category differences assessed using Chi-squared tests. All hypothesis tests were 2-sided and P values <.05 were considered statistically significant. Stata v16.0 (StataCorp, College Station, TX, USA) was used for analysis.

3 | RESULTS

Baseline characteristics are summarized in Table 2. At baseline 55% were female and the mean age was 48.5 ± 15.2 years. The median time to AS diagnosis from the index symptom experience was 4 years (IQR: 1-10). Sixty-eight percent of enrolled subjects had documented sacroiliitis (SI) with bilateral SI reported in 58%. In Cohort 2, 69% were employed, 8% were current and 34% ex-smokers. Baseline medication use was collated from Cohort 2 (Table 2). Eighty-five percent were taking analgesics, 82% nonsteroidal anti-inflammatories (NSAIDS), 7% sulfasalazine, 12% methotrexate and 32% biological disease-modifying antirheumatic drugs (bDMARDS). Baseline disease activity, quality of life and follow up are illustrated in Table 3 and Figures 2 and 3. Baseline disease activity measured by BASDAI was mild to moderate with a mean (95% CI) of 5.27 (4.99-5.56) (Table 3).

Longitudinal measures using continuous data were collated for the combined group and are presented in Table 3 and Figures 2 and 3. Pain scores measured as nocturnal back pain and total back pain improved at 6 and 12 months ($P < .001$) (Table 3, Figure 2). Patient AS-specific global well-being (BAS-G) improved at 3, 6 and 12 months ($P < .001$) and PGDA at 6 ($P = .012$) and 12 ($P < .001$) months (Table 3, Figure 2).

In the composite indices of activity and function in AS (Table 3, Figure 2), BASDAI improved at 6 weeks, 3, 6 and 12 months ($P < .001$). The BASDAI mean improved from moderate to minimal disease activity (4.17 with cut-off 4) and a mean (95% CI) improvement of −1.10 (−1.41, −0.80). The composite index of function, BASFI, improved by 12 months ($P < .001$) from a mean (95% CI) of 3.83 (3.53, 4.14) to 3.34 (2.99, 3.69) with a mean (95% CI) change from baseline of −0.49 (−0.74, −0.24).

MAF-GFI improved by 6 months ($P = .003$) and was sustained at 12 months ($P = .009$) (Table 3, Figure 2). Anxiety and depression scale, HADS ($P = .002$) (Table 3, Figure 2) and subscale for anxiety, HADS-A ($P = .001$) (Table 3) improved by 12 months. Health distress score, HDQ improved by 3 months ($P = .020$) and at 6 and 12 months ($P < .001$) (Table 3, Figure 3).

ASQoL (Table 3, Figure 3) improved at 3, 6 and 12 months by greater than 1 point at all time points ($P = .001$). Total EASi-QoL ($P = .001$) and all 4 EASi-QoL domains: PF, DA, EWB and

TABLE 3 Longitudinal ankylosing spondylitis outcome measures

Outcome	Follow up	Estimated mean (95% CI)	Mean change from baseline	*P* value
Nocturnal back pain (0-10)	Baseline	4.45 (4.06-4.85)		
	6 wk	4.03 (3.63-4.42)	−0.43 (−0.74, −0.11)	.008
	3 mo	4.11 (3.67-4.56)	−0.34 (−0.71, 0.03)	.070
	6 mo	3.83 (3.39-4.26)	−0.63 (−0.98, −0.27)	.001
	12 mo	3.57 (3.12-4.02)	−0.88 (−1.26, −0.50)	<.001
Total back pain (0-10)	Baseline	5.15 (4.76-5.53)		
	6 wk	4.85 (4.46-5.24)	−0.29 (−0.64, 0.06)	.012
	3 mo	4.83 (4.38-5.27)	−0.32 (−0.73, 0.09)	.129
	6 mo	4.38 (3.95-4.81)	−0.77 (−1.16, −0.37)	<.001
	12 mo	4.21 (3.76-4.67)	−0.93 (−1.35, −0.51)	<.001
PGDA (0-10)	Baseline	4.90 (4.53-5.27)		
	6 wk	4.91 (4.54-5.28)	0.01 (−0.34, 0.36)	.972
	3 mo	4.66 (4.23-5.09)	−0.24 (−0.65, 0.17)	.246
	6 mo	4.40 (3.99-4.81)	−0.50 (−0.90, −0.11)	.012
	12 mo	4.09 (3.65-4.52)	−0.81 (−1.23, −0.40)	<.001
BAS-G (0-10)	Baseline	5.60 (5.25-5.96)		
	6 wk	5.42 (5.06-5.78)	−0.18 (−0.48, 0.11)	.215
	3 mo	4.85 (4.44-5.25)	−0.76 (−1.10, −0.42)	<.001
	6 mo	4.60 (4.21-4.99)	−1.01 (−1.34, −0.68)	<.001
	12 mo	4.12 (3.71-4.53)	−1.48 (−1.83, −1.13)	<.001
BASDAI (0-10)	Baseline	5.27 (4.99-5.56)		
	6 wk	4.86 (4.57-5.14)	−0.42 (−0.64, −0.19)	<.001
	3 mo	4.54 (4.20-4.89)	−0.73 (−1.03, −0.43)	<.001
	6 mo	4.48 (4.19-4.78)	−0.79 (−1.03, −0.55)	<.001
	12 mo	4.17 (3.82-4.52)	−1.10 (−1.41, −0.80)	<.001
BASFI (0-10)	Baseline	3.83 (3.53-4.14)		
	6 wk	3.96 (3.65-4.26)	0.13 (−0.05, 0.31)	.166
	3 mo	3.67 (3.32-4.01)	−0.16 (−0.41, 0.08)	.187
	6 mo	3.70 (3.38-4.01)	−0.13 (−0.33, 0.06)	.180
	12 mo	3.34 (2.99-3.69)	−0.49 (−0.74, −0.24)	<.001
ASQoL (0-18)	Baseline	8.41 (7.42-9.41)		
	6 wk	8.10 (7.10-9.09)	−0.32 (−0.97, 0.34)	.347
	3 mo	7.27 (6.24-8.29)	−1.15 (−1.85, −0.44)	.001
	6 mo	7.27 (6.24-8.30)	−1.14 (−1.85, −0.44)	.001
	12 mo	6.94 (5.90-7.98)	−1.47 (−2.20, −0.74)	.001
GFI (MAF) (1-50)	Baseline	26.47 (23.89-29.05)		
	6 wk	27.14 (24.55-29.73)	0.67 (−1.10, 2.43)	.460
	3 mo	24.61 (21.94-27.29)	−1.86 (−3.75, 0.03)	.054
	6 mo	23.56 (20.88-26.24)	−2.91 (−4.81, −1.01)	.003
	12 mo	23.85 (21.13-26.57)	−2.62 (−4.57, −0.67)	.009
HADS (0-42)	Baseline	14.40 (13.21-15.58)		
	6 wk	14.27 (13.08-15.46)	−0.13 (−0.97, 0.71)	.761
	3 mo	14.01 (12.66-15.36)	−0.39 (−1.44, 0.67)	.471
	6 mo	13.95 (12.73-15.17)	−0.45 (−1.34, 0.44)	.324
	12 mo	12.63 (11.25-14.01)	−1.77 (−2.86, −0.67)	.002

(Continues)

TABLE 3 (Continued)

Outcome	Follow up	Estimated mean (95% CI)	Mean change from baseline	*P* value
HADS-A (0-21)	Baseline	8.29 (7.61-8.96)		
	6 wk	8.00 (7.33-8.68)	−0.28 (−0.81, 0.24)	.287
	3 mo	7.83 (7.05-8.61)	−0.46 (−1.11, 0.20)	.170
	6 mo	7.83 (7.14-8.53)	−0.45 (−1.00, 0.10)	.109
	12 mo	7.10 (6.30-7.90)	−1.19 (−1.87, −0.51)	.001
HADS-D (0-21)	Baseline	6.11 (5.47-6.75)		
	6 wk	6.26 (5.62-6.91)	0.15 (−0.31, 0.61)	.522
	3 mo	6.18 (5.45-6.91)	0.07 (−0.51, 0.64)	.824
	6 mo	6.12 (5.46-6.78)	0.00 (−0.48, 0.49)	.986
	12 mo	5.53 (4.78-6.27)	−0.58 (−1.18, 0.01)	.055
HDQ (0-5)	Baseline	2.40 (2.21-2.59)		
	6 wk	2.31 (2.12-2.50)	−0.09 (−0.26, 0.08)	.304
	3 mo	2.15 (1.91-2.38)	−0.25 (−0.47, −0.04)	.020
	6 mo	2.03 (1.83-2.23)	−0.37 (−0.55, −0.19)	<.001
	12 mo	1.99 (1.75-2.23)	−0.41 (−0.63, −0.19)	<.001
EASiQoL1_PF (0-24)	Baseline	7.72 (6.99-8.46)		
	6 wk	8.15 (7.41-8.88)	0.42 (−0.06, 0.90)	.084
	3 mo	7.58 (6.69-8.48)	−0.14 (−0.84, 0.56)	.695
	6 mo	7.11 (6.34-7.88)	−0.61 (−1.14, −0.08)	.024
	12 mo	6.39 (5.48-7.29)	−1.34 (−2.05, −0.62)	<.001
EASiQoL2_DA (0-16)	Baseline	7.69 (7.16-8.23)		
	6 wk	7.75 (7.21-8.29)	0.06 (−0.37, 0.48)	.788
	3 mo	7.23 (6.53-7.93)	−0.46 (−1.08, 0.16)	.142
	6 mo	6.91 (6.34-7.49)	−0.78 (−1.25, −0.31)	.001
	12 mo	6.90 (6.19-7.61)	−0.79 (−1.43, −0.16)	.014
EASiQoL3_EWB (0-20)	Baseline	7.16 (6.50-7.82)		
	6 wk	7.41 (6.74-8.07)	0.25 (−0.29, 0.78)	.365
	3 mo	6.20 (5.33-7.07)	−0.96 (−1.73, −0.19)	.015
	6 mo	6.36 (5.65-7.07)	−0.80 (−1.39, −0.21)	.008
	12 mo	5.47 (4.58-6.35)	−1.69 (−2.49, −0.90)	<.001
EASiQoL4_SP (0-20)	Baseline	7.49 (6.78-8.20)		
	6 wk	7.23 (6.52-7.94)	−0.26 (−0.83, 0.31)	.369
	3 mo	6.94 (6.02-7.87)	−0.55 (−1.37, 0.28)	.195
	6 mo	6.78 (6.03-7.54)	−0.71 (−1.34, −0.07)	.028
	12 mo	5.47 (4.58-6.35)	−1.53 (−2.37, −0.68)	<.001
Total EASi-QoL (0-80)	Baseline	30.07 (27.71 - 32.43)		
	6 wk	30.55 (28.18-32.91)	0.48 (−1.13, 2.09)	.560
	3 mo	27.96 (25.04-30.88)	−2.11 (−4.46, 0.24)	.079
	6 mo	27.14 (24.66-29.63)	−2.92 (−4.71, −1.13)	.001
	12 mo	24.71 (21.75-27.68)	−5.35 (−7.76, −2.94)	<.001

(Continues)

TABLE 3 (Continued)

Outcome	Follow up	Estimated mean (95% CI)	Mean change from baseline	*P* value
PSEQ (0-60)	Baseline	38.14 (35.71-40.56)		
	6 wk	37.36 (34.93-39.79)	−0.78 (−2.67, 1.12)	.420
	3 mo	38.27 (35.73-40.80)	0.13 (−1.89, 2.16)	.898
	6 mo	38.94 (36.40-41.48)	0.80 (−1.23, 2.84)	.438
	12 mo	41.41 (38.82-44.00)	3.28 (1.18, 5.37)	.002

Note: Lower scores in pain, PGDA, BAS-G, BASDAI, BASFI, ASQoL, GFI, HADS (HADS-A and HADS-D), HDQ and EASiQoLl indicates improvement. A higher score in PSEQ indicates improvement.

Abbreviations: ASQol, Ankylosing Spondylitis Quality of Life; BASDAI, Bath AS Disease Activity Index; BASFI, Bath AS Functional Index; BAS-G, Bath AS Global Score; CI, Confidence intervals; EASi-QoL, Evaluation of Ankylosing Spondylitis Quality of Life; Easiqol1_PF, Physical function; Easiqol2_DA, Disease activity; Easiqol3_EWB, Emotional well-being; Easiqol4_SP, Social participation; GFI-(MAF) = Global Fatigue Index-(Multi-Dimensional Assessment of fatigue); HADS, Hospital Anxiety and Depression Scale; HADS-A, HADS-Anxiety; HADS-D, HADS-Depression; HDQ, Health Distress Questionnaire; PGDA, Patient Global Disease Activity; PSEQ, Pain Self-Efficacy Questionnaire.

SP improved at 6 and 12 months (all $P < .03$) (Table 3, Figure 3). Pain self-efficacy score, PSEQ improved by 12 months ($P = .002$) (Table 3, Figure 3).

4 | DISCUSSION

Simple pharmacological treatments alone are not adequate to control many rheumatological conditions including AS. Patient understanding, action and involvement are key components to optimize care and outcomes. Adherence and persistence are major barriers to optimal outcomes and this relates partly to poor patient understanding and expectation of disease processes and progression. We have previously demonstrated improved outcomes in patients with osteoarthritis and RA enrolled in our inhouse-designed and implemented SM programs.[18,19] Here we designed and implemented a similar program which is patient-centered, problem-focused and action-oriented for patients with AS. The focus of this study was to enhance patient empowerment and participation in their disease management, utilizing educational, behavioral and cognitive strategies.[4-8]

Back pain, especially nocturnal rest pain affecting sleep and early morning stiffness are significant complaints in people with AS. We demonstrated a significant and early improvement in back pain by 6 months with sustained improvement at 12 months. There was a trend to improvement from as early as 6 weeks, that is, toward the end of the intervention period. This symptomatic improvement parallels the patient's global appreciation of improved disease activity measured by PGDA. This has translated into improvement of composite measures of disease activity (BASDAI) as early as 6 weeks and functional ability (BASFI) by 12 months which are more reliable than single-item reports and are utilized in follow up assessment of patients with AS to demonstrate response, efficacy and justify treatment continuation.

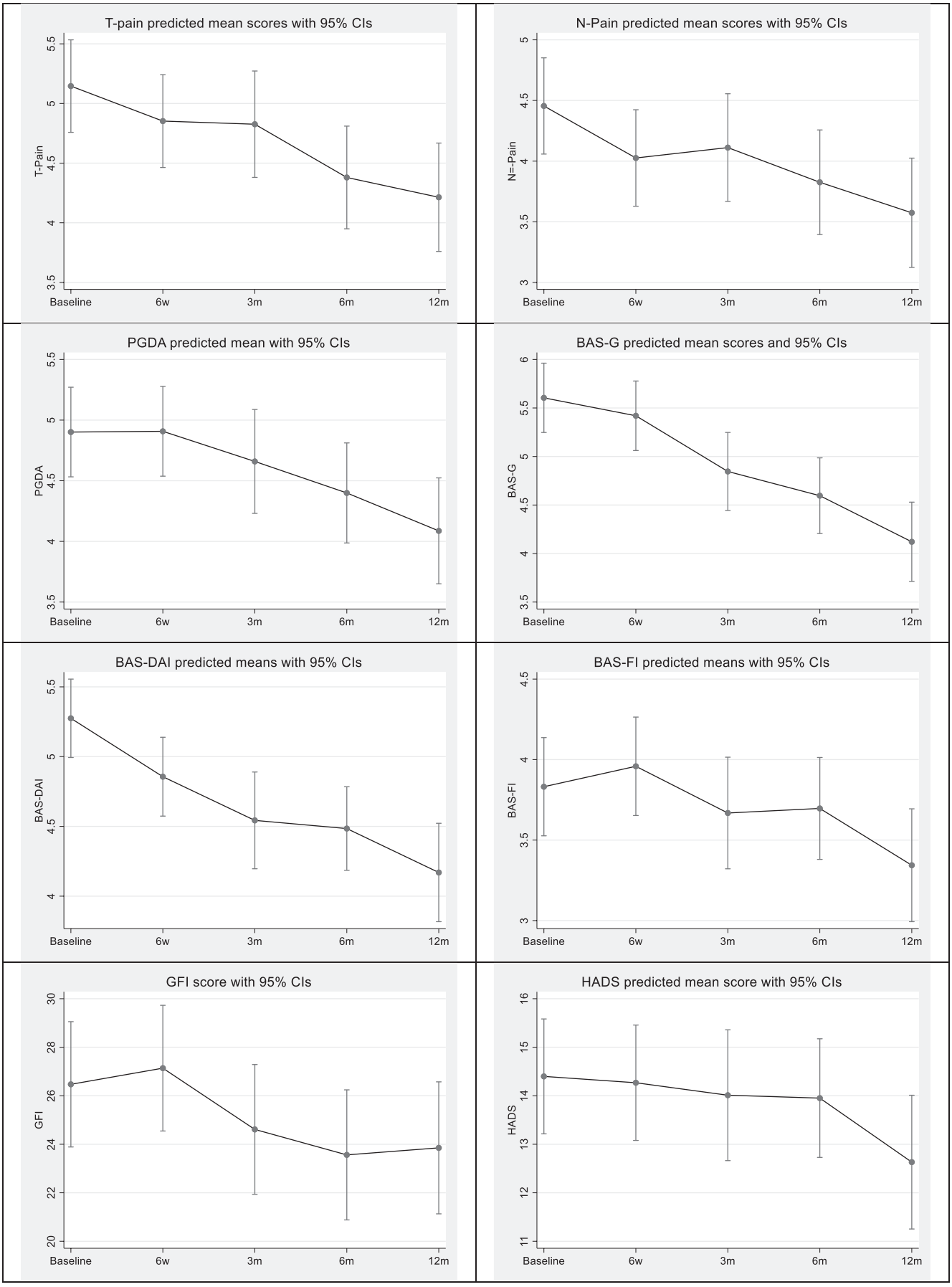
Quality of life measures are important in all populations with chronic disease but especially so in an AS population who are younger and are expected to be actively involved in the workforce and active social and recreational activities. The improvement in quality of life measures (ASQoL and EASi-QoL) suggests that patients in this program achieved these objectives as they improved in all domains, including physical function, emotional well-being and social participation in parallel with improvement in disease activity scores.

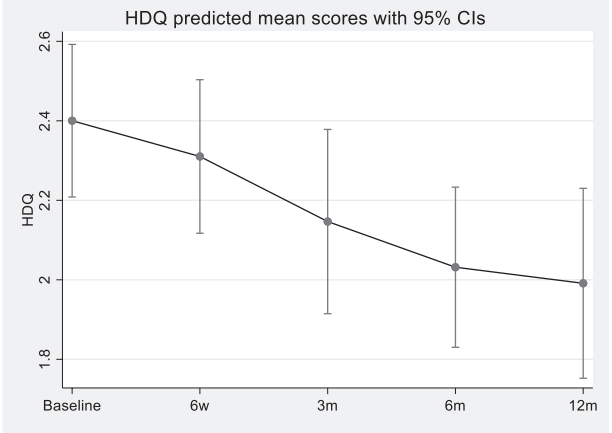
The presence of disabling fatigue, distress and poor health results in significant mood symptoms in the AS population. The improvement in the fatigue score (MAF), anxiety and depression score (HADS) and health distress score (HDQ) are important positive consequences of the physical and functional benefits resulting from the intervention program.

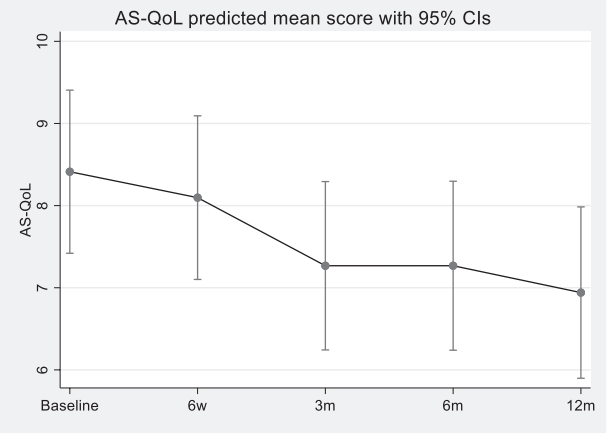
It is known that exercise is important but not adequate to control disease in the majority of patients, especially with disease progression and severity. Hence medications including anti-inflammatories and disease-modifying agents are important treatments. It is possible that involvement in the program has educated and may empower patients to proactively see their usual medical practitioners to optimize the medical management of their disease as well. There is a strong evidence base for the efficacy of treatment available including biological treatments.

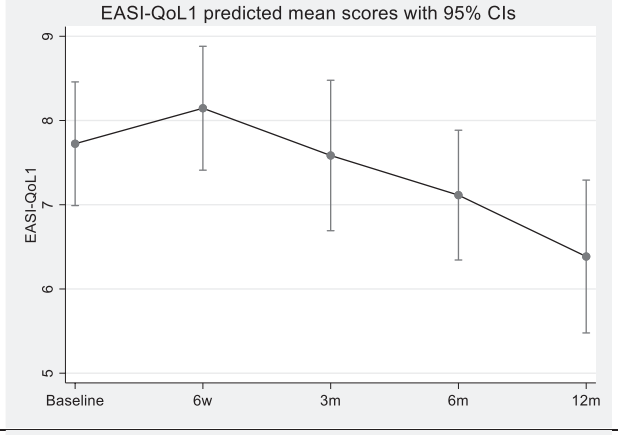
The main limitation of this study is that it is a single group repeated measures study rather than a randomized control study with a control group. A second limitation is the preponderance of females in the cohort when AS predominantly affects males. However, males were well represented. The third limitation is that patients had moderate disease activity at baseline. It is uncertain whether this program will achieve the same outcome in patients with more severe or milder disease. However, it may be prudent to target this program at patients with earlier disease so that proactive early management can be optimized to prevent progression.

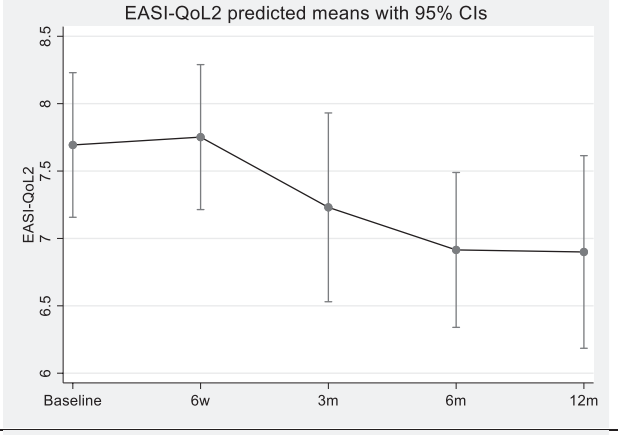
FIGURE 2 Physical and functional activity outcomes. BAS-DAI, Bath Ankylosing Spondylitis Disease Activity Index; BAS-FI, Bath Ankylosing Spondylitis Functional Index; BAS-G, Bath Ankylosing Spondylitis Global Score; GFI, Global Fatigue Index; HADS, Hospital Anxiety and Depression Scale; N-Pain, Nocturnal back pain; PGDA, Patient Global Disease Activity; T-pain, Total back pain

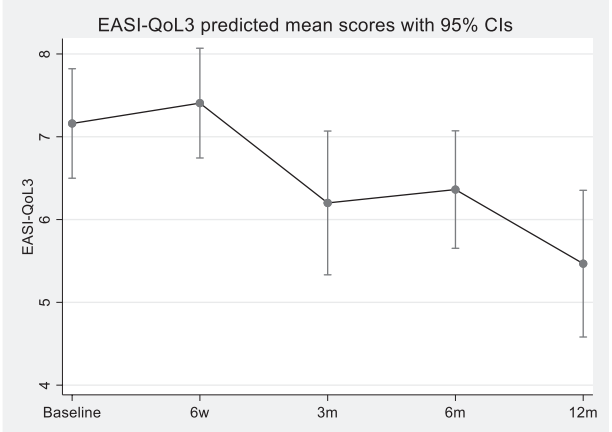

T-pain predicted mean scores with 95% CIs
T-Pain
N-Pain predicted mean scores with 95% CIs
N=-Pain
PGDA predicted mean with 95% CIs
PGDA
BAS-G predicted mean scores and 95% CIs
BAS-G
BAS-DAI predicted means with 95% CIs
BAS-DAI
BAS-FI predicted means with 95% CIs
BAS-FI
GFI score with 95% CIs
GFI
HADS predicted mean score with 95% CIs
HADS
Baseline
6w
3m
6m
12m

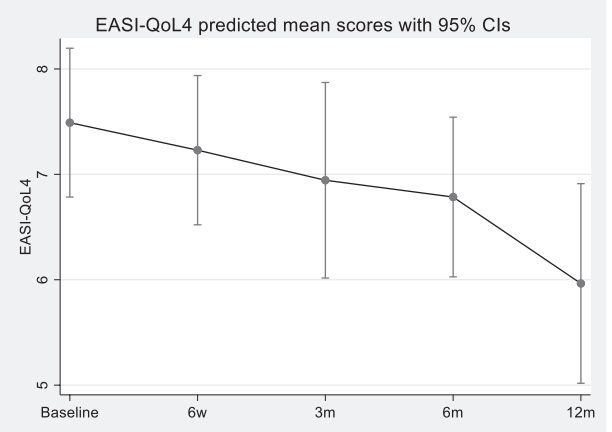

HDQ predicted mean scores with 95% CIs
HDQ
1.8
2
2.2
2.4
2.6
Baseline
6w
3m
6m
12m

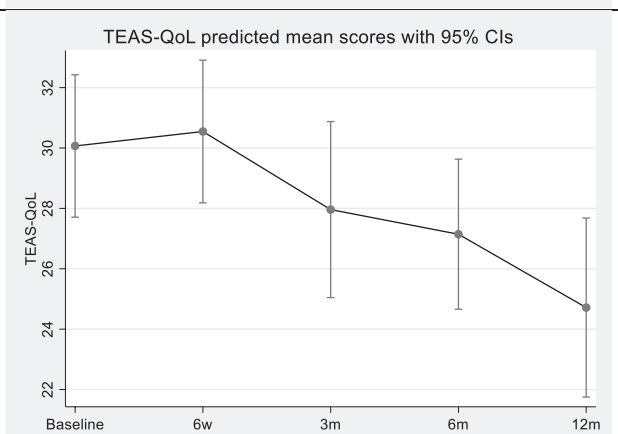

AS-QoL predicted mean score with 95% CIs
AS-QoL
6
7
8
9
10
Baseline
6w
3m
6m
12m


EASI-QoL1 predicted mean scores with 95% CIs
EASI-QoL1
5
6
7
8
9
Baseline
6w
3m
6m
12m


EASI-QoL2 predicted means with 95% CIs
EASI-QoL2
6
6.5
7
7.5
8
8.5
Baseline
6w
3m
6m
12m


EASI-QoL3 predicted mean scores with 95% CIs
EASI-QoL3
4
5
6
7
8
Baseline
6w
3m
6m
12m


EASI-QoL4 predicted mean scores with 95% CIs
EASI-QoL4
5
6
7
8
Baseline
6w
3m
6m
12m


TEAS-QoL predicted mean scores with 95% CIs
TEAS-QoL
22
24
26
28
30
32
Baseline
6w
3m
6m
12m

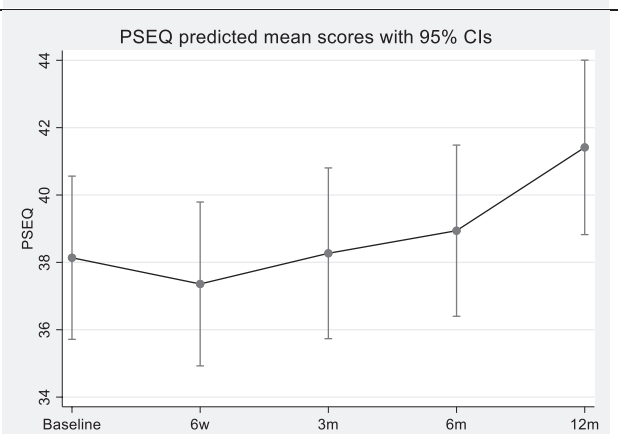

PSEQ predicted mean scores with 95% CIs
PSEQ
34
36
38
40
42
44
Baseline
6w
3m
6m
12m

FIGURE 3 Quality of life outcome measures. AS-QoL, Ankylosing Spondylitis Quality of Life; EASI-QoL1, Evaluation of Ankylosing Spondylitis Quality of Life - Physical function; EASI-QoL2, Evaluation of Ankylosing Spondylitis Quality of Life - Disease activity; EASI-QoL3, Evaluation of Ankylosing Spondylitis Quality of Life - Emotional well-being; EASI-QoL4, Evaluation of Ankylosing Spondylitis Quality of Life - Social participation; HDQ, Health Distress Questionnaire; PSEQ, Pain Self-efficacy Questionnaire; TEASI-QoL, Total Evaluation of Ankylosing Spondylitis Quality of Life

It is also difficult to determine the positive contribution of any particular aspect of this intervention. We do not have adequate information on medication change which may be a significant contributor. However, it may be reasonable to assume that more informed patients are more likely to be proactive in pursuing and complying with medical management. We know that simple contact with a healthcare provider or facility has a positive impact on patient care and quality of life (placebo effect). However, the persistence of benefit for up to 12 months suggests that it is the wider intervention, including education, empowerment and self-management, which may be important.

The strength of this study is that it is a customized program developed specifically for this patient disease group, was delivered by qualified healthcare professionals with an understanding of the condition and in a grouped supportive environment (support group benefit). Patients had median disease duration of 3 years with moderate disease activity, suggesting suboptimal management at baseline with a significant improvement following exposure to the program. However, there is a cost of this intervention that needs to be factored in.

5 | CONCLUSIONS

This custom-developed ASSMP has demonstrated significant and sustained benefit in terms of patient symptoms, disease activity measures and quality of life in a condition that results in significant impairment, disability and poorer quality of life. This program is reproducible in most other similar settings. A more general rollout would have significant positive impact on patient care and outcomes in a relatively young cohort. It has the potential to improve work productivity and contribution to society instead of increasing the burden of care and costs to society. The benefit of this program can be tested in a randomized controlled trial with cost effectiveness outcomes included.

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ORIGINAL ARTICLE

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The efficacy of manual soft-tissue mobilization in ankylosing spondylitis: A randomized controlled study

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Abstract

Aim: The aim of this randomized controlled study was to investigate the effect of soft-tissue mobilization in patients with ankylosing spondylitis (AS).

Method: Twenty-one patients (mean age 44.57 ± 10.40 years) were randomly divided into two groups. There were 13 patients (11 females, 2 males, age 43.69 ± 9.94 years) in the intervention group and 8 patients (5 females, 3 males, age 46.00 ± 11.67 years) in the control group. In the intervention group, soft-tissue mobilization therapy and 20 spinal mobility exercises were applied. The control group received only 20 spinal mobility exercises. The Bath AS Disease Activity Index (BASDAI), Bath AS Functional Index (BASFI), and Bath AS Metrology Index (BASMI) were used for assessment of disease activity, functional level, and mobility, respectively. Nottingham Health Profile (NHP) for quality of life and Roland Morris Disability Questionnaire (RMDQ) were used to determine disability levels.

Results: We found significant differences between pretreatment and post-treatment scores of BASDAI ($P = 0.049$); BASFI ($P = 0.009$; lateral lumbar flexion ($P = 0.005$), maximal intermalleolar distance ($P = 0.001$) and total score ($P = 0.001$) of BASMI; pain subtest ($P = 0.036$) and total score ($P = 0.036$) of NHP; and RMDQ score ($P = 0.004$) in the intervention group. However, in the control group the BASMI score ($P = 0.049$) was observed to worsen significantly. Delta values were compared and differences in BASFI ($P = 0.039$), and in lateral lumbar flexion ($P = 0.027$), maximal intermalleolar distance ($P = 0.045$) and total score ($P = 0.001$) of BASMI were significant in favor of intervention group. Only tragus-to-wall distance ($P = 0.039$) of BASMI was observed to worsen significantly in the control group.

Conclusion: We recommend the use of soft-tissue mobilization in addition to the exercises to treat AS patients.

KEYWORDS

ankylosing spondylitis, exercise, manual therapy

1 | INTRODUCTION

Ankylosing spondylitis (AS) is a chronic immuno-inflammatory disease characterized by inflammation of sacroiliac joints and inflammatory back pain, mainly affecting the axial skeleton by causing pain and limitations in thoracic and spinal mobility.[1,2]

Structures and soft tissues in the low back, hip, and pelvic region are suitable areas for the occurrence of symptoms in AS.[3] The

most common and characteristic symptom of AS is insidious onset inflammatory low back pain. It is usually felt in the deep gluteal and sacroiliac region.[4] Sacroiliac pain can also cause pain in the back, buttocks, groin, and lower extremity areas. Pain may initially be unilateral or intermittent, but later becomes continuous and bilateral.[5]

The progression of AS occurs with progressive stiffness in the spine, decrease in lumbar lordosis, and increase in thoracic kyphosis. Some patients with AS complain of a feeling of musculotendinosis stiffness and/or sensitive points. Pathological changes occur in tendons, attachment points of ligaments to bone, and cartilaginous and synovial joints.[5] The thoracic and spinal joints become stiffer, meld, and progressively lose their mobility. As a result of all these influences, disability increases.[6,7]

Physiotherapy interventions have an essential role in the treatment of AS and in the prevention of musculoskeletal deformities. The aim of physiotherapy interventions in AS is to maintain and/or improve overall functionality and quality of life.[8] In many studies, with physical therapy, it has been emphasized that many patients with AS have had adequate reduction in symptoms (decreased pain and stiffness, maintained or improved posture and muscle strength), improved mobility, functionality, and overall health.[9-14]

One of the commonly used physiotherapy interventions is manual therapy. The term manual therapy covers joint manipulation/mobilization, myofascial relaxation techniques, soft tissue mobilization, and various massage treatments.[15] Manual treatment techniques are performed to increase soft-tissue flexibility and joint movement, mobilize soft tissues, relieve pain, and reduce swelling and inflammation in soft tissues, and are applied with hands. They are characterized by soft, rhythmic, passive or active assisted movements for each spinal segment.[16,17]

A review in Cochrane points out that randomized controlled trials on physiotherapy interventions other than exercise, such as manual therapy in AS, are few and insufficient. It is recommended that other commonly used physiotherapy interventions, such as manual therapy, should be compared with exercise in future studies.[18]

The aim of this randomized controlled trial was to examine the effect of soft-tissue mobilization applied in addition to exercise on disease activity, functional level, mobility, quality of life, and disability level in patients with AS.

2 | MATERIALS AND METHODS

The effects of soft-tissue mobilization in patients with AS were compared with the control group in our study, which was planned as a randomized controlled parallel group. The participants were randomly divided into the intervention group and the control group. Randomization was carried out by a researcher through a computer program (SPSS.v.22; IBM, Armonk, NY, USA) in charge of allocation. The intervention group underwent 20 spinal mobility exercises and soft-tissue mobilization therapy for the problems of soft tissue as a result of the individual evaluation of each participant in this group. The control group underwent only 20 spinal mobility exercises. All treatment was performed 3 days a week and for 4 weeks for a total of 12 sessions. All evaluations were performed before and after treatment. The disease-specific outcome measures were conducted by a physiotherapist who is specialized in rheumatological rehabilitation. Soft-tissue assessment was performed separately for each participant and for the problems seen as a result of this evaluation, each participant was treated by another physiotherapist experienced in soft-tissue mobilization therapy. Spinal mobility exercises were performed under the supervision of another physiotherapist experienced in rheumatological rehabilitation.

2.1 | Participants

In all, 21 volunteer patients (mean age 44.57 ± 10.40 years) were included in the study. Participants were diagnosed with AS by the same rheumatologist according to the modified New York criteria and referred to the Rheumatological Physiotherapy and Rehabilitation Clinic. In the clinic, participants were screened for eligibility.

Inclusion criteria were: (a) being diagnosed with AS according to the Modified New York criterion; (b) being volunteer for the study; (c) being aged between 20 and 65 years; and (d) regular use of disease-modifying anti-rheumatic drugs, including methotrexate, sulfasalazine, and anti-tumor necrosis factor agents, for 3 months or more, or non-steroidal anti-inflammatory drugs and/or corticosteroids, at a stable dosage for at least 4 weeks. In order not to affect the results of the study, attention was paid to the regular use of the drugs.

Exclusion criteria were: (a) exercising regularly during the last 3 months; (b) the presence of a history of osteoporosis or fracture secondary to osteoporosis; (c) the presence of cardiovascular, pulmonary, orthopedic, and neurological problems that may interfere with exercise (uncontrollable hypertension, heart attack or history of coronary revascularization, history of syncope or exercise-related arrhythmia, decompensated type 1 diabetes mellitus, hip and/or knee arthroplasty); (d) having undergone any surgery in the last 6 months for both groups; (e) communication problems for both groups; and (f) not being able to participate in at least 75% of the treatment. The data of the patients who made any changes in drug treatment during the study were not included in this study and the treatment of the participant was terminated.

Ethical approval of the study was obtained from the local ethics committee. All patients were informed verbally and informed consent forms were signed. The followed procedures were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

2.2 | Evaluations

Evaluations were performed before and after treatment. All evaluations were performed by the same experienced physiotherapists, according to standardized testing protocols and in the same environment where the same conditions were provided. Before starting the tests, patients were allowed to adapt by the same therapists.

Demographic data, habits and disease information of the patients were recorded on the assessment form using the face-to-face interview method. Then, Bath AS Disease Activity Index (BASDAI), Bath AS Functional Index (BASFI), and Bath AS Metrology Index (BASMI) were used to assess disease activity, functional level, and mobility, respectively. Nottingham Health Profile (NHP) was used to assess quality of life and Roland Morris Disability Questionnaire (RMRS) was used to determine disability levels. In addition, the soft-tissue assessment of pelvic, spinal, and neck regions was performed for each patient in the intervention group.

2.2.1 | Bath Ankylosing Spondylitis Activity Disease Activity Index (BASDAI)

This index, developed to assess disease activity, consists of six VAS measurements. These are measurements of fatigue, spine and peripheral joint pain, sensitivity, and morning stiffness.[19]

2.2.2 | Bath Ankylosing Spondylitis Functional Index (BASFI)

This index consists of eight questions about daily activities and two questions that evaluate the patient's ability to cope with daily life. Patients mark the degree of difficulty they experienced in performing the specified tasks on the 10-cm VAS. The total score is calculated by taking the average of the score from 10 questions ranging from 0 to 10.[20]

2.2.3 | Bath Ankylosing Spondylitis Metrology Index (BASMI)

The BASMI has five components (lateral lumbar flexion, tragus-to-wall distance, lumbar flexion (modified Schober), maximal intermalleolar distance, cervical rotation). The scale score for each component ranges from 0 to 10. Then the total score is divided by five.[21]

2.2.4 | Nottingham Health Profile (NHP)

This consists of six subtests that assess the emotional, social, and physical health problems perceived by the patient. The survey consists of a total of 38 questions that require a yes/no answer. Positive answers to questions have a predetermined score, and the sum of these scores gives the total score. The total score of each subtest is 100. The sum of the subtest scores can be given as a profile.[22,23]

2.2.5 | Roland Morris Disability Questionnaire (RMDQ)

In the questionnaire that consists of 24 sentences, patients are asked to answer each sentence in the form of yes if it fits their situation and no if it does not. A total score of 0-24 is calculated by giving 1 point to "yes" answers and 0 points to "no" answers in the evaluation form, which consists of 24 items related to functional inadequacies. A high score expresses further disability.[24,25]

2.2.6 | Soft-tissue assessment (pelvic, spinal, and neck regions)

Soft-tissue assessment was performed in the prone and/or supine positions as the muscles could be palpated more easily. It was first started from the pelvic area. Muscles located in this region, such as m. gluteus maximus, m. gluteus medius and m. piriformis were evaluated with palpation for pain, spasm, and sensitivity. Evaluation continued by palpation of lumbar region paravertebral muscles, m. quadratus lumborum, and m. iliopsoas. Lumbosacral fascia mobility was assessed. Thoracic and cervical paravertebral muscles, m. rhomboideus, m. levator scapulae, and m. trapezius were assessed by palpation for pain, spasm, and sensitivity. Cervico-thoracic fascia was assessed for mobility. Following spinal evaluation, the lower and upper extremity muscles were evaluated with palpation for pain, spasm, and sensitivity. Hamstring muscles and iliotibial band were evaluated for tonus and sensitivity in tendon points.

2.3 | Intervention

2.3.1 | Control group (spinal mobility exercises)

Patients in this group underwent 20 spinal mobility exercises, lasting approximately 30 minutes, aimed solely at spine mobility and flexibility. These 20 spinal mobility exercises consisted of cervical, thoracic, and lumbar spine flexibility exercises, shoulder complex, hamstring, quadriceps, and erector spinal muscle stretching and abdominal, back, and proximal muscle strengthening with diaphragmatic breathing and chest expansion exercises. All sessions were conducted under the supervision of a physiotherapist. The exercises were conducted 3 days a week for 4 weeks.

2.3.2 | Intervention group (soft-tissue mobilization + spinal mobility exercises)

Patients in this group were prescribed soft-tissue mobilization treatment that lasted 30 minutes for each patient for problems seen as a result of the individual soft-tissue evaluation. According to manual therapists, manual mobilization techniques were applied to increase spinal mobility.[17] During the course of treatment, the patient's current condition was improved by ensuring the equilibrium between increased pain and increased mobility. As described by Chamberlain, Cyriax's friction massage technique was applied to muscles that were found to be painful, under spasms or sensitive.[26] Friction massage was applied transversely to the specific tissue-involving muscles. Superficial massage (fascial stretch) was applied to fascia fibers longitudinally as described by Manheim.[27] Lewit and Simon's post-isometric relaxation techniques were applied for muscles, such as paravertebral muscles, m. Trapezius, and m. iliopsoas.[27] Active stretching was applied for long muscles, such as hamstring.[28] These patients also performed 20 spinal mobility exercises, which lasted approximately 30 minutes, following the same protocol as the training applied to the control group. All sessions were conducted under the supervision of a physiotherapist. The treatment was conducted 3 days a week for 4 weeks.

2.4 | Statistical analysis

The data were analyzed with the SPSS (version 21.0) package program. Continuous variables were given as mean ± standard deviation and categorical variables were given as numbers and percentages. Independent *t* test test was used to compare the differences between the two averages when parametric test assumptions were provided, while the Mann-Whitney *U* test was used to compare the differences between the groups when parametric test assumptions were not provided. In dependent group comparisons, paired sample *t* test was used when parametric test assumptions were provided; and the Wilcoxon signed-rank test was used when parametric test assumptions were not provided. A *P* value less than 0.05 was accepted for statistical significance.

3 | RESULTS

This study started with 32 patients with AS. Four patients declined to participate. A total of 28 patients with AS who met the inclusion criteria of the study were randomly separated into two groups as the intervention group (n = 14) and the control group (n = 14). One patient from the intervention group stopped treatment because his wife had given birth prematurely. In the control group, two patients could not get permission from work and dropped out, three patients dropped out because the distance between the treatment place and the house was too much, and one patient did not continue treatment because he was a teacher and had a lot of classes. The study was therefore completed with a total of 21 patients, 13 patients (11 women, 2 men, age = 43.69 ± 9.94 years) in the intervention group and 8 patients (5 women, 3 men, age = 46.00 ± 11.67 years) in the control group. Figure 1 shows a flow chart of the study design.

The demographic information of the patients was recorded before the evaluations. Demographics are shown in Table 1.

In this study with 21 patients, no problems were reported during evaluations and training. There was no statistical difference between the demographic data of the groups except for height (in meters), because of the higher presence of females in the intervention group ($P > 0.05$) (Table 1).

In the comparison of the data between pretreatment and post-treatment, the difference in BASDAI ($P = 0.049$), BASFI ($P = 0.009$), and lateral lumbar flexion ($P = 0.005$), maximal intermalleolar distance ($P = 0.001$) and total score ($P = 0.001$) of BASMI, pain subtest ($P = 0.036$) and total score ($P = 0.036$) of NHP and RMDQ score ($P = 0.004$) were significant in the intervention group. In the control group, the BASMI score ($P = 0.049$) was found to worsen significantly (Table 2).

The delta values of the participants were calculated by subtracting the pretreatment result from the post-treatment result. Delta values were compared and the differences in BASFI ($P = 0.039$) and lateral lumbar flexion ($P = 0.027$), maximal intermalleolar distance ($P = 0.045$), and total score ($P = 0.001$) of BASMI were significant in favor of intervention group. Tragus-to-wall distance ($P = 0.039$) of BASMI worsened significantly in the control group (Table 3).

4 | DISCUSSION

At the end of a short period of 4 weeks, soft-tissue mobilizations in addition to the spinal mobility exercises provided positive improvements in quality of life by increasing mobility and functional level and reducing disease activity and disability. It was observed that spinal mobility exercises alone were insufficient in preventing and / or correcting the negative clinical picture caused by AS, especially in terms of mobility.

A systematic review reported that there is insufficient evidence to support or refuse the application of physiotherapy interventions involving manual therapy.[29] There was only one controlled study examining the efficacy of manual therapy in patients with AS, and the other studies were mostly case reports. Manual therapy involves joint manipulation / mobilization, myofascial relaxation techniques, and various massage treatments.[15] In these studies, manual therapy was applied in combination with soft-tissue mobilization, spine and joint mobilization, or / and manipulation. To the best of our knowledge, our study is the first study that examines the effectiveness of soft-tissue mobilization techniques in the treatment of AS.

In the only controlled study examining the efficacy of manual therapy in the literature, 16 sessions of self and manual mobilization were compared with the control group without any treatment. Initially, they applied vibratory and gentle mobility exercises to the

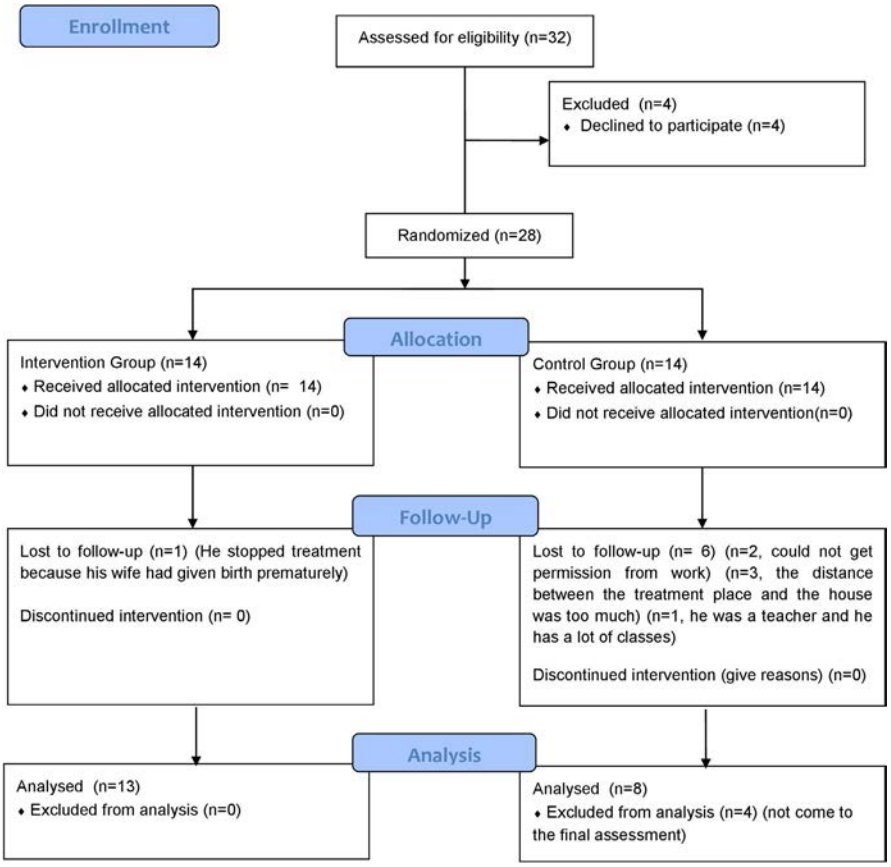

FIGURE 1 Flow chart of the progress through phases of the study [Colour figure can be viewed at wileyonlinelibrary.com]

soft tissues of the back muscles, followed by active and passive mobility exercises for the joints in the spinal column and chest wall. They then used the contracting-relaxing method for stretched tight muscles, and finally a manual massage of the soft tissues of the neck after relaxation exercises. In addition, three exercises, which can be prescribed individually, were given to the patients as a home program. As a result, improvement in chest expansion measured from processus xiphoideus level was achieved in the self and manual mobilization group, but no change in vital capacity was observed. Therefore, the authors stated that they partially confirmed the hypothesis that treatment could improve chest expansion in patients with AS because they could not improve pulmonary function. The authors also stated that patients had more accurate postures and increased mobility of the spine in the flexion direction rather than extension by achieving improvement in the neutral position in the thoracic and lumbar spine.[30]

Gyurcsik et al conducted an individualized complex physical exercise program in 10 AS patients. This program included general posture reeducation, manual mobilization exercises of the spine, pelvis, and upper and lower extremities, stretching with joint prevention strategies, and functional exercises. After 3 months of individualized complex physical therapy, improvement was achieved in several subjective and functional parameters. Pain intensity and spine stiffness in particular were reduced with this treatment. The authors reported that this program may be useful in AS patients in order to maintain and increase spine mobility, preserve functional capacity, and decrease pain and stiffness.[31]

Mengshoel and Robinson applied 12 sessions of specific spinal mobilization techniques to the area that presents stiffness, identified as a result of clinical evaluations in six AS patients. As a result, spinal stiffness and perceived stiffness decreased in five of the six patients and improvement was obtained in BASFI in one patient. In the light of the findings, it was stated that specific spinal mobilization can reduce spinal stiffness. However, it has been reported that this has different meanings for the patients and that the experiences and learnings of the individuals during the therapy process are the main points. It was also suggested that more patients should be studied to obtain more generalizable results.[32]

In the case series study in which Cornelson et al treated three patients with chiropractic care, the first had neck pain and stiffness, the second had low back and left hip pain, and the third had low back pain. Chiropractic care consisted of instrument-assisted spinal manipulation, diversified spinal and soft-tissue manipulation, interferential electric stimulation, trigger point therapy, and Cox flexion-distraction. Stretching and rehabilitation exercises were also assigned to patients. As a result, pain reduction and improvement in daily activities were achieved in all three patients. It has been reported that chiropractic manipulation and rehabilitation are beneficial in reducing symptoms and improving musculoskeletal function, and it is a potential method for additional treatment or complementary therapy in similar situations.[33]

McDermaid and Mior treated a patient (one of two AS patients) with sacroiliac, back, and low back pain and stiffness by applying soft-tissue therapy to the lumbar spine and gluteal musculature

TABLE 1 Demographic characteristics of the patients

Variables	Intervention group (n = 13) Mean ± SD	Control group (n = 8) Mean ± SD	*P* value
Age (years)	43.69 ± 9.94	46.00 ± 11.67	.634[b]
Body weight (kg)	76.30 ± 15.02	80.12 ± 10.61	.539[b]
Height (m)	1.59 ± 0.08	1.66 ± 0.08	**.029**[a]
Body mass index (kg/m^2)	30.19 ± 5.61	29.44 ± 6.12	.776[b]
Duration of disease (years)	7.87 ± 8.39	6.88 ± 8.06	.860[a]
Education duration (years)	10.60 ± 3.97	10.37 ± 4.62	.913[b]
Morning stiffness (min)	38.50 ± 37.86	33.33 ± 49.32	.442[a]
	n	n	
Gender			
Female/Male	11/2	5/3	.248[c]
Family history			
Yes/No	11/2	5/3	.248[c]
Type of drug used			
NSAIDs/DMARD/Anti-TNF	8/2/3	0/3/5	.019[c]

Abbreviations: Anti-TNF, anti-tumor necrosis factor; DMARD, disease-modifying anti-rheumatic drugs; NSAIDs, non-steroidal antiinflammatory drugs; SD, standard deviation.

[a]Mann-Whitney *U* test; significant values are shown in bold.

[b]Independent samples test.

[c]χ^2 test.

and a self-directed exercise program. As a result, symptoms decreased. There is a lack of knowledge of manual therapy in AS, so it is emphasized that careful documenting of treatment results is needed.[34]

Rose and Kim applied manipulation (grade 5) to thoracic spine and mobilization (grade 3) to lumbar and cervical spine in a physical therapy program for 12 weeks to a 30-year-old man with moderate to severe local back and neck pain and limited mobility. As a result, improvement of quality of life and spinal flexibility was obtained. Some temporary localized soreness has been reported as a side effect after treatment. The aim of this study was to develop practical treatment protocols for field practitioners who try to improve quality of life and prevent disability. Chiropractic treatment, including manipulation and mobilization, was shown to have some positive effects in advanced AS. The authors used Short Form-36 for assessment of quality of life and a tape measure to determine spinal mobility. They stated that their limitation was not using outcome measures, such as BASDAI, which enables a more comprehensive evaluation of patients with AS. They reported that in order to better determine the effectiveness of these treatments, future studies are needed in which BASDAI, developed specifically for AS, is used as outcome measure.[35]

In a case report by Rutherford et al, the treatment of AS patients with complaints of upper back pain and stiffness and low back pain was started with soft-tissue treatment of the cervical and thoracic paraspinal muscles and spinal manipulation of the lower cervical, thoracic, and lumbar spine. Then, diversified rotary manual procedures were used for rib mobilizations and manipulations, interferential current, and lower cervical and lumbar spine manipulation. As a result of the study, improvement was achieved in disease activity (BASDAI), functional index (BASFI), spinal flexibility, and chest expansion. These results have been reported to be favorable and a noteworthy outcome for longstanding AS patients. The authors also reported that the use of outcome measures developed and validated specifically for function and disease activity in AS provides unprecedented support in the investigation of the effectiveness of manipulative therapy and improves the assessment of the results obtained from the studies. As the results of these studies guide the clinical management of AS more accurately, more intensive research using these instruments on patients with AS undergoing manipulative therapy was suggested by the authors.[36]

In a case study conducted by Chunco (2011), soft-tissue massage, kneading, stretches, proprioceptive neuromuscular facilitative stretching, and mobilization of non-ankylosed joints are proposed as appropriate therapy. The reduction in morning stiffness and increased mobility in this case study were the most prominent gains.[37]

In studies investigating the effectiveness of new treatment options in patients with AS, the use of disease-specific validated instruments may increase the validity and reliability of the results obtained.[35,36] Taking into account the recommendations in the literature, in this study, in which we examined the effectiveness of soft-tissue mobilization in patients with AS, we used validated and reliable disease-specific BASFI, BASMI, and BASDAI instruments to evaluate the efficacy of treatment in AS. We applied soft-tissue mobilization therapy, which is one of the manual treatment methods, as an additional method to exercise therapy. We

TABLE 2 The comparison of pretreatment and posttreatment results within groups

	Intervention group (n = 13)			Control group (n = 8)		
	Pretreatment	Post-treatment		Pretreatment	Post-treatment	
Variables	Mean ± SD	Mean ± SD	*P* value	Mean ± SD	Mean ± SD	*P* value
BASDAI	4.59 ± 1.73	3.52 ± 1.88	**.049**[a]	4.83 ± 2.69	4.16 ± 1.73	.354[a]
BASFI	4.32 ± 2.20	2.38 ± 1.95	**.009**[b]	4.20 ± 3.13	3.71 ± 2.45	.212[a]
BASMI total	3.44 ± 0.98	2.72 ± 0.97	**.001**[a]	3.94 ± 2.87	4.15 ± 2.55	**.049**[a]
Lateral lumbar flexion	4.69 ± 2.05	3.46 ± 2.06	**.005**[b]	4.71 ± 2.81	4.87 ± 2.94	.581[b]
Tragus-to-wall distance	2.53 ± 1.56	2.15 ± 1.46	.187[b]	2.62 ± 3.15	3.12 ± 2.53	.157[b]
Lumbar flexion (modified Schober)	2.92 ± 1.75	2.38 ± 2.02	.252[a]	4.57 ± 3.86	4.87 ± 3.87	.172[a]
Maximal intermalleolar distance	3.00 ± 1.35	2.30 ± 1.18	**.001**[a]	3.85 ± 3.02	3.75 ± 3.10	1.000[a]
Cervical rotation	4.07 ± 1.32	3.30 ± 1.10	.077[b]	4.14 ± 3.02	4.12 ± 2.79	.736[a]
NHP total	292.38 ± 170.02	218.90 ± 125.01	**.036**[a]	207.57 ± 154.33	226.80 ± 137.12	.271[a]
Energy level	65.60 ± 42.68	49.13 ± 41.83	.206[b]	60.53 ± 38.35	62.00 ± 28.73	.774[a]
Pain	67.71 ± 37.39	43.07 ± 37.55	**.036**[a]	48.32 ± 42.85	54.00 ± 42.40	.278[a]
Emotional reactions	45.37 ± 34.59	34.46 ± 26.84	.117[a]	26.22 ± 34.01	23.99 ± 28.59	.659[a]
Social isolation	35.31 ± 43.69	33.63 ± 43.01	.180[b]	10.77 ± 26.40	19.21 ± 24.50	.655[b]
Sleep	42.30 ± 35.23	33.03 ± 31.07	.369[a]	36.70 ± 34.26	36.78 ± 30.97	.287[a]
Physical abilities	36.08 ± 26.28	25.55 ± 17.96	.141[a]	25.00 ± 15.84	30.80 ± 20.62	.660[a]
RMDQ	15.58 ± 5.80	9.66 ± 6.78	**.004**[b]	12.00 ± 9.59	12.00 ± 6.44	.246[a]

Abbreviations: BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; NHP, Nottingham Health Profile; RMDQ, Roland-Morris Disability Questionnaire; SD, standard deviation.

[a]Wilcoxon test, significant values are shown in bold.

[b]Paired simple *t* test.

did not do this study only at the level of the case report but using an increased sample number.

In a recent literature review, Sharan and Rajkumar proposed a four-step rehabilitation protocol, phase one including pain reduction and improving mobility, and phase two including restoring flexibility and postural re-education.[38] It has been suggested that hip joint, hamstrings, and low back can be supported by mobilization and stretching in AS. In AS, fibrotic changes may be greater than expected because of atrophy in the paraspinal muscles.[39] Metrology measures, such as spinal flexion, and occiput-to-wall distance, are measures related to disease severity and deformity rather than disease activity.[17]

In addition to spinal mobility exercises, we used only soft-tissue mobilization in the intervention group, which was one of the manual therapy techniques, and we started the treatment from the pelvic region and applied to certain muscles and fascia of the spine including the neck. Because of the presence of pain and other symptoms in soft tissues in the deep gluteal, low back, hip, and sacroiliac regions in patients with AS,[3,4] we focused on these areas mostly in soft-tissue mobilization treatment. As a result of our treatment in the intervention group; we observed increased mobility in the lateral lumbar flexion and maximal intermalleolar distance components of BASMI, which evaluated the mobility of the tissues and joints in the hip, gluteal, low back, and sacroiliac regions, and the total score. This increased activity increases functionality and decreases disease activity; hence, we believe that as patients' disability decreases, their active participation in life increases, with an overall positive effect on quality of life. Nava underlined the mechanism of postural changes over time because the pathology of AS influences a sensory input and creates a new postural equilibrium. We think that the sensory input we provide with fascia stretches and the post-isometric relaxation techniques that we apply within the scope of manual therapy techniques will be effective in regulating this improper postural equilibrium.[40]

Contrary to the use of combined joint mobilization / manipulation and soft-tissue mobilizations in the literature, we believe that the positive results obtained by investigating the effectiveness of only soft-tissue mobilization will contribute to the literature.

Patients with AS have acute inflammatory joints and chiropractic practice guidelines have reported that manipulation should not be used in acute inflammatory joints.[41] Osteoporosis can occur in the early stages of AS and this makes patients more prone to vertebral compression and the formation of traumatic spinal cord fractures of the cervical spine.[42] In addition, it is estimated that the risk of traumatic spinal cord compression is 11 times higher in the AS population compared with the healthy population.[43] There are two case reports that report paraplegia[44] and incomplete quadriplegia[45] after chiropractic manipulation in a patient with AS. In our study, where

TABLE 3 The comparison of delta values between groups

Variables	Intervention group (n = 13) ΔMean ± SD	Control group (n = 8) ΔMean ± SD	*P* value
BASDAI	−1.34 ± 2.21	−0.66 ± 1.89	.481[a]
BASFI	−1.94 ± 1.95	−0.49 ± 1.01	**.039**[a]
BASMI total	−0.72 ± 0.51	0.25 ± 0.27	**.001**[a]
Lateral lumbar flexion	−1.23 ± 1.01	0.28 ± 1.49	**.027**[b]
Tragus-to-wall distance	−0.38 ± 0.96	0.50 ± 0.92	**.039**[b]
Lumbar flexion (modified Schober)	−0.53 ± 1.61	0.28 ± 0.48	.138[b]
Maximal intermalleolar distance	−0.69 ± 0.48	0.00 ± 0.81	**.045**[b]
Cervical rotation	−0.76 ± 1.36	0.14 ± 1.06	.144[a]
NHP total	−73.48 ± 106.52	−29.68 ± 58.79	.367[a]
Energy level	−16.46 ± 34.51	−4.66 ± 37.68	1.000[b]
Pain	−24.64 ± 35.78	−9.65 ± 19.42	.357[a]
Emotional reactions	−10.90 ± 22.20	−2.69 ± 14.07	.639[b]
Social isolation	−1.67 ± 5.57	0.40 ± 13.50	.607[b]
Sleep	−9.26 ± 34.27	−10.96 ± 22.57	.914[a]
Physical abilities	−10.52 ± 22.99	−2.10 ± 11.01	.639[b]
RMDQ	−5.91 ± 4.46	−2.60 ± 4.27	.090[b]

Abbreviations: BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; NHP, Nottingham Health Profile; RMDQ, Roland-Morris Disability Questionnaire; SD, standard deviation.

[a]Independent samples test.

[b]Mann-Whitney *U* Test, Δ, post-treatment-pretreatment, significant values are shown in bold.

soft-tissue mobilizations were used rather than risky manipulation applications, significant improvement was achieved in disease activity (BASDAI), functional level (BASFI), mobility (BASMI), quality of life, and disability. These results have shown that soft-tissue mobilization is an effective and useful treatment in AS, and that the severity of the disease as well as deformities can be preserved and / or improved with this treatment. We think that soft-tissue mobilization techniques are safer than and preferable to chiropractic spinal manipulation.

ASAS/EULAR (2017) and the American College of Rheumatology / Spondylitis Association of America / Spondyloarthritis Research and Treatment Network (2019) strongly recommended physical therapy and regular exercise in their recommendations for AS treatment. However, there is no consensus on which exercise method is more effective in the treatment of AS.[46,47] As a result of our study, we found that spinal mobility exercises consisting of stretching and strengthening exercises alone were not sufficient in the treatment of AS or in stopping the progression of symptoms. We think that a physical therapy program planned for AS treatment may consist of spinal mobility exercises and soft-tissue mobilization techniques, instead of spinal mobility exercises only.

The strengths of our study are that in the literature, although combined treatments were performed in addition to exercise in patients with AS, we examined the effectiveness of only soft-tissue mobilization therapy.

The main limitation of our study is the low number of samples in the control group. Another limitation is that while investigating the therapeutic effect of soft-tissue mobilization, no evaluation was made with the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS), which determines radiographic severity. In future studies, we recommend the use of mSASSS to examine the effect of soft-tissue mobilization on radiological findings.

In our study, the groups were heterogeneous in terms of the types of drugs the participants used (disease-modifying anti-rheumatic drugs, anti-tumor necrosis factor, non-steroidal anti-inflammatory drugs). We think that in future studies, it could be investigated whether the use of different types of drugs makes any difference in the effectiveness of soft-tissue mobilization in addition to the spinal mobility exercises.

5 | CONCLUSION

We found that spinal mobility exercises consisting of stretching and strengthening exercises alone were not sufficient in the treatment of AS or in stopping the progression of symptoms. Patients with AS had significant improvement in disease activity, functional level, mobility, lateral lumbar flexion, maximal intermalleolar distance, quality of life, and disability level with soft-tissue mobilization in addition to spinal mobility exercises. Hence, soft-tissue mobilization have been shown to be an effective and useful additional treatment method. Achieving these positive results in a short period like 4 weeks can be advantageous in choosing specific soft-tissue mobilization as an effective treatment method compared with long-term treatments in AS. Indeed, there is a general idea in the literature that at least 8 weeks of training are required to see changes in muscle structure, such as strength and flexibility,[48,49] and the most common reason for drop out in treatment programs is the lack of time,[50-52] something that can be overcome by the comprehensive treatments we are proposing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

EGK and BBC designed the study. EGK searched databases and performed the selection of studies; EGK, MO, and VC collected data; EGK and MO wrote the manuscript; BBC analyzed the data; BBC and VC contributed to writing and critically appraising the manuscript and approved the last version.

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What is the effect of shock wave therapy in rotator cuff disease with or without calcification? A Cochrane Review summary with commentary

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The aim of this commentary is to discuss the published Cochrane Review "Shock wave therapy for rotator cuff disease with or without calcification"[1] by Surace SJ, Deitch J, Johnston RV, Buchbinder R[a], under the direct supervision of Cochrane Musculoskeletal Group. This Cochrane Corner is produced in agreement with *International Journal of Rheumatic Diseases* by Cochrane Rehabilitation.

1 | BACKGROUND

Shoulder disorders are common, with a reported prevalence ranging from 7% to 26% in adults.[2]

Rotator cuff disease is the most common cause of shoulder pain seen by physicians.[3] About 40% of cases persist for longer than 1 year with rates of recurrence and chronicity of shoulder pain rated as moderate to high.[2] Many people with shoulder pain do not experience a complete resolution of symptoms, and 51% reported recurrence after 26 weeks and 41% reported recurrence after 12-18 months.[4] There are different conservative approaches to relieve pain and restore movement and function of the shoulder. Extracorporeal shock wave therapy (ESWT) has been introduced for treating various musculoskeletal disorders, including chronic tendinopathies since the 1990s. Shock waves are single sonic pulses with a steep pressure rise, high peak pressure, short duration, followed by a low tensile amplitude.[5,6] According to the energy flux density there are low-energy shock waves (less than 0.1 mJ/mm^2) and high-energy shock waves (0.2 mJ/mm^2-0.4 mJ/mm^2)[7] Radial shock waves show a lower peak pressure, longer rise time and the focal point of energy is not centered on a target zone but on the tip of the applicator.[5-7] The possible mechanism of their effect in tendinopathies is overstimulation of pain nerve fibers, the reduction of pain-conducting C-fibers, and induction of the healing process of the tendon.[5] The new functional proteins induced by ESWT promote a chondroprotective effect, neovascularization, anti-inflammation, anti-apoptosis, and tissue and nerve regeneration.[8]

2 | SHOCK WAVE THERAPY FOR ROTATOR CUFF DISEASE WITH OR WITHOUT CALCIFICATION

(Stephen J Surace, Jessica Deitch, Renea V Johnston, Rachelle Buchbinder, 2020).

2.1 | What is the aim of this Cochrane Review?

The aim of this Cochrane Review was to determine the benefits and harms of shock wave therapy for rotator cuff disease, with or

[a]This summary is based on a Cochrane Review previously published in the Cochrane Database of Systematic Reviews 2020, Issue 3. Art. No.: CD008962. https://doi.org/10.1002/14651858.CD008962.pub2 (see www.cochranelibrary.com for information). Cochrane Reviews are regularly updated as new evidence emerges and in response to feedback, and Cochrane Database of Systematic Reviews should be consulted for the most recent version of the review.

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

without calcification, and to establish its usefulness in the context of other available treatment options.

2.2 | What was studied in the Cochrane Review?

The population addressed in this review was adults with rotator cuff disease (rotator cuff tendinitis or tendinopathy, supraspinatus, infraspinatus or subscapularis tendinitis, subacromial bursitis or rotator cuff tears) with or without calcific deposits. Participants with a history of significant injury or systemic inflammatory conditions such as rheumatoid arthritis were excluded.

The interventions studied were radial and focused shock wave therapy. The intervention was compared to placebo, or another treatment, or of varying types and dosages of ESWT. Trials that included co-interventions were eligible for inclusion provided if co-interventions were given to both experimental and control groups. The major outcomes studied were: participant-reported pain relief of 30% or greater; mean pain score, or mean change in pain score on a visual analog scale (VAS) or Numerical Rating Scale (NRS) or categorical rating scale; disability or function/Shoulder Pain And Disability Index (SPADI); Shoulder Disability Questionnaire (SDQ); Constant score; Disabilities of the Arm, Shoulder and Hand (DASH); Health Assessment Questionnaire (HAQ); composite endpoints measuring "success" of treatment such as participants feeling no further symptoms; quality of life; number of participant withdrawals due to intolerance or adverse events; the number of participants experiencing any adverse event. The minor outcomes were: proportion of participants achieving pain score below 30/100 mm on VAS; range of movement active preferred over passive measures; for participants with calcification, the effect of ESWT on the size of the calcification and the number of participants with complete or partial resolution of calcific deposits.

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

The review authors searched for studies published up to November 2019 in Ovid MEDLINE, Ovid Embase, CENTRAL, ClinicalTrials.gov and the World Health Organization International Clinical Trials Registry Platform.

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

The review included 32 randomized controlled trials (2281 participants), mean age from 48 years to 56.2 years; mean duration of symptoms ranged from 7.1 to 60 months; 61% of participants were female. Most trials (25) included participants with rotator cuff disease and calcific deposits, 5 trials included participants with rotator cuff disease and no calcific deposits, and 2 trials included a mixed population of participants with and without calcific deposits. Twelve trials compared shock wave therapy to placebo, 11 trials compared high-dose shock wave therapy (0.2 mJ/mmN to 0.4 mJ/mmN and above) to low-dose shock wave therapy. Single trials compared shock wave therapy to ultrasound-guided glucocorticoid needling, ultrasound-guided hyaluronic acid injection, transcutaneous electric nerve stimulation (TENS), no treatment or exercise, dual session to single session therapy.

The main comparison was shock wave therapy versus placebo and results are reported for the 3-month follow-up. All trials were susceptible to bias, including selection (74%), performance (62%), detection (62%), and selective reporting (45%) biases.

The review shows the following results concerning the major outcomes:

- *Pain relief ≥ 50% (low-quality evidence, downgraded for bias and imprecision, 1 study, 74 participants).* Shockwave therapy may provide no improvement in the number of participants with a pain reduction of 50% or more. At 3 months follow-up 14/34 participants with shock wave therapy, compared to 15/40 with placebo reported pain relief of 50% or greater (risk ratio [RR] 1.10, 95% confidence interval [CI] 0.62-1.94);
- *Pain (moderate quality evidence, downgraded for bias, 9 studies, 608 participants).* Shockwave therapy probably results in little or no clinically important improvement in pain. At 3 months follow-up mean pain (0-10 scale, higher scores indicate more pain) was 3.02 points in the placebo group and 0.78 points better in the shock wave group (ranging from 0.17 better to 1.4 better; clinically important difference was 1.5) (95% CI −1.4 to −0.17);
- *Function (moderate quality evidence, downgraded for bias, 9 studies, 612 participants).* Shockwave therapy probably results in little or no clinically important improvement in function. At 3 months follow-up mean function (scale 0-100, higher scores indicate better function) was 66 points in the placebo group and 7.9 points better in the shock wave group (ranging from 1.6 better to 14 better, clinically important difference 10 points);
- *Participant-reported success (low-quality evidence downgraded for bias and imprecision, 6 studies, 287 participants).* Shockwave therapy may provide no improvement in the number of participants reporting treatment success. Success was reported by 58/150 people in the shock wave therapy group compared with 35/137 people in placebo group (RR 1.59, 95% CI 0.87-2.91);
- None of the trials measured *quality of life*;
- *Number of participant withdrawals (low-quality evidence downgraded for bias and imprecision, 7 studies, 581 participants).* There is uncertainty if shockwave therapy increases withdrawal rates due to the small number of events. There were 11/34 withdrawals in the ESWT group compared with 13/40 withdrawals in the placebo group (RR 0.75, 95% CI 0.43-1.31);
- *Number of participants experiencing any adverse effect (low-quality evidence downgraded for bias and imprecision, 5 studies, 295 participants)* There is uncertainty if shockwave therapy increases adverse events. There were 41/156 adverse events with ESWT compared with 10/139 adverse events in the placebo group (RR 3.61, 95% CI 2.00-6.52);

- Subgroup analyses indicated there were no between-group differences in pain and function outcomes in participants who did or did not have calcific deposits in the rotator cuff.

2.4.1 | Minor outcomes

Shock wave therapy was associated with an increased rate of complete resolution of calcium deposits by the end of the trial, but this was of uncertain clinical significance.

There was very low-certainty evidence that high-dose shock wave therapy may provide a clinically important benefit compared with low-dose shock wave therapy at the end of the trial with respect to treatment success and function.

Higher doses also had a benefit of uncertain clinical significance with respect to range of movement and reduction of calcific deposits.

High-dose therapy had a higher risk of adverse events but not withdrawals.

Evidence was downgraded due to the risk of selection, detection or reporting bias, or a combination of these biases, as well as imprecision or heterogeneity.

2.5 | What did the authors conclude?

The authors concluded that based upon the currently available low- to moderate-certainty evidence, the review indicates few clinically important benefits of shock wave therapy compared with placebo, ultrasound-guided needling, transcutaneous electrical nerve stimulation, supervised exercises or percutaneous lavage for the treatment of rotator cuff disorders with or without calcific deposits. There is also uncertainty regarding its safety. Due to the wide clinical heterogeneity and varying treatment protocols it is unknown whether "subtherapeutic" doses were tested in some trials underestimating any potential benefits.

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

Extracorporeal shock wave has few clinically important benefits for the treatment of rotator cuff disorders with or without calcific deposits, based on low-to-moderate-certainty evidence. Further trials of ESWT for rotator cuff disease should be based upon a strong rationale and consideration of whether or not they would alter the conclusions of this review. A standard dose and treatment protocol should be decided upon before further research is conducted.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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CORRESPONDENCE

Chondroprotectives used for the treatment of knee osteoarthritis: A comment

Chondroitin is a glycosaminoglycan (GS); glucosamine is an aminosaccharide acting as a substrate for the synthesis of GS. Chondroitin undergoes hydrolysis in the intestine; taken orally, it may act as a precursor of GS. Intra-articular hyaluronic acid (IAHA) is GS used for injections. Oral and intra-articular GS are distinct treatment modalities united under the term "chondroprotectives" and are applied for the treatment of osteoarthritis (OA). The oral preparations have been designated as symptomatic slow acting drugs in osteoarthritis (SYSADOA),[1] although these drugs are not symptomatic in the narrower sense as they were primarily aimed not at a symptomatic relief but at the compensation of a supposed deficiency of GS and their precursors. The evidence is controversial; there has been skepticism in the scientific community.[2-4] A meta-analysis concluded that chondroitin, glucosamine, and their combinations have no clinically relevant effect on the joint space width and perceived pain.[5] According to a Cochrane review, trials of mostly low quality reported that chondroitin, alone or in combination with glucosamine, was superior to placebo against pain among OA patients in a short-term research.[6] Indeed, there are many studies and reviews reporting the efficiency of chondroprotectives compared to placebo, but reliability is sometimes questionable. The research quality, funding of studies and treatment duration should be taken into account when evaluating data.[7] According to the unique "Systematic Review of Systematic Reviews,"[7] limited benefits were observed with glucosamine, chondroitin and IAHA; however, when only publicly funded trials were examined, the results turned out to be insignificant.[7] Positive results were mostly represented by alleviation of subjective symptoms, whereas a placebo effect is not excluded. Potential bias related to the inadequate masking of study agents was also discussed.[8] According to another Cochrane review, if only best designed studies were included, for example those with adequate allocation concealment, the benefit from glucosamine in the joint function and pain was no longer present.[9] Despite numerous trials and widespread practical use of glucosamine and chondroitin, their place in the treatment of OA is still under debate.[4] This letter is focused on the knee joint because it is the most prevalent OA location in the lower limbs, most frequently leading to disability,[10] although conclusions are relevant to OA in general.

The theoretical basis of the supposed chondroprotection remains unclear. GS and their precursors are not irreplaceable; they are produced also in vegetarians, who consume no immediate GS precursors. It appears doubtful that oral supplementation of chondroitin and/or glucosamine can shift the balance between the cartilage synthesis and degradation in the whole body insomuch that it would be significant for the structure and function of joints. Furthermore, the raw materials such as chitin and fungi for glucosamine preparations, fish and bird cartilage for chondroitin as well as possible contaminations may impart unpredictable properties to the drugs and dietary supplements.[11,12] GS that are present in food may be metabolically more adequate (the diet is discussed below). On the other hand, poor-quality preparations available on the market may blur the effect of other compounds. For example, patented crystalline glucosamine sulfate has demonstrated better pharmacokinetics along with more supportive evidence of clinical effectiveness compared with other glucosamine preparations.[10,13]

The use of IAHA remains controversial because of conflicting data regarding its efficacy reached by reviews.[14] A meta-analysis and systematic review concluded that IAHA in patients with knee OA were associated with small, clinically insignificant benefits and an increased risk of adverse effects.[15,16] Consequences of a single IAHA injection are similar to those of multiple procedures in terms of the pain relief,[16] which is indicative of a placebo effect. Pain measurements in clinical trials are difficult. Pain, stiffness and other studied endpoints are largely subjective, which indicates that at least a part of reported benefits can be attributed to the placebo effect. The evidence remains generally inconsistent and controversial.[17] According to a Cochrane review on IAHA for ankle OA, it is unclear whether there is a benefit or harm compared to placebo. Inconclusive results were also obtained by comparing IAHA with other treatment modalities.[18] It is known that invasive procedures may have a pronounced placebo effect. By definition, a placebo must cause no harm; otherwise it is called pseudo-placebo.[19] Among OA patients treated by viscosupplementation, withdrawals from the treatment due to adverse events were consistently observed.[7] A recent review evaluated results of repeated IAHA injections and found that the most common side effects were joint swelling and arthralgia.[20] In particular, the IAHA treatment of hip OA is associated with risks due to the proximity of vital anatomical structures.[21,22]

Mechanisms of the reportedly longstanding effectiveness of IAHA is hardly comprehensible.[23] Both pre- and post-injection viscosity was found to be compatible with the norm.[24] No explanation has been found for the discrepancy between the short intra-articular

half-life of hyaluronic acid preparations and reported duration of the carry-over effect. Presumably, the rheological effect of IAHA lasts ≤24 hours.[25] The intra-articular half-life of Hyalgan (sodium hyaluronate) is ~17 hours. The low molecular weight component of Synvisc (~90% of the preparation) has a half-life of 1.5 days, while another component with a higher molecular weight has a half-life of 8.8 days.[24] In contrast, the carry-over effect after the treatment was reported to last from 3 months with oral preparations to 6-9 months after IAHA.[26,27] The half-life of a hyaluronic acid preparation with artificial cross-linking was reported to last up to 4 weeks;[28] however, there are no reasons to expect a much longer carry-over effect. It is possible that IAHA has a longer effect by virtue of other mechanisms beyond the viscosupplementation or lubrication. For example, a short-term functional improvement due to the enhanced viscosity of synovia may reinforce the placebo effect over the long term through a conditioned reflex. Among other potential mechanisms are the reduction of chondrocyte apoptosis and increasing proliferation, anti-inflammatory effects, inhibition of chondrodegenerative enzymes and pain mediators, deactivation of nociceptors, which in turn may contribute to the patients' mobility and protection of cartilage.[20,27,29,30] Admittedly, it is not clear which of these mechanisms are effective and clinically relevant.[27] Hyaluronic acid, chondroitin and glucosamine were primarily chosen to compensate for a supposed deficiency of GS. Therefore, a probability of their specific action on the cellular or molecular level would be a priori not superior to that of substances taken at random. As for molecular mechanisms studied in vitro, their clinical relevance is often questionable because of higher concentrations of tested substances in laboratory experiments compared to in vivo conditions. Furthermore, hyaluronic acid is a biopolymer; according to the law of mass action, its local enrichment would displace the chemical equilibrium toward low molecular precursors, that is reduction of viscosity. Therefore, suppositions about an increased synthesis of endogenous GS after injections of the same or similar substances[27] seem to be unfounded.

In the Russian Federation, chondroitin, glucosamine and IAHA are known as chondroprotectors. These drugs are prescribed to OA patients including aged people with low incomes. Many patients purchase the medicines for a prolonged use.[31] Chondroprotector-containing ointments and preparations for intra-articular injections have been patented.[21,32] Certainly, if the medicines alleviate sufferings of OA patients, this alone would justify their use. However, it might be more or less equivalent to recommend to the patients a diet rich in natural GS: animal joints, chicken limbs, minced cartilage and so on. Such a diet may have similar efficacy to a more expensive pharmaceutical treatment. To support the placebo effect, patients can be informed that the diet would supply their bodies with GS precursors similar to drugs and dietary supplements. Assumptions that the diet can improve the symptoms of OA have appeared in recent publications.[31,33] The overall appreciation of GS administration (diet or not), along with theoretic arguments discussed here, suggest a conclusion of an efficacy in OA that is not far beyond a placebo effect. Additional information could be obtained from studies of the OA prevalence among vegetarians and in populations consuming predominantly vegetable food, thus not ingesting much GS and their direct precursors. Effectiveness of a dietary supplementation of natural GS (minced cartilage) compared to pharmaceutical preparations of chondroitin and glucosamine can be tested in animals, for example canine OA. A recent review concluded that the efficiency of glucosamine and chondroitin in dogs with OA could neither be confirmed nor denied. Unfortunately, not only human but also animal studies are sometimes influenced by conflicts of interest.[34]

CONFLICT OF INTEREST

The author has no conflicts of interest to declare.

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Autoantibodies in severe COVID-19-related acute respiratory distress syndrome: Just innocent bystanders?

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1 | INTRODUCTION

Ever since coronavirus disease 2019 (COVID-19) was declared a global public health emergency, more than 60 million confirmed cases and more than one million confirmed deaths have been recorded globally. Although most patients have minimal flu-like symptoms, some of them develop a severe pneumonia, which may lead to multi-organ failure and death.[1] Systemic hyperinflammation and a procoagulant state play a major pathophysiological role in these severe forms.[2] Autoimmune diseases are also characterized by inflammation and often by a procoagulant state, and viruses may be involved in their development,[3] so a link between COVID-19 and autoimmunity has recently been postulated. A report found that up to 35% of hospitalized patients with less severe COVID-19 have antinuclear antibodies, suggesting a possible role of autoimmune mechanisms, which may potentially imply specific treatments.[4] At the time of data collection, no reports had been published that focused on the most severe patients, ie those undergoing mechanical ventilation. During the revision process, however, two papers were published on autoimmune aspects of severe COVID-19 patients.[5,6] The present study aimed to assess the prevalence and clinical outcomes associated with the presence of autoantibodies in critically ill, mechanically ventilated patients admitted to the intensive care unit (ICU) for COVID-19-related respiratory failure.

2 | MATERIALS AND METHODS

Consecutive patients aged at laest 18 years admitted to the ICU at Ospedale San Carlo Borromeo with confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection were enrolled. Patients previously diagnosed with autoimmune diseases were excluded. The study was approved by the Hospital Ethics Committee; informed consent was obtained according to Italian regulations. At ICU admission, every patient was tested for the presence of anti-nuclear, anti-mitochondrial, anti-smooth muscle cell, and anti-neutrophil cytoplasmic antibodies. The primary outcome was hospital mortality. Secondary outcomes were the duration of mechanical ventilation, the ICU and hospital lengths of stay, the number of ventilator-free days in the first 28 days of ICU stay, and the proportion of patients who developed ventilator-associated pneumonia or bacteremia. Continuous variables are reported as mean ± standard deviation or as median (1st; 3rd quartile), as appropriate; categorical variables as number and percentages. Analysis was performed with Stata 13.0 (Statacorp, College Station, TX, USA); $P < 0.05$ was considered statistically significant.

3 | RESULTS

Twenty-eight patients were enrolled in the present analysis; 15 (53.6%) of them had autoantibodies. Table S1 shows the demographic and clinical characteristics, comorbidities, treatment received before ICU admission, blood biochemistry, gas exchange and respiratory physiology at ICU admission. All patients were intubated and mechanically ventilated at ICU admission. No significant differences were found between the groups for any of the variables recorded, with the exception of a higher organ failure score at admission for patients who had autoantibodies. Of the patients with

autoantibodies, 3 (20%) had anti-nuclear antibodies, 1 (7%) had anti-mitochondrial antibodies, 12 (80%) had anti-smooth muscle cell antibodies, and 1 (7%) had anti-neutrophil cytoplasmic antibodies. Table S2 shows the lymphocyte subset counts and complement components. Both groups were lymphopenic; however, we were unable to find any difference in any lymphocyte subset or in complement components C3 or C4 between the two groups of patients.

Table 1 compares the clinical outcomes of patients with and without autoantibodies: no differences were found for hospital mortality, duration of mechanical ventilation, the ICU and hospital length of stay, the number of ventilator-free days in the first 28 days of ICU stay, and the proportion of patients who developed ventilator-associated pneumonia or bacteremia. Figure S1 shows the Kaplan-Meyer curves for hospital mortality in patients with and without autoantibodies (Log-rank test $P = 0.2548$).

4 | DISCUSSION

In a small cohort of mechanically ventilated, adult COVID-19 patients, more than half of patients had autoantibodies, but we could not identify any specific risk factor associated with this finding. Patients with autoantibodies had a similar degree of disease severity as patients who did not have autoantibodies, and their clinical outcome was comparable. This is the first report on the finding of autoantibodies among COVID-19 patients receiving invasive ventilation. Autoimmunity develops as a result of a multifactorial interplay of genetic, hormonal, immunological, and environmental factors. Viral infections play a substantial role in triggering autoimmunity in predisposed patients.[3] The viral disruption of self-tolerance can be caused by molecular mimicry, epitope spreading, bystander activation, stimulation of inflammasome platforms, or polyclonal immune activation. Of note, all these mechanisms have been found in the setting of COVID-19.[7] Moreover, it was hypothesized that COVID-19 pneumonia can be worsened by an autoimmune response, and that the presence of autoantibodies may be a surrogate marker of severity and poor prognosis.[8] However, no such studies were performed in the most critically ill patients.

In 29 unselected critically ill, COVID-19 patients, several systemic autoimmune reactivities were found in 70% of the patients, suggesting a post-SARS-CoV-2 or para-SARS-CoV-2 infectious autoimmune activation.[6] In a cohort of COVID-19 patients hospitalized both in the ICU and in the medical ward, antibodies against nuclear, vasculitis-associated, and phospholipid antigens were detected in 30% of the patients. Notably, similar levels of inflammatory markers and total immunoglobulin levels in autoantibody-positive versus autoantibody-negative patients were found, as well as a similar outcome.[5]

In SARS, immune-mediated mechanisms were described, and both autoimmune responses and a cross-reaction between viral antigens and autoantibodies were found.[9] Some of the extensive cellular damage associated with COVID-19 may be the result of viral antigenic mimicry with human tissue, as the immune responses against SARS-CoV-2 showed cross-reaction with various tissue antigens.[10] Indeed, a causal link between SARS-CoV-2 and the appearance of autoinflammatory diseases has not yet been firmly established; however, the temporal association with the current pandemic highly suggests this possibility. Of note, the pathogenicity of these autoantibodies is unknown, as well as the chance to induce autoimmune disorders in the long term. It is well-known that autoantibody seropositivity may also be found in healthy individuals;[11] however, the prevalence we found is higher than reported in other western countries.[12] Nevertheless, despite a higher Sequential Organ Failure Assessment score and hence a more severe clinical presentation of patients with autoantibodies, the similar outcome of patients with and without autoantibodies, and the lack of a clear risk factor for

TABLE 1 Clinical outcomes in patients with and without autoantibodies[a]

	No autoantibodies (N = 13)	Autoantibodies (N = 15)	*P*
ICU mortality	7 (53.9)	6 (40)	.464
Duration of mechanical ventilation (days)	13 (4; 26)	10 (8; 17)	.7119
Duration of pressure support ventilation (days)	2 (1; 5)	3 (1; 6)	.9815
ICU length of stay (days)	17 (5; 26)	11 (8; 23)	.6280
Hospital length of stay (days)	27 (23; 37)	26 (21; 35)	.7967
Ventilator-free days (days)	0 (0; 24)	11 (0; 20)	.7891
Patients who developed VAP	7 (53.9)	8 (53.3)	.978
Number of VAP per patient	2 (1; 4)	2 (1; 2)	.4579
Patients who developed bacteremia (%)	6 (46.2)	7 (46.7)	.978
Number of bacteremias per patient	1 (1; 2)	1 (1; 2)	.8053

Abbreviations: ICU, intensive care unit; VAP, ventilator-associated pneumonia.

[a]Values are given as number (percentage) or as median (1st; 3rd quartile).

their development, seem to suggest that autoimmunity is not yet to be considered the hallmark of COVID-19. Many of the other reported immune-mediated disorders in SARS-CoV-2 may just represent transitory epiphenomena accompanying a viral infection.

Immunosuppressive therapies at least short term, such as dexamethasone and tocilizumab, showed benefit in COVID-19 patients in the ICU. Whether this depends on an effect on autoimmunity is not clear. It would be interesting to note whether the rates of autoantibodies in these RCTs were any different between the placebo and control arms. Whether the use of immunosuppressive therapies may prove of benefit in such cases is a fascinating perspective, but definite evidence in support is still lacking.

The limitations of this study relate to its small-size and single-centre, retrospective, observational nature. Autoantibodies were only tested at ICU admission, and we cannot exclude a possible late appearance in some cases, nor did we assess any long-term consequence of our findings. In conclusion, we found that autoantibodies were present in more than half of critically ill patients undergoing mechanical ventilation for COVID-19. Their presence was not associated with a worse clinical outcome; however, we cannot exclude that, because of the small sample size, our study might be underpowered to detect such a difference. Further studies are needed to elucidate whether the presence of autoantibodies only represents a transitory epiphenomenon accompanying a viral infection or if it is associated with any different clinical outcome.

CONFLICT OF INTEREST

None declared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.**How to cite this article:** Umbrello M, Nespoli S, Pisano E, Bonino C, Muttini S. Autoantibodies in severe COVID-19-related acute respiratory distress syndrome: Just innocent bystanders?. *Int J Rheum Dis*. 2021;24:462–464. https://doi.org/10.1111/1756-185X.14077

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International Journal of Rheumatic Diseases WILEY

ERRATUM

The publisher would like to draw the readers' attention to an error in the following article:
Chowdhary, VR (2020) Retraction statement: When doing the right thing is wrong: Drug efflux pumps in steroid-resistant nephrotic syndrome. *Int. J. Rheum. Dis* **23**: 1261-1261.

The *International Journal of Rheumatology* was named in error in the following sentence:
The retraction has been agreed due to an error which caused the article to be published in the *International Journal of Rheumatology* after its original publication in the *Indian Journal of Rheumatology.*

The correct journal should be *International Journal of Rheumatic Diseases* and the sentence should read:
The retraction has been agreed due to an error which caused the article to be published in the *International Journal of Rheumatic Diseases* after its original publication in the *Indian Journal of Rheumatology.*

The online version has been corrected with the above changes after first online publication.

The publisher apologizes for this error and any confusion this may have caused.

DOI: 10.1111/1756-185X.14086

International Journal of Rheumatic Diseases

WILEY

Your help is needed in the fight against COVID-19: Please contribute to the COVID-19 Global Rheumatology Alliance Registry

The COVID-19 Global Rheumatology Alliance is a global collaboration of rheumatologists, scientists, patients and organisations all committed to addressing the issues in rheumatology created by the COVID-19 global pandemic. To date the alliance has published important data on the effect of COVID-19 infection on outcomes and the effect of rheumatic medications on COVID-19 outcomes.

We currently have 3520 cases from all over the world but we still need to collect many more cases and we need cases from all around the world including the Asia-Pacific region. We are hoping for more cases from the Asia-Pacific region because this is currently under-represented in the registry.

To contribute we ask that you provide details of the case, rheumatic diagnosis details, treatments, and the outcome of the case.

You can join the mailing list for the COVID-19 Global Rheumatology Alliance by signing up on our webpage (top right hand corner)

For more information please visit our website at www.rheum-covid.org, if you have questions or issues and would like to know more information please email rheum.covid@gmail.com.

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 has been launched. Interested applicant may get in contact with APLAR's Member National Organisation for more information and application form. Application information has been made available through the Member National Organisations of APLAR. Application is now closed. Look for updates by visiting the APLAR website.

APLAR Grants – Opened for application now!

Closing date: 31st March 2020

APLAR FELLOWSHIP GRANT

APLAR Fellowship Grant supports Science and Medical graduates to undertake intensive or advanced study in clinical aspects or research methodology of either adult or paediatric rheumatology in a rheumatic disease unit outside his own country, preferably at an APLAR Center of Excellence, or others, for a minimum period of six (6) months. The APLAR Executive Committee will consider the option of extending the Fellowship duration to twelve (12) months if there is a clinical need.

Applicant 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 to cover accommodation and subsistence costs.

APLAR RESEARCH GRANT

APLAR Research Grant gives science and medical graduates the opportunity to start and do research within their own country of residence. It hopes 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.

APLAR-COPCORD GRANT

The Asia Pacific League of Associations for Rheumatology (APLAR) invites applications from physicians from APLAR member societies for its APLAR Community Oriented Program for Control of Rheumatic Diseases (COPCORD) research grant. 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.

APPLICATION FOR APLAR GRANTS 2021

The APLAR Grants is now **opened for application** from **1st January to 31 March 2021**. We are pleased to inform you that up to 3 grants each, will be awarded for the APLAR Fellowship Grant and APLAR Research Grant, while up to 2 grants will be awarded for the COPCORD Grant.

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For information **on eligibility, criteria, and application requirement,** kindly visit our official website at https://www.aplar.org/education/.