ISSN 1756-1841 VOLUME 25 NUMBER 12 2022

International Journal of Rheumatic Diseases

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

WILEY

International Journal of Rheumatic Diseases

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International Journal of Rheumatic Diseases

Volume 25 | Number 12 | December 2022

Contents

Received: 27 May 2022 | Revised: 8 August 2022 | Accepted: 7 September 2022

DOI: 10.1111/1756-185X.14444

International Journal of Rheumatic Diseases

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REVIEW

Factors associated with cytomegalovirus infection in antineutrophil cytoplasmic antibody-associated vasculitis: A narrative review

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Abstract

Patients with antineutrophil cytoplasmic antibody-associated vasculitis (AAV) are vulnerable to opportunistic infections, including cytomegalovirus (CMV) infection. This narrative review aims to identify factors associated with CMV infection in patients with AAV. The literature review was conducted on Cumulative Index to Nursing and Allied Health Literature (CINAHL), Cochrane, PubMed, Scopus, and Web of Science. The start date of the literature search was unrestricted and the end date was February 2022. CMV infection was defined as (a) CMV pp65 antigenemia or positive CMV DNA viral load by polymerase chain reaction or CMV detection on histological specimens, with associated signs and symptoms compatible with CMV infection; (b) presence of CMV clinical syndrome (defined as presence of compatible symptoms and signs and documentation of CMV by biopsy by virus isolation, rapid culture, immunohistochemistry, or DNA in biopsy material as defined by the CMV Drug Development Forum); and (c) CMV infection as coded by the International Statistical Classification of Diseases and Related Health Problems, 10th revision with at least one prescription for CMV treatment. We identified 4505 articles, of which three (2327 patients with AAV) were included. All studies were retrospective and only one of the three studies included only patients with AAV. Low or decreasing lymphocyte counts and higher prednisolone usage were associated with CMV infection in patients with AAV. Patients with AAV with lymphopenia and on high doses of prednisolone should be monitored closely for signs and symptoms of CMV infection, and might benefit from CMV prophylaxis. Prospective studies are urgently needed to better identify causes of CMV infections in patients with AAV.

KEYWORDS
antibodies, antineutrophil cytoplasmic antibody-associated vasculitis, ANCA, cytomegalovirus, granulomatosis with polyangiitis, opportunistic infections

1 | INTRODUCTION

Antineutrophil cytoplasmic antibody-associated vasculitis (AAV) is an autoimmune disease entity characterized by small-vessel inflammation and necrosis, particularly in pulmonary and renal vascular beds, that can lead to end-organ damage. This group of systemic vasculitides comprises granulomatosis polyangiitis, eosinophilic granulomatous polyangiitis, and microscopic polyangiitis and is associated with significant mortality and morbidity, particularly in the first year of diagnosis.[1–6] This is a corollary of the high burden of infectious sequelae that plague the induction of remission regimens.

Compared with the general population, AAV patients have a mortality ratio of 2.6[7] and the second highest mortality of the primary systemic vasculitides.[1] More patients die from infection in the first year (48%) than from active vasculitis (19%).[7] In one of the largest reported studies of AAV using data from four European Vasculitis Study Group studies, it was found that more deaths in the first year of therapy were from therapy-associated adverse events than from active vasculitis (59% of deaths compared with 14% of deaths, respectively).[8] Cause of death of these patients was mostly due to infection (85%). These induction regimens often use high doses of glucocorticoids (either 1 mg/kg per day of oral prednisolone or intravenous methylprednisolone 0.5-1 g per day for 3 days), followed by a slow taper of prednisolone until remission is achieved, in conjunction with immunosuppressants including methotrexate, cyclophosphamide (CYC) or rituximab.[2,9,10] Induction therapy is usually continued for 3-6 months. Thereafter, patients are placed on maintenance therapy, usually with rituximab, azathioprine, or methotrexate and low-dose glucocorticoids, or mycophenolate mofetil in patients where azathioprine and methotrexate are contraindicated.[2]

Broadly speaking, AAV patients have up to seven times higher risk of infection than the general population and the overall risk remains significant,[11] even after 8 years of follow up.[3] This is, in part, a result of the heavy immunosuppression required to control disease. One long-term study for major infections in granulomatosis polyangiitis patients found that 71.7% of infectious episodes occurred during induction therapy or relapses.[12] A study based on the Polish Vasculitis Registry data also found infection to be the most frequently seen treatment side effect (38.8% of all cases).[13]

Viral infections remain a significant cause of morbidity and mortality in AAV patients, representing up to 35.8% of all major infections.[12] Cytomegalovirus (CMV), a latent virus usually of low pathogenicity that has come to be known as a noteworthy opportunistic infection in immunocompromised individuals, is a significant contributor to treatment-related fatality.[6] CMV accounted for 7.5% of all major infectious episodes in one long-term study following patients with AAV.[12] Studied extensively in transplant populations, it can be difficult to determine when CMV proceeds from asymptomatic antigenemia or viral shedding to clinically significant tissue invasive disease. Its seroprevalence in the adult population is in the range of 40%-100% depending on geographic location.[14] It is a prominent masquerader, with varied manifestations in AAV patients from the typical classical CMV pneumonia[15–22] and viral pneumonitis[6,23] to retinitis[24–28] and gastrointestinal disease[29–36] and even case reports of CMV endocarditis[37] and oesophagitis.[38] For the consistent reporting of CMV outcomes, the CMV Drug Development Forum has published definitions for CMV clinical syndromes and probable syndromes.[39]

Cyclophosphamide and corticosteroids have been frequently implicated as a cause of higher rates of any infection.[12,15,22,40–42] Factors including older age, lymphopenia and renal impairment are also frequently cited as risk factors for infectious complications in patients with rheumatic disease.[4,5,11,43–46] There has been no literature review to date examining the risk factors for CMV infection in patients with AAV. We aim to review the literature and identify risk factors for CMV infection in patients with AAV.

2 | MATERIALS AND METHODS

2.1 | Study design

The narrative review was limited to studies that included patients with AAV. CMV infection was defined as (a) CMV pp65 antigenemia or positive CMV DNA viral load by polymerase chain reaction or CMV detection on histological specimens, with associated signs and symptoms compatible with CMV infection; (b) presence of CMV clinical syndrome (defined as presence of compatible symptoms and signs and documentation of CMV by biopsy by virus isolation, rapid culture, immunohistochemistry, or DNA in biopsy material as defined by the CMV Drug Development Forum); and (c) CMV infection as coded by the International Statistical Classification of Diseases and Related Health Problems, 10th revision with at least one prescription for CMV treatment, assuming physician judgment in these cases arrived at an assessment of symptomatic CMV clinical syndrome.

2.2 | Identification and selection of studies

Literature search was conducted on Cumulative Index to Nursing and Allied Health Literature (CINAHL), Cochrane, PubMed, Scopus, and Web of Science. The search strategy included MeSH terms anti-neutrophil cytoplasmic antibody-associated vasculitis, antibodies, antineutrophil cytoplasmic, granulomatosis with polyangiitis, Churg-Strauss syndrome, microscopic polyangiitis, cytomegalovirus, and opportunistic infections, and free-text terms granulomatosis, antineutrophil cytoplasmic antibody, antineutrophil, vasculitis, polyangiitis, Churg-Strauss, cytomegalovirus, and infections. The search strategies can be found in Appendix 1. Subsequent hand searches were conducted from the references of relevant studies. The start date of the literature search was unrestricted and the end date was February 2022. Two authors, AL and WF, independently reviewed

the articles and applied the inclusion criteria. Disagreements were resolved through discussion.

2.3 | Inclusion and exclusion

Original peer-reviewed publications were included in this literature review. Exclusion criteria included those articles not written in English, systematic reviews, conference abstracts, and those whose full text was not readily available. We also excluded case reports and case series (with10 or fewer participants), systematic reviews, meta-analyses, studies where the diagnostic criteria for labeling of CMV infection were not clearly defined, studies where it was unclear if those participants with CMV infection had an underlying AAV diagnosis as opposed to any other rheumatic disease, and studies that had no comparator or control arm for development of CMV infection and that performed no analysis on risk factors for developing any infection. Gray literature such as opinion pieces, letters to the editor, abstracts, comments, and conference proceedings were excluded.

2.4 | Data extraction

Data relating to CMV infection in AAV patients were extracted. This included author, number of AAV patients with CMV infection in the study, proportion of AAV patients included in the study, study type, study duration, country, patient demographics, criterion for how CMV infection was diagnosed in the study, diagnostic criteria, and clinical features of AAV, laboratory characteristics, and steroid and immunosuppressant regimens.

3 | RESULTS

We reviewed 3925 studies and 3512 were excluded after title screening. Of the remaining 413 studies, 106 were excluded after abstract reading. Following this, 307 full-text articles were downloaded for review. Of these, three studies were finally included in the qualitative synthesis. Reasons for exclusion of studies are detailed in Figure 1.

Across the primary studies, 2327 AAV patients were included in this literature review. Only one of the three studies included for review dealt with a population comprised solely of AAV patients; the remaining two assessed patients with rheumatic disease, with the proportion of AAV patients in the study population ranging from 11% in Xue et al's study[47] to 16% in Kaneshita et al's study.[48] The studies included a preponderance of female patients (68.4% of patients studied) and median ages reported were 49 to 73 years. Characteristics of studies looking at factors associated with the development of CMV infection in AAV patients are reviewed in Table 1. A summary of the main findings from the three selected studies can be found in Table 2.

3.1 | Risk factors for CMV infection

3.1.1 | Patient demographics

Sakai et al[49] found age by decade to be significantly associated with the development of any opportunistic infection (including treated CMV infection) in their cross-sectional study of 2299 AAV patients (odds ratio [OR] 1.24, 95% confidence interval [CI] 1.12-1.36, $P<0.001$). However, increasing age was not significantly associated with CMV infection in two of the three studies included in this review. Xue et al,[47] in their reporting of factors associated with development of CMV infection in patients with any underlying rheumatic disease, found that age was not significant ($P=0.34$). These findings were similar in the study by Kaneshita et al[48] ($P=0.41$). However, it is noteworthy that both studies did not only include patients with AAV in their analyses.

Xue et al[47] found male patients with any underlying rheumatic disease to be at significant risk of developing CMV infection ($P<0.01$), while sex was not associated with CMV infection in any rheumatic disease in the study by Kaneshita et al ($P=0.053$).[48]

3.1.2 | Laboratory characteristics

Xue et al[47] found lower lymphocyte counts to be significantly associated with those patients with any underlying rheumatic disease that went on to develop symptomatic CMV pneumonia, with lymphocyte counts of 69 asymptomatic patients being median 1.2 (0.1-5.7)$\times 10^9$/L compared with 0.6 (0.1-4.0)$\times 10^9$/L for the 73 patients with symptomatic CMV infection ($P<0.05$). Kaneshita et al[48] too found that decreases in the lymphocyte count (decrement/week) 4 weeks before first detection of CMV antigenemia independently predicted for CMV infection (OR 1.96, 95% CI 1.09-3.54, $P=0.025$).

Gradient serum albumin (decrement/week) and serum albumin <30 g/L ($P=0.03$ and $P<0.01$ respectively) were found to be significantly associated with development of CMV infection in any underlying rheumatic disease in the study by Kaneshita et al.[48] They did not find gradient serum C-reactive protein or serum immunoglobulin G to be significant ($P=0.93$ and $P=0.70$ respectively).

Higher lactate dehydrogenase and blood urea nitrogen measurements were also identified as predictors for the development of CMV infection ($P<0.01$ and $P<0.05$ respectively), as was lower $CD4^+$ T-cell count ($P<0.01$) in the study by Xue et al.[47] They also found $CD4^+$ T-cell count to be associated with CMV load (relative risk 1.95, 95% CI 1.15-2.66).

3.1.3 | Comorbidities

Xue et al[47] in their study of patients with any underlying rheumatic disease found raised creatinine ($P<0.05$), suggestive of renal impairment, to be significantly associated with those patients

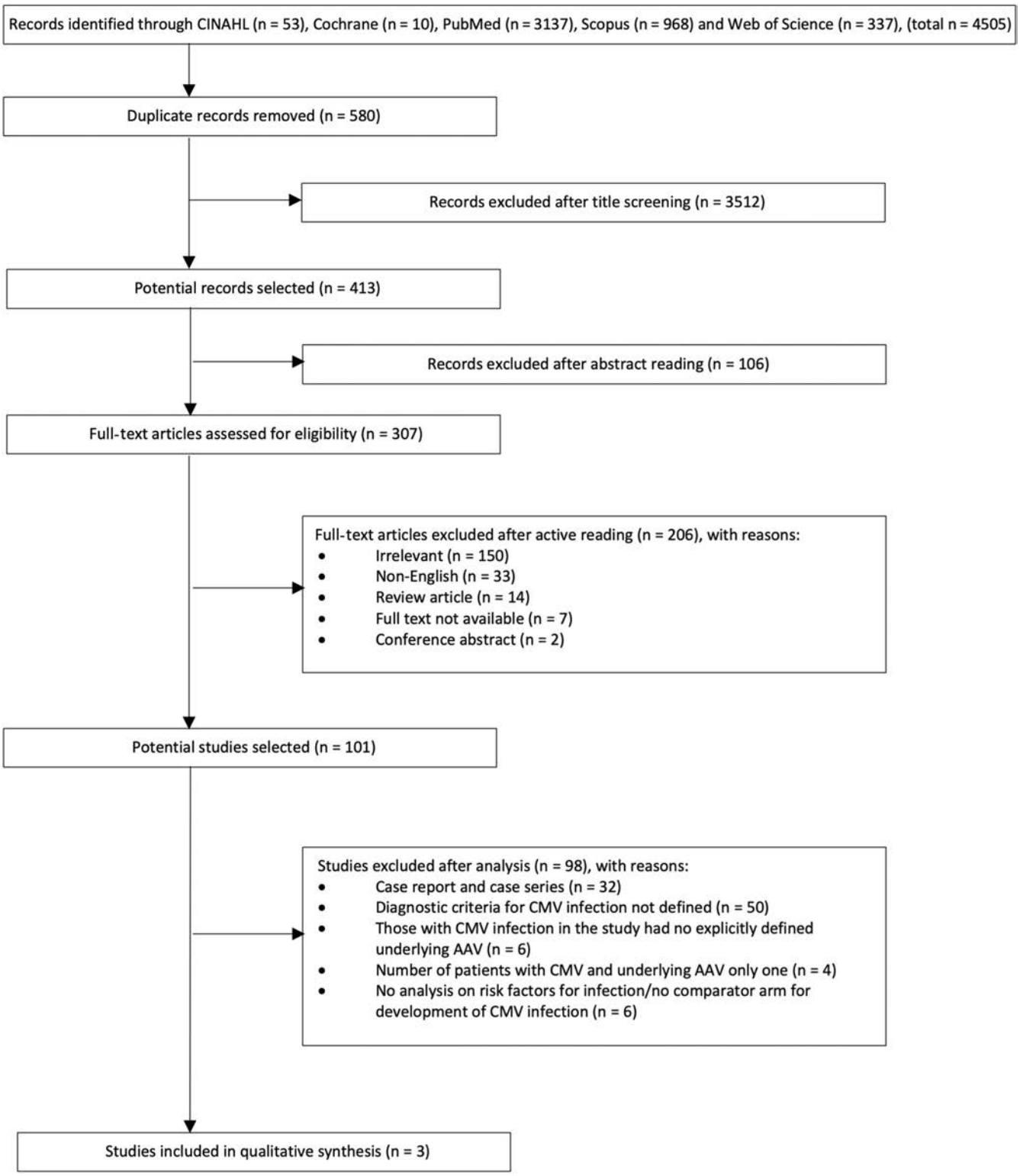

FIGURE 1 Selection of articles

who developed CMV infection. Oral candidiasis ($P = 0.01$) was a significant risk factor for the development of CMV infection in patients with underlying rheumatic disease in Kaneshita et al's study.[48]

3.1.4 | Disease characteristics of AAV

Xue et al[47] found shorter disease duration ($P < 0.05$) to be significantly associated with CMV infection in any underlying rheumatic disease.

TABLE 1 Characteristics of studies examining risk factors for CMV infection in patients with ANCA vasculitis

Author	Year of publication	Number of AAV patients with CMV infection, n (%)	Number of AAV patients in study population, n (%)	How AAV was diagnosed	How CMV infection was diagnosed	Study type	Study duration (years)	Country	Sex (% male)	Age (years)	Significant risk factors	Non-significant factors
Xue et al[47]	2016	Unknown	15/142 (11%)	Not reported	CMV DNA viral load by PCR with CMV pneumonia on chest X-ray film or high-resolution computed tomography and compatible symptoms	Retrospective analysis	7	China	19	Median: 49 (16-87)	CMV in any rheumatic disease • Male ($\chi^2 = 9.280$, $P < 0.01$) • Shorter disease duration (Z value = −2.571, $P < 0.05$) • Dose of PSL correlated with CMV viral load (Spearman coefficient = 0.315, $P < 0.01$) • PSL use for recent 3 months (Z value = −3.069, $P < 0.01$) • Higher doses of average PSL dose (Z value = −3.865, $P < 0.01$) • Use of immunosuppressants ($\chi^2 = 7.654$, $P < 0.01$) • Use of cyclosporine ($\chi^2 = 15.176$, $P < 0.01$) • Use of CYC ($\chi^2 = 4.407$, $P < 0.05$) • Use of mycophenolate mofetil ($\chi^2 = 4.856$, $P < 0.05$) • Lower lymphocyte counts (Z value = −2.314, $P < 0.05$) • Higher ALT, ALP, GGT (Z values = −2.923, −2.877, −3.704, $P < 0.01$, $P < 0.01$, $P < 0.01$) • Higher LDH, BUN (Z values = −3.174, −2.450, $P < 0.01$, $P < 0.05$) • Higher creatinine level (Z value = −2.072, $P < 0.05$) • Lower $CD4^+$ T-cell count (Z value = −3.216, $P < 0.01$) • $CD4^+$ T-cell count associated with CMV load (relative risk = 1.95, 95% CI 1.15-2.66)	CMV in any rheumatic disease • Age (t value = 0.963, $P = 0.34$) • Duration of PSL therapy (Z value = −1.017, $P = 0.31$) • WBC count (Z value = −0.609, $P = 0.54$) • Neutrophil count (Z value = −1.464, $P = 0.14$) • AST (Z value = −1.262, $P = 0.21$) • $CD8^+$ T-cell count (Z value = −0.533, $P = 0.59$)

(Continues)

TABLE 1 (Continued)

Author	Year of publication	Number of AAV patients with CMV infection, n (%)	Number of AAV patients in study population, n (%)	How AAV was diagnosed	How CMV infection was diagnosed	Study type	Study duration (years)	Country	Sex (% male)	Age (years)	Significant risk factors	Non-significant factors
Sakai et al[49]	2019	122 (5.3%)	2299/2299 (100%)	ICD-10 code	ICD-10 code for CMV and at least one prescription for anti-CMV treatment	Retrospective analysis	9	Japan	44.8	Median: 73 (IQR 65-80)	Any opportunistic infection • Age by decade, OR 1.24 (95% 1.12-1.36), $P<0.001$ • Maximum daily PSL dosage in the index month per 10 mg increment, OR 1.16 (95% CI 1.08-1.25), $P<0.001$ • Corticosteroid pulse therapy, OR 1.29 (95% CI 1.04-1.60), $P = 0.023$]	Any opportunistic infection Immunosuppressive drugs or rituximab, OR 1.11 (95% CI 0.89-1.39), $P = 0.338$
Kaneshita et al[48]	2020	Unknown	13/80 with CMV pp65 antigenemia (16%)	Not reported	CMV pp65 antigenemia with symptoms or end-organ disorder	Retrospective analysis	9	Japan	31	Median: 65 (IQR 51.5-74.0)	CMV in any rheumatic disease • Oral candidiasis, OR 8.82 (95% CI 1.64-47.30), $P = 0.01$ • Serum albumin <30 g/L, OR 0.81 (95% CI 0.69-0.95), $P<0.01$ • CMV pp65-positive cell count >5.6/10^5 PMNs, OR 1.26 (95% CI 1.05-1.50), $P = 0.01$ • Gradient serum albumin (95% CI decrement/week), OR 2.02 (95% CI 1.07-3.8), $P = 0.03$ • Gradient lymphocyte count (decrement/week), OR 1.96 (95% CI 1.09-3.54), $P = 0.025$	CMV in any rheumatic disease • Age, OR 1.02 (95% CI 0.97-1.08), $P = 0.41$ • Sex, OR 4.74 (95% CI 0.98-22.9), $P = 0.053$ • Gradient serum CRP, OR 0.96 (95% CI 0.82-1.12), $P = 0.58$ • Serum IgG, OR 1.05 (95% CI 0.82-1.34), $P = 0.70$ • Lymphocyte count, OR 0.91 (95% CI 0.79-1.06), $P = 0.22$ • PSL dose, OR 0.92 (95% CI 0.54-1.56), $P = 0.76$

Abbreviations: AAV, antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CI, confidence interval; CMV, cytomegalovirus; CRP, C-reactive protein; CYC, cyclophosphamide; GGT, gamma-glutamyl transpeptidase; ICD-10, International Statistical Classification of Diseases and Related Health Problems 10th Revision; IQR, interquartile range; LDH, lactate dehydrogenase; OR, odds ratio; PCR, polymerase chain reaction; PMN, polymorphonuclear leukocytes; PSL, prednisolone; WBC, white blood cell.

TABLE 2 Summary of the main findings from the three selected studies

	Significant risk factors for CMV infection in patients with ANCA vasculitis
Patient demographics	Age (for any opportunistic infection)[49] Male[47]
Laboratory characteristics	Lymphopenia or decreases in lymphocyte count over 4 weeks[47,48] Albumin <30 g/L or decreases in albumin over 4 weeks[48] Lower $CD4^+$ T-cell count[47] Higher LDH, BUN levels[47]
Comorbidities	Renal impairment[47] Oral candidiasis[48]
Disease characteristics of AAV	Shorter disease duration[47]
Treatment	Higher doses of prednisolone[47,49] Use of immunosuppressants, cyclosporine, CYC or mycophenolate mofetil[47]

Abbreviations: AAV, antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis; BUN, blood urea nitrogen; CMV, cytomegalovirus; CYC, cyclophosphamide; LDH, lactate dehydrogenase.

3.1.5 | Treatment

Prednisolone use was identified as a risk factor for CMV infection.[47,49] In their multivariate analysis, Sakai et al[49] found maximum daily prednisolone dosage in the index month and corticosteroid pulse therapy (OR 1.16, 95% CI 1.08-1.25, $P < .001$ and OR 1.29, 95% CI 1.04-1.60, $P = 0.023$, respectively) to be significantly associated with the development of any opportunistic infection. Xue et al[47] found prednisolone use in the recent 3 months to be significantly associated with symptomatic CMV in patients with any underlying rheumatic disease ($P < 0.01$). They also found that their patients with symptomatic CMV infection received higher average prednisolone doses compared with their non-symptomatic counterparts (median 32, range 4-100 mg/day vs. median 20, range 1-50 mg/day, $P < 0.01$). They also found that the dose of prednisolone correlated with CMV viral load (Spearman coefficient = 0.315, $P < 0.01$). They did not, however, find duration of prednisolone therapy (measured in months) to be significantly different between the two groups ($P = 0.31$). Kaneshita et al[48] did not find prednisolone dose to be a significant risk factor for CMV infection ($P = 0.76$) in their study of patients with any underlying rheumatic disease.

Xue et al[47] found use of immunosuppressants, use of mycophenolate mofetil, cyclosporine, or CYC (all at least $P < 0.05$) to be significantly associated with development of CMV infection. Conversely, use of immunosuppressive drugs or rituximab (OR 1.11, 95% CI 0.89-1.39, $P = 0.338$) were not implicated in the development of CMV infection in Sakai et al.[49]

4 | DISCUSSION

This literature review identified higher doses of glucocorticoid use and lymphopenia as being associated with the development of CMV infection in patients with AAV.

Glucocorticoid therapy exerts its anti-inflammatory effects by inhibiting a diverse range of leukocyte populations including neutrophils, lymphocytes, and monocytes.[40,50] This inhibition may allow CMV infection to develop. Watanabe-Imai et al[22] found that an initial prednisolone dosage of more than 0.8 mg/kg/day predicted serious infection in their study of Japanese patients with AAV ($P = 0.002$ and $P = 0.001$ for two models used in their prospective study). Of the 63 serious infections reported in their study, there were four cases of CMV pneumonia and nine of CMV viremia.

Glucocorticoid therapy has been shown to be a risk factor for CMV infection in other diseases. Takizawa et al[51] reported that the use of pulsed methylprednisolone significantly increased mortality rate ($P = 0.03$) in patients with rheumatic disease with CMV infection. They included a broad range of rheumatic disease in their study, with systemic lupus erythematosus (SLE), microscopic polyangiitis, and dermatomyositis being the most common conditions. In another study, into which of SLE, microscopic polyangiitis, rheumatoid arthritis, dermatomyositis, and polymyositis featured most prominently, a significantly higher initial dose of prednisolone was found to predict for CMV antigenemia (1.06 ± 0.15 vs. 0.91 ± 0.25 mg/kg of body weight/day, $P < 0.05$).[52] In their systematic review of patients with SLE, Choo et al[53] identified prednisolone as a significant risk factor for CMV infection. Santos et al[54] demonstrated that higher doses of daily oral corticosteroids were a predictor for mortality ($P = 0.07$) in their study of patients with autoimmune rheumatic disease, of whom 59% of patients found to have CMV antigenemia had SLE. In allogeneic stem cell transplant populations, patients with intermediate risk (donor and recipient CMV serostatus D+/R+ and D+/R−) receiving prednisone were found to have a higher incidence of CMV infection ($P = 0.01$) and higher CMV recurrence rates ($P = 0.02$).[55] In a study of lung transplant recipients in South Korea, multivariate analysis identified steroids at more than twice the standard dose as a risk factor for CMV reactivation (hazard ratio 8.07, $P < 0.001$).[56]

Low or decreasing lymphocyte counts may be a factor associated with the development of CMV infection. However, it should be noted that conclusions drawn from Xue et al[47] are limited because they studied a population of any rheumatic disease and not AAV in particular, with no subgroup analysis performed on the AAV patients included in their cohort. Their participants comprised mostly patients with SLE (37%) and dermatomyositis (37%), followed by AAV (11%). One study evaluating patients with connective tissue diseases complicated by CMV antigenemia found the peripheral blood lymphocyte counts in CMV antigenemia-positive patients to be significantly lower than in their antigenemia-negative counterparts ($812 \pm 475/\mu L$ and $2066 \pm 1338/\mu L$ respectively, $P = 0.01$).[57] This study included mostly patients with SLE but also polymyositis and dermatomyositis and one patient with microscopic polyangiitis. Elsewhere, lymphopenia has not been found to be a significant predictor for CMV infection in AAV populations.[58,59] In a liver transplant population, multivariate analysis demonstrated pretransplant lymphopenia to be the strongest independent predictor of CMV infection (hazard ratio 5.52, 95% CI 2.31-13.1, $P = 0.001$).[60] In one Chinese cohort of patients with AAV, it was reported that $CD4^+$ T-cell count had a higher predictive value than lymphocyte count for overall infections.[61] Prospective studies should be performed to elucidate if lymphopenia is a definite risk factor for CMV infection.

$CD4^+$ T cells are integral to antiviral immunity and to the resolution of symptomatic disease in primary CMV infection as well as potential maintenance of latently infected cells. CMV infection has been shown to alter the composition of the $CD4^+$ T-cell compartment, inducing the accumulation of short-lived, multifunctional $CD4^+CD45RA^+CD27^-$ T cells[62] and, following long-term carriage, generating large expansions in the $CD4^+$ T-cell memory response.[63] Following CMV infection, there should be a robust $CD4^+$ T-cell response, and poorer $CD4^+$ T-cell responses result in a prolonged course of viral shedding and more severe disease.[64] In the first months after kidney transplantation, a decrease in CMV-specific $CD4^+$ T-cell frequencies preceded the clinical symptoms of CMV disease.[65] Similarly, in adults transplanted with cord blood, CMV reactivation by day 60 was associated with lower $CD4^+$ T-cell counts.[66]

Hypoalbuminemia and its association with the development of CMV infection may be reflective of high disease activity of the patient's underlying AAV, causing the individual to be susceptible to opportunistic infection as serum albumin is a negative inflammatory biomarker showing an inverse correlation with disease activity in AAV patients. Albumin levels show a strong correlation with the Birmingham Vasculitis Activity Score and have been found to increase following treatment.[67]

It was interesting to note that shorter duration of the underlying rheumatic disease showed a significant association with CMV infection in the current review[47] as it would follow that a longer disease duration would have a cumulatively greater exposure to glucocorticoids and immunosuppressive treatment, and by extension, immunocompromised state and susceptibility to opportunistic infection. In the study in question by Xue et al,[47] the shorter duration might have occurred because seven of the 73 patients with CMV infection died, with 42.9% of them having concomitant fungal infection.

There was no consensus in the studies included in this review on whether other immunosuppressant use was implicated in CMV infection in AAV. In two randomized controlled non-inferior trials comparing rituximab to the previous standard of care CYC, for induction of remission in AAV patients, the RAVE and RITUXVAS trials, there was no observed difference between incidence of adverse events.[68,69] In another randomized controlled trial comparing CYC with methotrexate for induction of remission, there was no significant difference in the incidence of infections for either group, though there was mention of one death from CMV infection in the CYC group.[6] Number and incidence of infections did not differ between induction with CYC or rituximab in a review studying induction remission treatment regimens for AAV patients.[9]

The risk factors for CMV infection identified in this review are similar to those studied in SLE populations. As mentioned previously, Choo et al[53] found that prednisolone use predicted CMV infection in SLE populations. However, in contrast to the current review on AAV patients, they did find other immunosuppressant use such as mycophenolate mofetil and azathioprine use to be predictors for CMV infection in patients with SLE, and not lymphocyte or leukocyte count. As an aside, they did not find age to be associated with development of CMV infection.

To the best of our knowledge, this is the first literature review to study data for risk factors significant to the development of CMV infection in patients with AAV. However, there are limitations in our study. All the studies were conducted retrospectively, and we were therefore unable to ascertain the temporal relationship between the factors and development of CMV. Also, there was only a single study that included only patients with AAV, whereas the other two cohorts included patients with other rheumatic diseases, and the number of patients with AAV in these cohorts were small, so conclusions could only be inferred. Further, one of the three study's outcomes was that of opportunistic infections, and although CMV infection was reported to be their most frequently occurring opportunistic infection, there was no analysis performed specifically for risk factors of CMV infection alone. In one of the three studies, Xue et al[47] did observe that 23.2% of its symptomatic CMV cohort had co-infections. These patients were not excluded from the study. They were still included in the current literature review however, because co-infections with CMV commonly occur in the real world. Other infectious complications accompanying CMV infection have been reported as 34%[51] and up to 72.7% in other studies.[54]

Another significant hurdle is the lack of consensus on definitions for CMV infection, where many studies were found to define CMV infection as antigenemia alone, which was a definition that was not accepted in this review. As mentioned earlier, it is proposed that future research efforts extrapolate the CMV Drug

Development Forum's published definitions for CMV clinical syndromes for transplant patients to patients with rheumatic diseases for the consistent reporting of CMV outcomes.[39] More research, with particular attention to the issues highlighted above, is needed in this area.

5 | CONCLUSION

Low or decreasing lymphocyte counts and higher doses of prednisolone are associated with CMV infection in patients with AAV. Such information would be useful to clinicians in risk stratification of AAV patients and as a guide for whom chemoprophylaxis for CMV infection might be indicated.

AUTHOR CONTRIBUTIONS

All listed authors have contributed significantly in all of the following: (a) substantial contributions to the conception or design of the work, or the acquisition, analysis, or interpretation of data for the work; (b) drafting the work or revising it critically for important intellectual content; (c) final approval of the version to be published; and (d) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ACKNOWLEDGEMENTS

We would like to thank librarian Wong Suei Nee of the National University of Singapore for her assistance in this narrative review.

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How to cite this article: Leong A, Fong W. Factors associated with cytomegalovirus infection in antineutrophil cytoplasmic antibody-associated vasculitis: A narrative review *Int J Rheum Dis*. 2022;25:1357-1367. doi: 10.1111/1756-185X.14444

APPENDIX 1

SEARCH STRATEGIES

CINAHL

Cytomegalovirus and vasculitis.

Cochrane

Cytomegalovirus and vasculitis.

PubMed

A AND B.

A) cytomegalovirus[Mesh] OR cytomegalovirus[Title/Abstract] OR opportunistic infections[Mesh] OR infections[Title/Abstract].

B) anti-neutrophil cytoplasmic antibody-associated vasculitis[Mesh] OR granulomatosis[Title/Abstract] OR antibodies, anti neutrophil cytoplasmic[Mesh] OR anca[Title/Abstract] OR microscopic polyangiitis[Mesh] OR antineutrophil[Title/Abstract] OR Churg-Strauss syndrome[Mesh] OR vasculitis[Title/Abstract] OR granulomatosis with polyangiitis[Mesh] OR polyangiitis[Title/Abstract] OR Churg-Strauss[Title/Abstract].

Scopus

Cytomegalovirus and vasculitis.

Web of Science

Cytomegalovirus and vasculitis.

Received: 14 January 2022 | Revised: 11 July 2022 | Accepted: 14 August 2022

DOI: 10.1111/1756-185X.14428

International Journal of Rheumatic Diseases WILEY

ORIGINAL ARTICLE

A prognostic analysis of antisynthetase syndrome-related interstitial lung disease

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Funding information
Chongqing Science and health joint medical research project, Grant/Award Number: 2020MSXM033

Abstract

Objective: To analyze prognostic factors of antisynthetase syndrome (ASS)-related interstitial lung disease (ILD).

Methods: We retrospectively collected the data of 77 inpatients with ASS-ILD at our hospital from January 1, 2018, to January 1, 2021. The improvement/stability group and deterioration/death group were defined according to their follow-up outcome. Clinical data of the 2 groups were compared. Univariate analysis was adopted to screen the possible prognostic factors and then logistic regression was used for multivariate analysis.

Result: After 6 to 42 months of follow-up, 52 patients (67.5%) were classified into the improvement/stability group, and 25 patients (32.5%) were classified into the deterioration/death group. According to the multivariate stepwise logistic regression analysis, respiratory failure (odds ratio [OR] = 6.71, 95% CI: 1.64–27.38, $P = .008$) and elevated muscle enzymes (OR = 4.31, 95% CI: 1.03–18.05, $P = .045$) were found to be independent risk factors, while mechanic's hands (OR = 0.06, 95% CI: 0.01–0.37, $P = .003$) and anti-Jo-1 antibody (OR = 0.24, 95% CI: 0.06–0.93, $P = .039$) were protective factors.

Conclusion: The prognostic assessment of ASS-ILD patients should be emphasized. Patients with a poor prognosis should be identified early based on their risk factors to guide clinical management decisions.

KEYWORDS
anti-aminoacyl-transferase RNA synthetase antibody, antisynthetase syndrome, interstitial lung disease, myositis, prognosis

1 | INTRODUCTION

Antisynthetase syndrome (ASS), which is characterized by the detection of positive anti-aminoacyl-transferase RNA synthetase (ARS) antibodies, manifests as interstitial lung disease (ILD), polymyositis/dermatomyositis (PM/DM), arthritis, mechanic's hands, Raynaud's phenomenon, and pyrexia of unknown origin.[1] Between 80% and 100% of ASS cases are associated with ILD, which is the major cause of death (mortality rate 4.8%–29.0%[2,3]) in ASS patients. ASS-ILD mainly has a chronic or subacute course, with a good response to

Xin Li and Qian Zhou contributed equally to this work.

glucocorticoids during initial therapy, and the short-term prognosis is generally good. However, 33.3% of ASS-ILD patients experience a relapse or deterioration in the long term.[4]

At present, few studies have investigated the prognosis of ASS-ILD patients, and most were small. The prognostic risk factors include non-anti-Jo-1 ARS antibodies, poor pulmonary function test (PFT) results, rapidly progressive ILD (RP-ILD), usual interstitial pneumonia (UIP) patterns, malignant tumors, male gender, elevated serum ferritin, fever, and decreased CD3+ CD4+ cell counts.[2,3,5–7] However, the prognostic factors of ASS-ILD patients are controversial and are still being researched.

2 | MATERIALS AND METHODS

2.1 | Study population

We screened the medical records of patients who were hospitalized at the First Affiliated Hospital of Chongqing Medical University between January 1, 2018 and January 1, 2021. Immunoblotting was used to detect anti-Jo-1, PL-7, PL-12, EJ, OJ, KS, Zo, and Ha antibodies. The laboratory-recommended cutoff values were used for determining positive, intermediate, and negative results as follows: <5, >10, and 5–10 AU/mL as negative, positive, and intermediate, respectively. Patients with intermediate results were rechecked after 8–12 weeks and were included if the result was positive. A total of 77 inpatients were diagnosed with ASS-ILD and followed up regularly by outpatient visits and telephone interactions until July 1, 2021.

The inclusion criteria were as follows: (a) inpatients with ILD diagnosed based on clinical manifestations, chest computed tomography (CT)/high-resolution computed tomography (HRCT), PFTs, or a pathological biopsy (only in a small number of patients); (b) inpatients diagnosed with ASS according to the 2011 Solomon criteria;[1] (c) regular follow-up and generally complete data on medical history and clinical information. The exclusion criteria were: (a) ILD with known causes, such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, Sjögren's syndrome, allergic pneumonia, pneumoconiosis, poisons, drugs, radiation damage, etc; (b) ILD combined with uncontrolled pulmonary infection.

2.2 | Clinical data

The clinical data, including demographics, symptoms, signs, serological data, chest CT/HRCT, PFTs, treatment information, and the follow-up data were collected.

2.3 | Diagnostic and assessment criteria

The Solomon criteria were adopted as the diagnostic criteria for ASS. The 2 major criteria are: (a) unexplained ILD; and (b) PM or DM, diagnosed based on the Bohan and Peter criteria.[8,9] The 3 minor criteria are: (a) arthritis; (b) Raynaud's phenomenon;[10] and (c) mechanic's hands.[11] The diagnosis of ASS is based on the presence of 2 major or 1 major plus 2 minor criteria, along with positive results for anti-ARS antibody testing. The diagnostic criteria of ILD refers to the 2013 American Thoracic Society/European Respiratory Society (ATS/ERS) standard criteria.[12] ASS-ILD was diagnosed and evaluated by a multidisciplinary team consisting of respiratory physicians, rheumatologists, and radiologists in our hospital. The CT/HRCT patterns of ILD referred to the imaging classification of idiopathic interstitial pneumonia proposed by ATS/ERS in 2013,[12] and the chest CT/HRCT images were independently read by experienced respiratory physicians and radiologists in our hospital. Disagreements, if any, were resolved through negotiation.

The clinical assessment of the condition of the ASS-ILD patients was based on the 2018 Chinese expert-based consensus statement.[13] As there is no recognized ASS disease activity assessment tool, the activity of ASS was evaluated by rheumatic immunologists on the basis of the physician's global assessment that was presented via visual analog scale (VAS) scores as follows: 0 = none; 1 to 3 = mild; 4 to 6 = moderate; 7 to 9 = severe; and 10 = intolerable. The outcome of ASS was classified as improved, stable, or deteriorated. Improvement and deterioration were respectively defined as a decrease or increase of at least 3 points, whereas stable disease was defined by changes of less than 3 points[14] (the VAS score at the last HRCT was compared with the baseline VAS score). The outcome assessment of ILD was evaluated by comparing the extent of imaging abnormalities at diagnosis with those at the last follow-up chest CT/HRCT and classified as improved, stable, or deteriorated. Improved and deteriorated states of ILD were respectively defined as a decrease or increase of at least 10% of the overall disease extent, whereas stable disease was defined as changes of less than 10%.[15] The final prognostic evaluation of ASS-ILD was made based on the integration of ASS and ILD. If both ASS and ILD were improved/stable, the ASS-ILD assessment was rated as improved/stable (52 cases). If both ASS and ILD had deteriorated/the patient died, the ASS-ILD assessment was reported as deteriorated/died (22 cases). If ASS improved/was stable and ILD deteriorated or ASS deteriorated and ILD improved/stabilized, the ASS-ILD assessment was graded as deteriorated (n = 2 and n = 1, respectively). The improvement/stability group was classified as the good-prognosis group (n = 52), and the deterioration/death group was classified as the poor-prognosis group (n = 25).

Respiratory failure was defined as a resting $PaO_2 < 60$ mm Hg or an arterial oxygen partial pressure (PaO_2) to fractional inspired oxygen (FiO_2) ratio < 300 mm Hg at sea level.

2.4 | Statistical analysis

SPSS 26.0 (IBM Corporation) was used for statistical analysis. Variables are expressed as number (percentage), median, or mean ± standard deviation. Comparisons of the categorical variables were performed using the Chi-square or Fisher's exact test,

and comparisons of the continuity variable were performed using Mann–Whitney U test, of which variables with $P < .1$ were screened for inclusion in the multivariate analysis. Stepwise logistic regression was used for the multivariate analysis, and a $P < .05$ was considered indicative of significant differences.

3 | RESULTS

3.1 | Demographics and clinical data

Among the 77 ASS-ILD patients, 26 were men (33.8%) and 51 were women (66.2%); the mean age was 58.87 ± 12.38 years, and the age at diagnosis ranged from 21 to 76 years. The duration from symptom onset to diagnosis of ASS-ILD ranged from 15 days to 6 years (median 8.0 months). The duration of follow-up ranged from 6 to 42 months.

3.2 | Demographics and clinical data at disease onset

Among the 77 participants, 26 (33.8%) had polymyositis and 16 (20.8%) had dermatomyositis. Arthritis, mechanic's hands, Raynaud's phenomenon, and pyrexia of unknown origin occurred in 39 (50.6%), 29 (37.7%), 28 (36.4%), and 2 (2.6%) patients, respectively.

All of the 77 participants had ILD, which had an acute/subacute or chronic/insidious onset in 36 and 41 participants, respectively. The initial manifestations at diagnosis included respiratory symptoms (n = 38; 49.4%), arthritis (n = 13; 16.9%), myositis (n = 10; 13.0%), mechanic's hands (n = 1; 1.3%), Raynaud's phenomenon (n = 3; 3.9%), and 2 or more of the above (n = 12; 15.6%).

Eight patients (10.4%) had complicated presentation with malignant tumors, among whom 6 had lung cancer, 1 had cervical cancer, and 1 had colon cancer. Among these 8 patients, we detected anti-PL-7 antibody in 4 patients, anti-Jo-1 antibody in 2 patients, anti-PL-12 antibody in 1 patient, and anti-EJ antibody in 1 patient.

3.3 | Laboratory results

The most common type of anti-ARS antibodies was anti-Jo-1 in 46 participants (59.7%), followed by anti-PL-7 in 11 participants (14.3%), anti-EJ in 11 participants (14.3%), anti-PL-12 in 8 participants (10.4%), and anti-OJ in 1 participant (1.3%). A total of 21 participants (27.3%) were positive for antinuclear antibody (ANA; high titer) and 45 (58.4%) were positive for the anti-Ro-52 antibody.

White blood cell count (normal range: $3.5–9.5 \times 10^9$/L) was increased in 29 participants (37.7%) and neutrophil% (N%; normal range: 40%–75%) was increased in 35 participants (45.5%). C-reactive protein level and erythrocyte sedimentation rate were elevated in 38 (51.4% [38/74]) and 50 participants (73.5% [50/68]), respectively. The muscle enzymes were elevated in 44 (61.1% [44/72]) participants. Serum level of C3 or C4 decreased in 26 participants (37.1% [26/70]).

3.4 | PFT

PFT was performed in 45 participants at diagnosis, and 40 (88.9%) showed restrictive ventilation dysfunction, whereas 40 (88.9%) showed a decline in diffusion capacity of carbon monoxide.

3.5 | CT/HRCT patterns of ILD

Patients were classified into 5 groups according to the ILD patterns displayed on chest CT/HRCT at diagnosis: nonspecific interstitial pneumonia (NSIP; n = 56), organizing pneumonia (OP; n = 10), NSIP with OP (n = 7), UIP (n = 2), and acute interstitial pneumonia (AIP; n = 2; Figure 1).

3.6 | Treatment

In total, 70 participants (90.9%) were treated with glucocorticoids; 6 participants refused glucocorticoid therapy because of fear that it might lead to tumor progression, and 1 patient refused because of the potential side effects. Among the 70 participants, 51 were treated with glucocorticoids combined with immunosuppressants (32 with cyclophosphamide, 6 with cyclosporine, 4 with azathioprine, 3 with methotrexate, 3 with hydroxychloroquine, 2 with mycophenolate mofetil, and 1 with tacrolimus). Only 14 participants (18.2%) received high-dose immunoglobulin therapy, and none of the participants received rituximab. A total of 19 participants had a complication of respiratory failure, including 6 who received noninvasive mechanical ventilation and 4 who received invasive mechanical ventilation; the remaining 9 participants improved after receiving supplemental oxygen via mask or nasal cannula.

3.7 | Prognostic analysis

After a 6- to 42-month follow-up, the outcome of ASS was defined as improved in 33 cases (42.9%), stable in 21 cases (27.3%), deterioration in 8 cases (10.4%), and death in 15 cases (19.5%). Furthermore, the outcome of ILD was classified as improved in 34 cases (44.2%), stable in 19 cases (24.7%), deteriorated in 9 cases (11.7%), and death in 15 cases (19.5%). The comprehensive prognostic assessment of ASS-ILD showed that 36 (46.8%) improved, 16 (20.8%) were stable, 10 (13.0%) deteriorated, and 15 (19.5%) died. Four patients died during their first hospitalization due to critical illness, among which 2 patients died of severe infection and 2 of severe ILD. The remaining 11 patients died during the follow-up, including 1 of

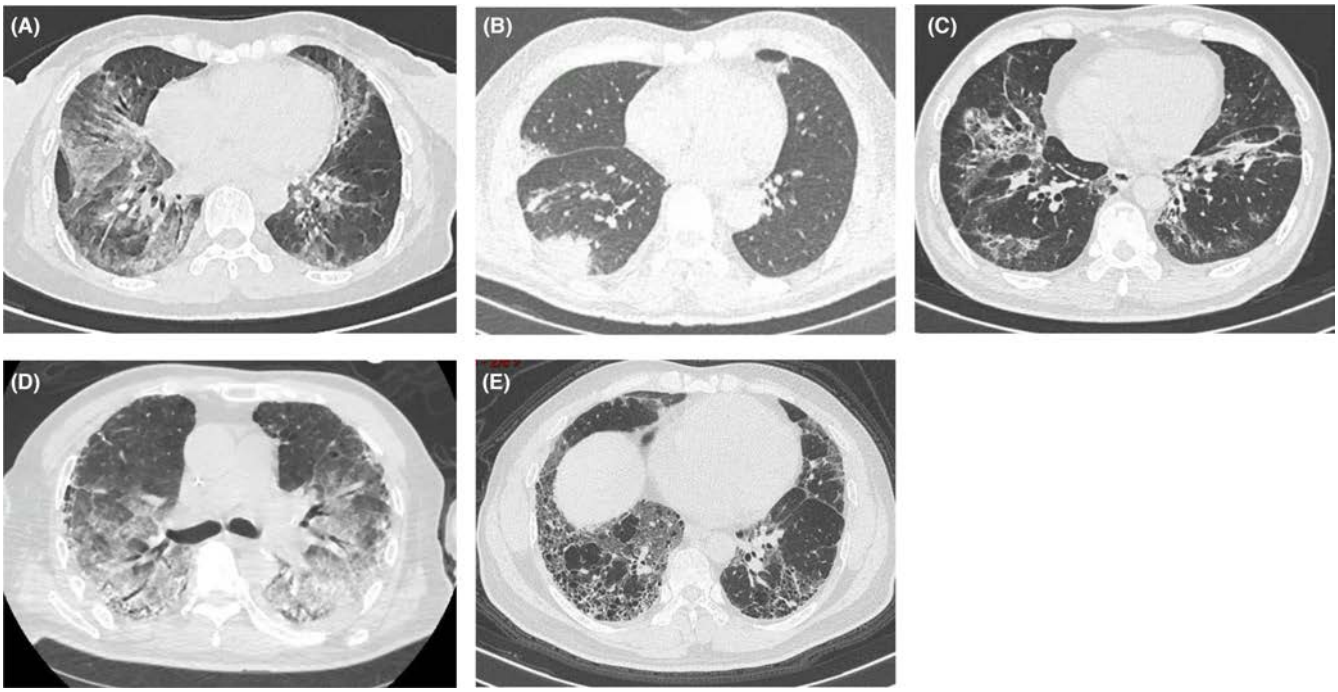

FIGURE 1 Chest high-resolution computed tomography patterns of antisynthetase syndrome -interstitial lung disease. Panels A–E are nonspecific interstitial pneumonia (NSIP), organizing pneumonia (OP), NSIP with OP, acute interstitial pneumonia, and usual interstitial pneumonia, respectively

cryptococcal meningitis, 2 of ILD exacerbation, 2 of lung cancer, and 6 of pneumonia.

We included 26 variables of clinical symptoms and signs, laboratory examinations, imaging characteristics, and treatment in the univariate analysis (Table 1). The results showed that gender, age ≥65 years, mechanic's hands, respiratory failure, anti-Jo-1 antibody, and elevated muscle enzymes were potential prognostic factors ($P < 0.1$). The above 6 variables were included in the multivariate logistic regression analysis (Table 2), and the results showed that respiratory failure (odds ratio [OR] = 6.71, 95% CI: 1.64–27.38, $P = .008$) and elevated muscle enzymes (OR = 4.31, 95% CI: 1.03–18.05, $P = .045$) were independent prognostic factors ($P < .05$), whereas mechanic's hands (OR = 0.06, 95% CI: 0.01–0.37, $P = .003$) and anti-Jo-1 antibody (OR = 0.24, 95% CI: 0.06–0.93, $P = .039$) were protective factors.

In our study, 19 participants (24.7%) had respiratory failure, of whom 6 (11.5%) were in the good-prognosis group and 13 (52.0%) were in the poor-prognosis group, respectively. Therefore, we further studied the 13 patients with respiratory failure in the poor-prognosis group. For the lung involvement degree of the 13 patients, 12 (92.3%) patients had lung involvement area ≥50% (the semi-quantitative CT analysis was applied to assess the area of lung involvement[16]). With regard to the onset of ILD[17] in the 13 patients, 9 cases (69.2%) had acute/subacute onset. The types of ARS antibodies in the 13 patients were identified as anti-Jo-1 in 5 patients, anti-PL-12 in 2 patients, anti-PL-7 antibodies in 3 patients, and anti-EJ in 3 patients.

4 | DISCUSSION

It is known that 80% to 100% of ASS patients are affected by ILD. Most ASS-ILD patients generally have a good prognosis in the short term; however, in the long term, 33.3% of patients undergo relapse or deterioration,[4] and their 5-year survival rate is 72.0% to 97.0%.[2–4,6,7] Clarifying the prognostic factors will be helpful for formulating individualized therapy and follow-up strategies. In our study, we analyzed the prognosis and prognostic factors of 77 patients with ASS-ILD followed up in the First Affiliated Hospital of Chongqing Medical University from January 1, 2018, to January 1, 2021. In our study, the population ratio of the good-prognosis group to the poor-prognosis group was about 2 vs 1, which was similar to the prior literature.[18] According to the multivariate logistic analysis, respiratory failure and elevated muscle enzymes were independent prognostic risk factors, while mechanic's hands and anti-Jo-1 antibody were protective factors in ASS-ILD.

The results of multivariate analysis showed that respiratory failure was an independent prognostic risk factor (OR = 6.71, $P < .05$). It has been reported that hypoxemia is an independent risk factor for death in patients with ILD,[19–21] and the occurrence of respiratory failure at diagnosis indicates a severe degree of lung involvement and respiratory function. Further analysis of the 13 patients with respiratory failure in the poor-prognosis group showed a possible relationship between lung involvement and a poor prognosis, but more research is required to validate this finding. Besides, it showed that patients with acute onset progressed rapidly and they were prone

TABLE 1 Comparison of clinical characteristics between the 2 groups, n (%)

Characteristics	Good-prognosis group (n = 52)	Poor-prognosis group (n = 25)	Chi-square value/*U* value	*P* value
Gender, woman	38 (73.1)	13 (52.0)	3.35	.07
Age ≥ 65 y	9 (17.3)	10 (40.0)	4.70	.03
Initial symptoms[a]				
Non-respiratory symptoms	22 (42.3)	5 (20.0)		.15
Respiratory symptoms	22 (42.3)	16 (64.0)		
Both	8 (15.4)	4 (16.0)		
Duration from symptom onset to diagnosis, mo	9.5	6.0	514.00	.14
Respiratory symptoms, entire follow-up period	44 (84.6)	23 (92.0)	0.30	.59
Onset of interstitial lung disease				
Acute/subacute	22 (42.3)	14 (56.0)	1.27	.26
Chronic/insidious	30 (57.7)	11 (44.0)		
Polymyositis/dermatomyositis	26 (50.0)	16 (64.0)	1.33	.25
Arthritis	26 (50.0)	13 (52.0)	0.03	.87
Mechanic's hands	27 (51.9)	2 (8.0)	13.87	<.01
Raynaud's phenomenon	18 (34.6)	10 (40.0)	0.21	.65
Pyrexia of unknown origin[a]	1 (1.9)	1 (4.0)		.55
Crackle	23 (44.2)	9 (36.0)	0.47	.49
Respiratory failure	6 (11.5)	13 (52.0)	14.87	<.01
Complicated with malignant tumors	4 (7.7)	4 (16.0)	0.52	.47
Positive antinuclear antibodies with a high titer	14 (26.9)	7 (28.0)	0.01	.92
Types of anti-aminoacyl-transferase RNA synthetase antibodies				
Anti-Jo-1 antibody	35 (67.3)	11 (44.0)	3.81	.05
Anti-PL-12 antibody	4 (7.7)	4 (16.0)	0.52	.47
Anti-PL-7 antibody	5 (9.6)	6 (4.0)	1.8	.18
Anti-EJ antibody	8 (15.4)	3 (12.0)	0.02	.96
Anti-OJ antibody[a]	0 (0)	1 (4.0)		.33
Positive anti-Ro-52 antibody	31 (59.6)	14 (56.0)	0.09	.76
White blood cell count > 9.5×10^9/L	18 (34.6)	11 (44.0)	0.63	.43
Neutrophil% > 75%	21 (40.4)	14 (56.0)	1.66	.20
C-reactive protein > 10 mg/L[b]	23 (46.0)	15 (62.5)	1.77	.18
Elevated erythrocyte sedimentation rate[b]	33 (71.7)	17 (77.3)	0.23	.63
Decreased serum level of C3 or C4[b]	15 (33.3)	11 (50.0)	2.27	.13
Elevated muscle enzymes[b]	25 (52.1)	19 (79.2)	4.94	.03
Computed tomography/high-resolution computed tomography patterns of interstitial lung disease[a]				
Nonspecific interstitial pneumonia	38 (73.1)	18 (72.0)		.28
Organizational pneumonia	7 (13.5)	3 (12.0)		
Nonspecific interstitial pneumonia with organizational pneumonia	6 (11.5)	1 (4.0)		
Usual interstitial pneumonia	1 (1.9)	1 (4.0)		
Acute interstitial pneumonia	0 (0.0)	2 (8.0)		
Treatment				
Glucocorticoid monotherapy	12 (23.1)	7 (28.0)	0.22	.64
Glucocorticoids combined with immunosuppressants	36 (69.2)	15 (60.0)	0.64	.42

Note: The definitions of the variables were:

Respiratory symptoms: cough, sputum, and dyspnea.

Non-respiratory symptoms: arthritis, mechanic's hands, Raynaud's phenomenon, unexplained fever or other skin changes, such as Gottron's sign, shawl sign, periorbital edematous purplish-red spots centered on the upper eyelid, "V"-shaped red rash on the anterior neck and upper chest, etc.

The onset of interstitial lung disease: according to the time from the beginning to the progression of respiratory symptoms, the clinical course of the onset of interstitial lung disease was divided into acute (within 1 mo), subacute (within 1 to 3 mo), and chronic (stable or slowly progressive for more than 3 mo), and insidious (no obvious respiratory symptoms).

Mechanic's hands: keratosis, crack, roughness on the lateral and palmar aspects of hands and fingers.

Raynaud's phenomenon: under cold or tension stimulation, the skin of the fingers (toes) turns pale, purple, and red successively, accompanied by transient local chills, paresthesia, and pain.

Elevated erythrocyte sedimentation rate: > 43 mm/h (man, age > 60 y) or > 21 mm/h (man, age ≤ 60 y) or > 38 mm/h (woman aged > 50 y) or > 26 mm/h (woman aged ≤ 50 y).

Decreased serum level of C3 or C4: serum C3 < 0.79 g/L or serum C4 < 0.16 g/L.

Elevated muscle enzymes: lactate dehydrogenase > 250 U/L or aspartate aminotransferase > 35 U/L or alanine aminotransferase > 35 U/L or creatine kinase > 200 U/L.

Positive antinuclear antibody with a high titer: the titer of nucleolar or centromeric pattern ≥1:100 or the titer of non-nucleolar or centromeric pattern ≥1:320.

[a]Fisher's exact test.

[b]There are missing values.

TABLE 2 Multivariate logistic regression analysis

Variable	β	Wald	*P* value	Odds ratio	95% CI
Respiratory failure	1.90	7.04	.008	6.71	1.64–27.38
Elevated muscle enzymes	1.46	4.01	.045	4.31	1.03–18.05
Mechanic's hands	−2.86	9.04	.003	0.06	0.01–0.37
Anti-Jo-1 antibody	−1.42	4.25	.039	0.24	0.06–0.93

to metabolic dysfunction for lacking enough time to compensate. In summary, respiratory failure at diagnosis indicates that the lungs are more likely to be seriously involved or to progress more rapidly, and thus, the outcome of such patients is generally poor.

Anti-ARS antibodies are specific to muscle involvement, and once muscle involvement happens, respiratory muscles and throat muscles could be involved, causing dyspnea, dysphagia, and so on.[22] Thus, we infer that the outcome of patients with myositis is worse than those without. However, prior studies have not found an association between myositis and the prognosis of ASS patients,[4,6,7] which is consistent with our findings. Interestingly, we found that elevated serum muscle enzymes, an important indicator of muscle involvement, was an independent risk factor for the prognosis of ASS-ILD patients (OR = 4.31, $P < .05$) in our study. We consider the possible factors are as follows. First, elevated muscle enzymes are an early and sensitive indicator of muscle involvement, earlier than the appearance of muscle performance and electromyographic changes.[23] Second, most ASS-ILD patients have respiratory symptoms as their first manifestation, whereas myositis appears slowly or insidiously, which may lead to a delayed diagnosis of PM/DM.[24] Can we consider that muscle involvement is a predictor of the outcome? However, there is no positive result due to the above limitations. Additional studies are required.

In our study, mechanic's hands were found in 29 patients (37.7%). Although mechanic's hands can also be found in other connective tissue diseases, it has a higher incidence in ASS, about 28%–60% of cases.[18,25–27] There were 27 cases (51.9%) with a good prognosis and 2 cases (8.0%) with a poor prognosis, and the multivariate analysis showed that mechanic's hands was a protective factor (OR = 0.06, 95% CI: 0.01–0.37). Some studies have revealed that mechanic's hands are related to an increased risk of ILD and systemic involvement.[28] However, the research by Sato et al[29] showed that DM patients with mechanic's hands have relatively less muscle involvement and a better prognosis. Local treatment is usually ineffective for mechanic's hands, but it can be improved through glucocorticoids or immunosuppressants, and the improvement of mechanic's hands is related to the improvement of the overall condition of ASS.[30] There is no relevant research on whether the presence of mechanic's hands indicates a high sensitivity to glucocorticoids. In general, there are few studies on mechanic's hands and the prognosis, and further exploration is warranted.

Anti-Jo-1 antibody was the most frequent type of anti-ARS antibodies (n = 46, 59.7%), and there were 35 (67.3%) and 11 cases (44.0%) observed in the good-prognosis group and the poor-prognosis group, respectively. According to the multivariate analysis, the prognosis of the non-anti-Jo-1 patients was 4.1 times worse than the anti-Jo-1 patients, which was similar to the prior literature.[3] Current studies generally showed that non-anti-Jo-1 ASS patients had a worse prognosis. In particular, anti-PL-7/PL-12 antibody is associated with severe ILD, a higher incidence of RP-ILD and UIP patterns, and a significantly poor response to glucocorticoids and immunosuppressants.[31,32] Moreover, non-anti-Jo-1 ASS patients are inclined to later suffer from myositis, arthritis, and other manifestations.[24,32] The symptoms of non-anti-Jo-1 ASS patients are not typical during the early stage, so clinical diagnosis and treatment are easily delayed. Overall, the prognosis of patients with different types of anti-ARS antibodies is heterogeneous, and the prognosis of anti-Jo-1 patients is relatively better than the non-anti-Jo-1 patients.

We found that 10.4% of the patients were complicated with cancers. Previous studies showed that the prevalence of cancer in ASS patients was 5.9%–13.9%,[5,26,33,34] which was consistent with our results. As for the prevalence of cancer in different anti-ARS antibody subtypes, Hamaguchi et al[26] showed there was no difference. However, we found that the incidence of tumor was high in patients with positive anti-PL-7 antibodies. Thus, studies are needed to clarify the prevalence of different anti-ARS antibody subtypes.

In our study, a total of 15 patients (19.5%) died, of which 11 patients (73.3%) died during the follow-up, and their main causes of death were severe infection, lung cancer, and aggravation of ILD. Therefore, whole-process management and regular follow-up are very important to improve the survival rate of ASS-ILD patients. During the follow-up, not only the condition of ILD but also the related complications and adverse effects of immunosuppressive therapy should be focused on to improve the survival rate.

This study has several limitations. First, due to the inherent limitations of retrospective studies, some examination data are missed. For example, the high missing rate of PFT and ferritin prevented further analysis. Second, because of the small sample size of the study, no further comparisons were made between patients with anti-PL-7, PL-12, EJ, and OJ antibodies, and the rare anti-ARS antibodies, such as anti-KS, anti-Zo, anti-Ha antibodies were not detected. Third, as the follow-up time was not long enough and the overall prognosis of ASS-ILD was good, the overall mortality of the sample was low, and thus a survival analysis was not performed. Fourth, only inpatients were admitted in our study, and there could be a small number of patients who were not hospitalized due to mild illness or other reasons, which may lead to tiny bias of the results. To conclude, more prospective and larger multicenter studies are needed to clarify the prognostic risk factors of ASS-ILD in the future.

In our study, the prognosis and the prognostic factors were analyzed among 77 patients with ASS-ILD. The results suggested that respiratory failure and elevated muscle enzymes at diagnosis were

risk factors, and mechanic's hands and anti-Jo-1 antibody positivity were protective factors. This provides some evidence for the whole-process management of ASS-ILD patients and the formulation of individualized therapy plans. For patients with adverse risk factors, the initial dose and maintenance time of corticosteroids should be appropriately adjusted.

AUTHOR CONTRIBUTIONS

Design: Li Xin, Qian Zhou, Xiaokui Tang. Clinical data collection: Xin Li, Qian Zhou, Pengchao Wu, Qian Chen. Statistical processing: Xin Li, Qian Zhou, Zhenyu Ren, Xue Yang. Writing original draft and revising: all authors. Supervision: Xiaokui Tang. Final approval of manuscript: all authors.

FUNDING INFORMATION

This study was funded by Chongqing medical scientific research project (Joint project of Chongqing Health Commission and Science and Technology Bureau) (NO.2020MSXM033).

CONFLICT OF INTEREST

No conflicts of interest.

ETHICS STATEMENT

This study was approved by the ethics committee in the First Affiliated Hospital of Chongqing Medical University (2020–294). Due to the retrospective nature of the study, informed consent was waived.

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How to cite this article: Li X, Zhou Q, Wu P, et al. A prognostic analysis of antisynthetase syndrome-related interstitial lung disease. *Int J Rheum Dis*. 2022;25:1368-1375. doi: 10.1111/1756-185X.14428

Received: 15 May 2022 | Revised: 9 June 2022 | Accepted: 21 August 2022

DOI: 10.1111/1756-185X.14431

International Journal of Rheumatic Diseases WILEY

ORIGINAL ARTICLE

An overview of the relationship between juvenile idiopathic arthritis and potential environmental risk factors: Do early childhood habits or habitat play a role in the affair?

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Abstract

Aim: The current study was undertaken to evaluate the influence of breastfeeding on the development and outcome measures of juvenile idiopathic arthritis (JIA). The second aim was to determine the consequences of particular sociodemographic and sociocultural characteristics and nutritional behavior of early childhood on JIA.

Methods: The study includes the patients diagnosed with JIA and regularly followed up at the Department of Pediatric Rheumatology in Istanbul University-Cerrahpasa. The comparison group consisted of healthy subjects and patients with juvenile systemic lupus erythematosus (jSLE). A face-to-face survey method was conducted with the parents of the participants between February 1, 2021, and September 1, 2021.

Results: The mean age of the JIA cohort (n = 324) was 12.2 ± 4.7 years, with a female ratio of 64.8%. The breastfeeding rate differed from the control groups (253 healthy subjects and 88 patients with jSLE) but was higher with a value of 94.8%. There was no difference between the groups ($P = .097$, $P = .064$) or within the subgroups of JIA ($P = .12$) regarding breastfeeding duration. Cow's milk introduction time ($P = .02$, $P = .0001$), household pet-keeping ($P = .001$), income level ($P = .0001$), maternal literacy ($P = 0.013$) made a statistical difference vs the control groups.

Conclusion: No relationship was established between the rate or duration of breastfeeding and the development or severity of JIA. The early introduction of cow's milk was found to be higher in the patient cohorts. The income level and maternal literacy appeared to be relevant with the high disability and damage scores, and frequent relapse rates. Secondhand smoking, higher in JIA, may prompt the basis of primary preventable strategies in JIA.

KEYWORDS
breastfeeding, early childhood exposures, environmental factors, juvenile idiopathic arthritis, outcome

1 | INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common chronic inflammatory rheumatic disease complex in childhood which encompasses 7 heterogenous subtypes, demonstrating a broad clinical distribution.[1–3] The disease, although not fully elucidated, arises with the interaction of genetic and environmental factors, leading to immune dysregulation and displaying both autoimmune and inflammatory features.[4] Advances in molecular medicine and elucidation of the cytokine network shed light on the etiopathogenesis for a better understanding of the disease. However, individual differences in the course of the disease strongly suggest the influence of external factors.

There seems to be an intricate interplay of genetic predisposition and environmental influences, with varying balances between ethnic groups and even individually at the root of autoimmune diseases.[5] The increase in the incidence of certain autoimmune disorders over the past few decades may well be associated with raised awareness, but is also a consequence of the potential impact of environmental alterations. Furthermore, the variation in clinical phenotypes of diseases is attributed to more than genetic divergence, strongly suggesting the growing role of environmental triggers.[6] Investigators have endeavored to determine the position of various environmental factors such as early childhood nutrition, lifestyle, microbiota, passive smoking, infections and medications in the etiology of largely known autoimmune diseases, such as diabetes mellitus, celiac disease, rheumatoid arthritis, inflammatory bowel disease (IBD).[5,7] Considering that they may have shared risks besides the overlapping genetic features with other autoimmune diseases, certain environmental factors have been focused on for JIA, but the heterogeneity in the definition and classification have brought methodological limitations to the studies. Among the environmental factors investigated, the most striking is the controversial protective effect of breastfeeding on the incidence and severity of JIA.[6,8] There is ample evidence respecting the fact that breastfeeding acts as a bridge to transfer the maternal immunological memory and essential compounds such as cytokines, and through this connection, supports building the infant's immune system as well as enriching the microbiome. Disruption of this balance is considered to lead to an increased risk of immune disorders, including autoimmune diseases.[9] On the other hand, the relationship between the early introduction of formula or cow's milk and the development of JIA is a matter of debate, with conflicting study results.[10–12]

The current study was undertaken to evaluate the influence of breastfeeding on the development and outcome measures of JIA according to the subtypes. The second aim of our work was to further broaden current knowledge of the consequences of particular sociodemographic and sociocultural characteristics and nutritional behavior of early childhood on JIA.

2 | MATERIALS AND METHODS

2.1 | Study design

The study was performed with patients with JIA who were diagnosed and regularly followed up at Department of Pediatric Rheumatology in Cerrahpasa Medical School in Istanbul University-Cerrahpasa, a tertiary care referral hospital in Turkey. The comparison group consisted of healthy subjects and patients with juvenile systemic lupus erythematosus (jSLE). A face-to-face survey method was conducted with the parents of the participants between February 1, 2021 and September 1, 2021.

2.2 | Identification of study groups

The study cohort consisted of patients who were diagnosed with JIA before the age of 16 and completed a year follow-up period. The patients were classified according to the International League of Associations for Rheumatology (ILAR) criteria and identified as oligoarthritis (oJIA), polyarthritis (pJIA) rheumatoid factor (RF) positive, polyarthritis RF negative, enthesitis-related arthritis (ERA), systemic juvenile idiopathic arthritis (sJIA), juvenile psoriatic arthritis (jPsA) and undifferentiated arthritis; each was evaluated separately in the study.[3] Nevertheless, patients in the category of undifferentiated arthritis were excluded due to insufficient number and oJIA was evaluated in 2 groups as extended and persistent.

The comparison group consisted of healthy relatives of the hospital staff and students from surrounding schools who did not have any known disease or use of any medication and patients diagnosed with juvenile systemic lupus erythematosus (jSLE), our largest cohort representing autoimmune diseases after JIA.

Inclusion and exclusion criteria were established to provide standardization among the participants. Subjects with a comorbid disease, a first-degree relative with an inflammatory rheumatic disease, and a familial genetic burden were excluded from the study. Patients with incomplete data regarding the content of the survey and those whose parents refused to participate in the study were also excluded. The patients with insufficient medical data or without regular follow-up were not included. Each participant and his/her legal representative approved the use of their information and informed consent was obtained from the legally authorized representatives of our patients prior to their inclusion in the study. The written consent and signature were obtained from the participants. Approval was obtained from the Ethics Committee of Cerrahpasa Medical School (approval: 09.01.2020-4116) for the study.

2.3 | Data collection and content of the survey

The patients were surveyed during their routine visits, their medical records were reviewed, and their inclusion and exclusion criteria were established. The content of the survey, consisting of open-ended and closed-ended questions, was formulated by the authors of the study. The subjects were surveyed for their sociodemographic and sociocultural characteristics, parental behaviors, gestation and breastfeeding period, and nutritional status in early childhood. The medical records of the patients diagnosed with JIA were reviewed for demographic data, clinical follow-up,

and treatment modalities. The disease characteristics including subtype, age at diagnosis, disease duration, medication, outcome measures were documented.

The number of active joints, functional ability, the number of joints with a limited range of motion, presence of systemic features such as fever, rash, serositis, splenomegaly, lymphadenopathy attributable to JIA, treatment responses, C-reactive protein level (normal <5 mg/L), erythrocyte sedimentation rate (normal ≤20 mm/h), pain and well-being assessments of the physician (PGA) and patient/parents (PtGA), recorded during the routine visits of the patients at 3-month intervals, were evaluated. Visual analog scale (VAS; 0 = no pain, 10 = worst pain) was used to access pain intensity.

Disease activity score of each patient was calculated individually according to disease subtype and disease duration. The Wallace criteria, Juvenile Arthritis Disease Activity Score (JADAS) 27, systemic JADAS (sJADAS), Juvenile Spondyloarthritis Disease Activity Index (JSpADA), Psoriasis Area Severity Index (PASI) were used for the assessments.[13–19] In the calculation of disease activity, JADAS for patients with extended and persistent oJIA, RF negative and positive pJIA; sJADAS for patients with sJIA; JSpADA for ERA; both PASI (skin involvement) and JADAS (joint involvement) for jPsA were used.

Juvenile arthritis damage index (JADI) and childhood health assessment questionnaire (CHAQ; 0 = normal, 3 = worst score) which were administered to the patients at 6-month intervals were used for the assessment of articular and extra-articular damage and functional disability, respectively.[20,21] In order to ensure standardization, the scores were calculated cumulatively according to disease duration. First clinical remissions (CR) determined according to Wallace clinical inactive disease criteria (CID) for oligoarticular, polyarticular and systemic subtypes, and relapse frequencies were recorded.[13] CR on medication was defined as CID for at least 6 months. CR off medication was defined as CID for 12 months after all medication was withdrawn. Since there is no standard evaluation method for patients with ERA and jPsA, the CID evaluation was based on the absence of active arthritis or rash or inflammatory pain. The state of the patient who did not meet the CID criteria in at least one visit was defined as a relapse.

2.4 | Statistical analysis

Statistical analyses were performed by using the IBM SPSS Statistics for Windows 25.0 software (Statistical Package for the Social Sciences) and Microsoft Excel. The visual (histograms, probability plots) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk's normality tests) were used to analyze the distribution of the variables. The demographic and clinical data were evaluated using descriptive analysis. Mean values with standard deviations (mean ± SD) or median (med) with minimum and maximum values (min-max) were given for demonstration of the data according to the distribution. Categorical variables were presented as counts or frequencies and compared using Chi-square test or Fisher test statistics. One-way analysis of variance or Student's *t* test was used for parametric distribution. Kruskal-Wallis test or Mann-Whitney *U* test was used for nonparametrically distributed variables. The statistical significance level was taken as .05.

3 | RESULTS

3.1 | Patient characteristics

Among the patients diagnosed with JIA, 26 were excluded owing to missing data or insufficient follow-up period, 21 due to comorbid diseases, while 47 patients were excluded because of a history of inflammatory rheumatic disease in their first-degree relatives. Ultimately, 324 patients met the inclusion criteria. Table 1 demonstrates the demographic and clinical characteristics of the patients in the JIA cohort.

3.2 | Comparison of the study groups

In the comparison group, 253 healthy subjects and 88 patients with jSLE were included. The JIA cohort and the healthy controls demonstrated statistical differences in maternal and paternal age, breastfeeding and formula feeding rate, cow's milk introduction period, preschool participation, household pet-keeping, passive smoking, and maternal literacy. The mean disease duration was 6.1 ± 4.1 years in JIA and 4.6 ± 2.6 years in jSLE. In the comparison of the JIA and jSLE cohorts, statistical differences were observed in terms of age, gender, breastfeeding rate, cow's milk introduction period, immunization status, household pet-keeping, and income level. Table 2 reflects the comparisons of the study groups by the early childhood exposures and environmental risk factors.

3.3 | The impact of potential environmental factors on disease outcome measures

Outcome measures of the JIA cohort according to the subtypes and disease duration are presented in Table 3, along with their current age and age at diagnosis. No statistical difference was observed when the subgroups (oJIA, pJIA, ERA, and sJIA) were compared as per the breastfeeding duration ($P = .12$). The reflections of the rate and duration of breastfeeding on the outcome measures were assessed and are presented in Table 4. In addition to breastfeeding, the impacts of immunization and certain socioeconomic and cultural factors, which differ from the control group, on disease outcome measurements were evaluated. The impacts of immunization, cigarette exposure, family income level, and maternal literacy on the disease were compared and are presented in Table 5.

TABLE 1 Clinical characteristics of patients in the juvenile idiopathic arthritis cohort.

Subtypes of JIA, n (%)	
Persistent oligoarthritis	100 (30.9)
Extended oligoarthritis	29 (9)
Seronegative polyarthritis	52 (16)
Seropositive polyarthritis	30 (9.3)
Psoriatic arthritis	16 (4.9)
Enthesitis-related arthritis	53 (16.3)
Systemic JIA	44 (13.6)
Age at diagnosis, y	
Mean ± SD	6.5 ± 4.1
Med (min-max)	6 (1-15)
Disease duration, y	
Mean ± SD	6.1 ± 4.1
Med (min-max)	5.0 (1-17)
Non-biologic DMARDs, n (%)	
Methotrexate	285 (88)
Sulfasalazine	6 (1.9)
Cyclosporine	5 (1.6)
Biologic DMARDs	
Anti-TNF	164 (50.6)
Anti-IL1	26 (8)
Anti-IL6	13 (4)
Biologic switching due to reactivation	64 (19.8)
First mo of clinical remission	5.8 ± 4.1
On medication	52 (16)
Off medication	32 (9.9)
Rates of relapse	
Mean ± SD	1.9 ± 1.8
Med (min-max)	2 (0-10)
CHAQ[a]	
Mean ± SD	1.26 ± 1.1
Med (min-max)	1.1 (0-4)
JADI[a]	
Mean ± SD	0.96 ± 1.6
Med (min-max)	0 (0-10)

Abbreviations: CHAQ, childhood health assessment questionnaire; DMARDs, disease-modifying anti-rheumatic drugs; JADI, juvenile arthritis damage index; JIA, juvenile idiopathic arthritis.

[a]Cumulative values are presented for the JADI and CHAQ scores.

4 | DISCUSSION

This study focuses on the possible role of early childhood exposures and environmental risk factors in JIA. Primarily the paper seeks to evaluate the influence of breastfeeding on the development and outcome measures according to the subtypes of the disease. While the early introduction of cow's milk was found to be higher in the patient cohorts, no relationship has been established between the rate or duration of breastfeeding and the development or severity of JIA. A few researchers addressed the issue in the early 1990s and revealed controversial results.[22,23] The small sample size, patient selection bias, and lack of a patient control group were the constraining factors in the studies. On the other hand, the modification in the classification and terminology suggested reinterpretation of the results as per the subtypes. A report including healthy controls as well as the patients with primary nephrotic syndrome as a comparison group underlined that although there was no difference respecting the breastfeeding rate within the groups, the breastfeeding duration in the oligoarticular type has been found shorter.[24] In our study, the breastfeeding rate of JIA differed vs jSLE and healthy individuals yet it was quite high with a value of 94.8%. Further, there was no difference between the 3 main groups or within the subgroups of JIA in terms of breastfeeding durations analyzed in 5 categories in our study. Alongside studies examining the role of breastfeeding in the development of JIA, Hyrich and co-workers discussed its association with the severity of the disease and concluded that breastfeeding was relevant to less occurrence, and milder or later onset of JIA yet without a comparison group as the major limitation. The study pointed out that breastfed children were more likely to be diagnosed at younger ages and had better physician or parent global pain and physical function scores, with longer durations of breastfeeding corresponding to progressively lower scores. Curiously, the authors have observed a higher rate of early disease onset in never-breastfed patients in JPsA and ERA.[25] Furthermore, in a report evaluating 203 patients with human leukocyte antigen B27 positive ankylosing spondylitis fulfilling the modified New York criteria, attention was drawn to the protective role of breastfeeding, referring to its effect on microbiota.[26] While the correlation between microbiota and ERA is currently on the agenda, these assumptions seem well-founded. Correspondingly, a recent report from Brazil observed a high prevalence of breastfeeding in the low-income JIA cohort, and breastfeeding over 6 months has been associated with less disease activity, CHAQ score, and less joint deformity.[27] Although there was no difference in outcome measures between the breastfed and non-breastfed groups, a reliable comparison could not be achieved in our study, since the breastfeeding rate was high in the entire JIA cohort, but the duration of breastfeeding did not create a statistical difference in this regard.

On top of the retrospective studies, 32 patients have been reached in a prospective study reported in accordance with the national registry from southeastern Sweden, and the 4-month time frame has been determined as the cutoff for breastfeeding duration. Ultimately it has been highlighted that the longer breastfeeding may be protective against JIA.[10] The same study has asserted that low parental literacy may influence breastfeeding,[10] but this assertion was not backed by other reports.[27] In our study, the rate of maternal literacy was lower vs the healthy control group, but did not negatively impact the breastfeeding rate. However, low maternal literacy was associated with high disease activity, frequent relapse, high CHAQ and JADI scores.

TABLE 2 Comparison of the juvenile idiopathic arthritis cohort with healthy subjects and juvenile systemic lupus erythematosus patients according to environmental risk factors.

Survey questions	JIA (n = 324)	Healthy (n = 253)	*P**	jSLE (n = 88)	*P***
Age, y					
Mean ± SD	12.2 ± 4.7	11.5 ± 4.1	.056	14.9 ± 3.3	.001
Med (min-max)	13 (2-18)	12 (2-18)		16 (6-18)	
Gender, n (%)					
Female	210 (64.8)	153 (60.5)	.298	69 (78.4)	.015
Male	114 (35.2)	100 (39.5)		19 (21.6)	
BMI, n (%)					
Underweight	12 (3.7)	9 (3.5)	.08	3 (3.4)	.06
Healthy weight	292 (90.1)	229 (90.6)		79 (89.8)	
Overweight	20 (6.2)	15 (5.9)		6 (6.8)	
Maternal pregnancy age					
<20	32 (9.9)	43 (17.0)	.002	9 (10.2)	.310
20-34	251 (77.5)	162 (64.0)		73 (83.0)	
≥35	41 (12.7)	48 (19.0)		6 (6.8)	
Paternal age					
<20	-	8 (3.2)	.001	-	.162
20-34	241 (74.4)	165 (65.2)		72 (81.8)	
≥35	83 (25.6)	80 (31.6)		16 (18.2)	
Birth order					
First	136 (42.0)	122 (48.2)	.07	33 (37.5)	.450
Second	99 (30.6)	84 (33.2)		33 (37.5)	
Third or later	89 (27.5)	47 (18.6)		22 (25)	
Mother's marital status					
Married	293 (90.4)	218 (86.2)	.11	78 (88.6)	.688
Divorced/single	31 (9.6)	35 (13.8)		10 (11.4)	
Smoking during pregnancy	66 (20.4)	38 (15.0)	.10	18 (20.7)	1.000
Major illness during pregnancy	28 (8.6)	22 (8.7)	1.000	7 (8.0)	1.000
Any medication during pregnancy	20 (6.2)	18 (7.1)	.736	6 (6.8)	.807
Alcohol during pregnancy	3 (0.9)	8 (3.2)	.066	2 (2.3)	.290
Has the child ever breastfed?	307 (94.8)	224 (88.5)	.008	76 (86.4)	.016
Breastfeeding duration, mo					
<6	62 (20)	31 (13.8)	.097	10 (13.2)	.064
6-12	44 (14.2)	40 (17.9)		20 (26.3)	
12-18	73 (23.5)	66 (29.5)		21 (27.6)	
18-24	85 (27.4)	64 (28.6)		15 (19.7)	
≥24	46 (14.8)	23 (10.3)		10 (13.2)	
Cow's milk introduction, mo					
<12	60 (18.5)	29 (11.5)	.020	39 (44.3)	.0001
>12	264 (81.5)	224 (88.5)		49 (55.7)	
Formula feeding	158 (48.8)	85 (33.6)	.001	43 (48.9)	1.000
Hospitalization due to infection in the first y of life?	54 (16.7)	36 (14.2)	.488	15 (17.0)	1.000
Immunization status					
Fully immunized	290 (89.5)	230 (90.9)	.674	85 (96.6)	.037
Partially immunized	34 (10.5)	23 (9.1)		3 (3.4)	

TABLE 2 (Continued)

Survey questions	JIA (n = 324)	Healthy (n = 253)	P*	jSLE (n = 88)	P**
Preschool/nursery	159 (49.1)	96 (37.9)	.009	40 (45.5)	.631
Household pet					
Cat-dog	21 (6.5)	31 (12.3)	.001	17 (19.3)	.001
Bird	34 (10.5)	17 (6.7)		5 (5.7)	
Hairless pet	2 (0.6)	12 (4.7)		2 (2.3)	
Household smoking	204 (63.0)	120 (47.4)	.0001	53 (60.2)	.710
Place of residence					
Urban area	305 (94.1)	241 (95.3)	.583	73 (83.0)	.624
Rural area	19 (5.9)	12 (4.7)		6 (6.8)	
Income level					
Below	184 (56.8)	122 (48.2)	.053	15 (17.0)	.0001
Minimum wage	125 (38.6)	110 (43.5)		69 (78.4)	
Above	15 (4.6)	21 (8.3)		4 (4.5)	
Maternal literacy					
Illiterate	65 (20.1)	28 (11.1)	.013	24 (27.3)	.085
Primary	134 (41.4)	101 (39.9)		44 (50.0)	
Secondary	89 (27.5)	78 (30.8)		15 (17.0)	
Higher	33 (10.2)	42 (16.6)		5 (5.7)	
Master's degree	3 (0.9)	4 (1.6)		-	
Paternal literacy					
Illiterate	29 (9.0)	16 (6.3)	.106	7 (8.0)	.682
Primary	153 (47.2)	98 (38.7)		42 (47.7)	
Secondary	97 (29.9)	90 (35.6)		30 (34.1)	
Higher	39 (12.0)	43 (17.0)		9 (10.2)	
Master's degree	6 (1.9)	6 (2.4)		-	

Abbreviations: BMI, body mass index; JIA, juvenile idiopathic arthritis; jSLE, juvenile systemic lupus erythematosus.
*P** represents comparison between JIA and healthy control group, and *P*** represents comparison between JIA and jSLE group.

TABLE 3 Outcome measures of the juvenile idiopathic arthritis cohort according to subgroups.

Subtypes of JIA	Current age	Age at diagnosis	Disease duration	Disease activity	CID	Relapse rate	CHAQ	JADI
Persistent oJIA (n = 100)	8 (3-18)	3 (1-15)	4 (1-15)	0.9 (0-14)	4 (1-24)	1 (0-6)	0.8 (0-3)	0 (0-4)
Extended oJIA (n = 29)	12 (5-18)	3 (1-10)	9 (2-17)	0 (0-8)	4 (0-9)	2 (0-4)	2 (0-4)	6 (3-20)
RF(−) pJIA (n = 52)	15 (7-18)	7 (1-15)	5 (1-15)	2 (0-18)	6 (1-24)	2 (0-8)	2 (0-3)	2 (0-8)
RF(+) pJIA (n = 30)	16 (10-18)	9 (2-15)	6 (1-17)	3 (0-12)	6 (3-16)	2 (0-7)	2.1 (0-4)	2 (0-10)
jPsA (n = 16)	12 (7-18)	8 (2-15)	5 (2-16)	2.5 (0.4-18.5)	6 (3-18)	2 (0-7)	1.7 (0-4)	1.5 (0-4)
ERA (n = 53)	16 (9-18)	10 (2-15)	5 (2-16)	1.5 (0-5)	6 (0-24)	1 (0-8)	1.8 (0-4)	0 (0-5)
sJIA (n = 44)	10 (2-18)	4 (1-14)	5 (1-15)	0 (0-3.9)	2 (1-12)	1 (0-10)	0 (0-3)	0 (0-8)

Abbreviations: CHAQ, childhood health assessment questionnaire; CID, clinically inactive disease; ERA, enthesitis-related arthritis; JADI, juvenile arthritis damage index; JIA, juvenile idiopathic arthritis; jPsA, juvenile psoriatic arthritis; oJIA, oligoarticular juvenile idiopathic arthritis; RF(−) pJIA, rheumatoid factor negative polyarticular juvenile idiopathic arthritis; RF(+) pJIA, rheumatoid factor positive polyarticular juvenile idiopathic arthritis; sJIA, systemic juvenile idiopathic arthritis.

Dietary intervention in infancy has been demonstrated to have a long-lasting effect on beta-cell autoimmunity markers, which may reflect an autoimmune process.[28] A recent review has stated that despite the shared roles of breastfeeding, maternal diet, and early nutrition in autoimmune diseases, each disease has specific dietary driver epigenetic mechanisms requiring further investigation.[29] The

TABLE 4 The influence of breastfeeding and breastfeeding duration on the outcome measures of the patients with juvenile idiopathic arthritis.

Breastfeeding as the risk factor	Disease duration	Disease activity	CID	Relapse rate	CHAQ	JADI
Rate						
Never breastfed	5 (1-16)	2.2 (0-7)	6 (1-16)	1 (0-4)	1.7 (0-3)	0 (0-4)
Breastfed	5 (1-17)	1.2 (0-18.5)	5 (0-24)	2 (0-10)	1 (0-4)	1 (0-10)
P	.171	.311	.869	.279	.798	.136
Duration, mo						
<6	5 (1-15)	1 (0-8)	6 (1-20)	2 (0-10)	1.1 (0-4)	1 (0-10)
6–12	4 (1-17)	2 (0-18)	5 (1-24)	2 (0-8)	1 (0-3)	0 (0-6)
12–18	6 (1-17)	1.2 (0-13)	5 (0-20)	2 (0-10)	1.4 (0-4)	1 (0-8)
18–24	4 (1-16)	1.2 (0-18.5)	4 (1-24)	2 (0-7)	1 (0-4)	0 (0-6)
>24	5 (1-15)	0.7 (0-6)	6 (1-24)	1 (0-6)	0.7 (0-4)	0 (0-5)
P	.398	.201	.139	.077	.098	.056

Abbreviations: CHAQ, childhood health assessment questionnaire; CID, clinical inactive disease; JADI, juvenile arthritis damage index.

TABLE 5 The influence of immunization, income, passive smoking and maternal literacy on the outcome measures of the juvenile idiopathic arthritis cohort.

Differentiating factors	Disease duration	Disease activity	CID	Relapse rate	CHAQ	JADI
Immunization						
Fully immunized	5 (1-17)	1.3 (0-18.5)	5 (0-24)	2 (0-10)	1 (0-4)	0 (0-10)
Partially immunized	5 (1-17)	1 (0-7.3)	6 (2-20)	2 (0-9)	1.4 (0-4)	0 (0-8)
P	.270	.531	.088	.139	.267	.050
Income level						
Below	5 (1-17)	1.5 (0-18.5)	6 (1-24)	2 (0-10)	1.4 (0-4)	1 (0-10)
Minimum wage	5 (1-17)	1.1 (0-18.0)	5 (0-24)	1 (0-10)	0.6 (0-4)	0 (0-8)
Above	5 (1-8)	0 (0-6)	4 (2-8)	1 (0-3)	0.6 (0-2)	0 (0-2)
P	.238	.080	.248	.004	.002	.009
Household smoking						
Yes	5 (1-17)	1.3 (0-18.5)	6 (0-24)	2 (0-10)	1.1 (0-4)	0 (0-10)
No	5 (1-15)	1.1 (0-18)	5 (1-20)	2 (0-10)	1 (0-4)	0 (0-8)
P	.453	.392	.559	.919	.543	.679
Maternal literacy						
Illiterate	6 (1-17)	3 (0-18)	6 (1-24)	2 (0-10)	2 (0-4)	2 (0-10)
Primary education	5 (1-17)	1.5 (0-18.5)	6 (1-24)	2 (0-8)	1.1 (0-4)	0 (0-8)
Secondary education	5 (2-15)	0.9 (0-8)	4 (0-20)	1 (0-6)	1 (0-3)	0 (0-8)
Higher education	3 (1-14)	0.6 (0-12)	3 (1-17)	1 (0-4)	0.6 (0-2)	0 (0-2)
P	.052	.0001	.0001	.0001	.0001	.0001

Abbreviations: CHAQ, childhood health assessment questionnaire; CID, clinical inactive disease; JADI, juvenile arthritis damage index.

Childhood Arthritis Risk Factor Identification Study (CLARITY) from Australia has supported the argument of neither breastfeeding duration nor the early introduction of cow's milk in determining JIA risk.[12] Nevertheless in another study, early introduction to the formula has been associated with an increased risk of JIA, while only a tendency of association has been detected concerning cow's milk.[10] Furthermore, few case reports have claimed that the removal of cow's milk, hence the allergens such as β-lactoglobulin and casein, from the diet in JIA patients improves the course of arthritis. The fact that early use of cow's milk was higher in both our patient cohorts vs the healthy controls may support this relationship and deserves further research.

Traditionally, the focus has always been on infections among the conspicuous environmental risk factors.[6] Given that joint involvement may accompany the course of an infection, the role of infectious factors in the development of the disease has been long argued. Streptococcus, Parvovirus B19 and Epstein-Barr virus were the main agents of concern.[30–34] Although a specific infectious agent

could not be revealed, the relationship between infections encountered in the first year of life and diseases such as type 1 diabetes mellitus and IBD has been identified.[35,36] Comparing a large cohort of patients with JIA vs a control group using Swedish registry data from 1973 to 2002, Carlens et al[37] have proposed a prospective association between hospitalization for any infection in the first year of life and the development of JIA. On the other hand, in a comprehensive study in which early-life risk factors were compared to playmate-matched controls, neither breastfeeding nor hospitalization has been associated with an increased risk.[11] Our experiments were consistent with Shenoi et al's findings. Thus, the results of our study do not support the relationship between a moderate–severe infection requiring hospitalization and the development of JIA. Research has tended to focus on infections requiring hospitalization, for the sake of easy recall and documentation. However, children may be faced with many infections in the early stages of their lives, and it seems challenging to evaluate the role of past infections in etiology as a whole. Conversely, there are also claims regarding a protective role of infection on the risk of autoimmune diseases by supporting the maturation of the immune system, particularly early in life, under the concept of the hygiene hypothesis.[38] The CLARITY study has assessed the impacts of sibling exposure on JIA with a comprehensive set of data including birth order and the number of siblings. The cumulative sibling exposure has been associated with a negative correlation which proposes that increased microbial exposure in childhood may confer protection against the development of the disease.[39] A case–control study evaluating perinatal and maternal characteristics according to the subgroups further supported the concept of hygiene or microchimerism hypotheses and declared a decreased risk with increasing parity.[40] However, possible inaccuracies in reporting maternal reproductive history and deficiencies in birth records have been indicated as the weakness of the study. Hence, the straight or consequential influences of infections are one of the controversial points in the etiopathogenesis of JIA. A meticulous screening with a larger sample size is essential in order to reach an explicit conclusion. Correspondingly, factors that have been associated with microbial exposure in early childhood, such as birth order, number of siblings, preschool attendance, and household pet-keeping were surveyed in our study. Among these variables, the rate of household pet-keeping in JIA was lower compared to the control group. Unexpectedly, in another study, contact with farm animals or pet contact during infancy has not been associated with oJIA, but with the development of IBD and SLE.[41] Since the overall cohort is heterogeneous, studies focusing on a subgroup and a straightforward factor may reflect more reliable results. While the comparisons were between the main groups in our study, the limitation of subgroup analyses due to numerical inadequacy was one of the drawbacks.

As a result of a systematic review and meta-analyses on environmental factors, one of the most intriguing correlations was with maternal smoking.[42] In the early 2000s, Jaakkola and co-workers examined the relationship between maternal smoking and the development of JIA in the first 7 years of life, based on Finnish birth registry records, and noticed 31 cases. They revealed a likely association between smoking 10 or more cigarettes per day during pregnancy and the development of JIA in females.[43] The results of the study, which were criticized methodologically, have not been confirmed by more extensive studies.[11,12,44] Although maternal smoking did not seem to pose a risk, according to our study results, the patients with JIA had higher indoor smoking exposure vs the healthy control group. Outcome measures demonstrated no association with cigarette exposure. However, considering that smoking is one of the unfavorable factors in the etiology of autoimmune diseases such as RA,[45,46] further investigations are needed to estimate the association between passive smoking and JIA.

Few researchers have addressed the question of whether several socioeconomic factors are associated with JIA.[47] The probability of developing JIA has been found to be higher in children from families with high incomes and living in urban dwellings by establishing a link with a hygienic early-life habitat. Our study results do not appear to corroborate their observations, and while the income levels of our patients with JIA did not differ from the healthy controls, their incomes were lower than the jSLE cohort. Moreover, income levels below the minimum wage were associated with frequent relapses, higher disability and damage. Our findings were consistent in that low socioeconomic status negatively affected the control of the disease.

Ultimately, we obtained comprehensive results that will shed light on our understanding of the relationship between breastfeeding and particular environmental factors and JIA. Compelling measurement and comparison of environmental data, the likely recall bias in the past inquiry, and the reflections of geographical and cultural discrepancies may render the results of studies in this field speculative. On the other hand, we know that many individual factors and confounders may come into play during the emergence and course of JIA, and clinical phenotypes, immunogenetic interactions and treatment processes may determine long-term outcomes. The strong point of our analysis in this respect was the binary comparison, including the healthy and patient groups. Further, categorized questions were asked to the parents face-to-face by the same researcher. The outcome measurements of each patient were calculated individually and cumulatively. Breastfeeding duration and striking differences between the main groups were compared according to these measures. Nevertheless, the overall high rate of breastfeeding in our study group prevented a robust assessment of outcome measures as planned in the breastfed and non-breastfed groups. Further, the decrease in the number of samples when divided into categories limited the analysis of the subgroups.

5 | CONCLUSION

Our observations have several implications for further research in determining the relationship between alternative environmental factors and autoimmunity by confirming the influence of the early introduction of cow's milk and suggesting a role for formula feeding

as a trigger for some autoimmune disorders. The results emphasize that socioeconomic factors such as income level and sociocultural aspects such as maternal literacy, as well as passive smoking, may affect the pecuniary and intangible dimensions of JIA and may reveal the necessity of determining preventive strategies. Although relevant environmental factors vary, many seem to intersect by disrupting microbiome diversity and immune balance in etiopathogenesis. The environmental factors examined so far require further investigation in well-designed studies with satisfactory samples to determine their position in the disease risk. While creating pertinent studies and interpreting the results, the heterogeneity of the disease itself and the fact that the interaction of genetic and environmental factors constitutes the infrastructure should not be overlooked. Environmental factors will inevitably soon become the issue of more research, under the influence of global climate changes.

AUTHOR CONTRIBUTIONS

All the authors contributed to the manuscript. Professor OK was responsible for the conception and design. All the authors were responsible for the acquisition, analysis and interpretation of data. Assistant Professor OK and Professor OK participated in drafting the work and all authors contributed to criticism of the manuscript. All authors read the manuscript and approved the submission. All authors of the study agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ACKNOWLEDGEMENTS

We would like to thank our patients and their families for their sincere participation in our survey and study.

CONFLICT OF INTEREST

No conflict of interest.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

ETHICAL APPROVAL

Informed consent was obtained from the legally authorized representatives of our patients. Approval was obtained from the Ethics Committee of Cerrahpasa Medical School (approval: 09.01.2020-4116) for the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

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How to cite this article: Koker O, Aliyeva A, Sahin S, et al. An overview of the relationship between juvenile idiopathic arthritis and potential environmental risk factors: Do early childhood habits or habitat play a role in the affair? *Int J Rheum Dis*. 2022;25:1376-1385. doi: 10.1111/1756-185X.14431

Received: 16 April 2022 | Revised: 2 August 2022 | Accepted: 21 August 2022

DOI: 10.1111/1756-185X.14433

International Journal of Rheumatic Diseases WILEY

ORIGINAL ARTICLE

Direct and indirect health-related costs of systemic sclerosis in New Zealand

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Abstract

Aim: To study the economic impact of systemic sclerosis (SSc) in the patients attending Rheumatology clinics in Waikato Hospital, Hamilton, New Zealand (NZ). There is currently no bottom-up data on this in NZ.

Methods: This is a retrospective cross-sectional questionnaire-based study, including demographics, costs related to SSc, quality of life measures including the short-form survey (SF-36) the scleroderma health assessment questionnaire-visual analog scale (SHAQ-VAS), the NZ index of Deprivation (NZiDep), and work limitations questionnaire (WLQ). Direct health costs include patient-reported costs and costs incurred by the public health system. Indirect costs include calculated loss of work productivity. Comparisons were made between age, gender, disease duration, and disease subtype (diffuse, limited, and overlap syndromes).

Results: Participants fulfilled the 2013 ACR/EULAR criteria for SSc. The study was completed by 86 (65.5%) patients, 77 (90%) were females, 19 (22%) had diffuse cutaneous systemic sclerosis (dcSSc), 72 (83%) were NZ European (NZE), seven(8%) were Māori or NZE/Māori. Seventy-six (41.8%) were employed. The average total costs for 6 months were NZ$ 444.50 with the highest costs in the dcSSc sub-group at NZ$ 598.00. The costs incurred by the Hospital for the 2018/2019 fiscal year was NZ$ 3091 per patient. The SF-36 score was lower compared with the general population, mean SHAQ was 0.82. Mean summative WLQ scores were: Time management 21.7, Physical demands 62.5, Interpersonal 23.6, Output demands 23.8. The calculated percentage productivity loss was 46.5%.

Conclusions: This study has shown high health-related costs of SSc in NZ, with reduction in employment, work productivity, and quality of life. The contributors to the costs included physical disability and loss of productivity.

KEYWORDS
direct health costs, health economics, systemic sclerosis

1 | INTRODUCTION

Systemic sclerosis (SSc) is a chronic multisystem autoimmune disease characterized by generation of autoantibodies against various cellular and intracellular antigens, inflammation, and widespread autoimmune vascular injury leading to progressive fibrosis of the skin and internal organs.[1] Common manifestations include skin thickening and fibrosis, joint inflammation and contractures,

Raynaud phenomenon, digital ulcers, interstitial lung disease, and renal insufficiency.

Subtypes include diffuse cutaneous (dcSSc) and limited cutaneous (lcSSc) disease based on the extent of skin involvement. In lcSSc, skin sclerosis is restricted to distal parts of the limbs, including hands and face, while dcSSc is characterized by involvement of skin proximal to the elbows and knees.[2] The third subtype, systemic sclerosis sine scleroderma lacks skin involvement but has relevant serology and features of internal organ involvement.[3] Scleroderma-overlap syndromes (SOS) are described that share features of SSc with overlapping features of other connective tissue disease. The internal organ involvement contributes to significant morbidity and mortality associated with SSc.

The prevalence of SSc varies from three to 24 per 100 000 population.[4] Women are affected more than men with a sex ratio of 4:1–6:1. Morbidity and mortality in patients with SSc is higher than that in the general population. Pulmonary involvement is the leading cause of mortality.[5] Scleroderma renal crisis and cardiovascular involvement are also significant factors of reduced survival.[6]

The multi-system involvement can be a source of significant disability and impacts on physical, psychological, and social well-being.[7] Other impacts are potential reduced work productivity (presenteeism), early retirement, and reduced hours of work leading to financial strain. This has an overall impact on healthcare costs and costs of lost productivity.[8] Studies have shown that SSc causes progressive and significant functional decline.[9] This is a rare condition, but the functional disability is higher than in other chronic and rheumatic conditions.[10] Although there are treatments for some complications of SSc, like pulmonary hypertension, there are no treatments available at present targeting the underlying disease process in SSc.[11] The lack of specific treatments and progressive physical disability together increase the healthcare and societal cost burdens. There have been only a few studies on the disability, functional decline, and the impact on healthcare costs, most of which are from data that were collected from Health Registries and databases.

There is a lack of bottom-up data on the health economic impact and quality of life in chronic rheumatic conditions in New Zealand. In 2018, Arthritis New Zealand released a report on the economic burden of arthritis in New Zealand, which acknowledges this limitation.[12] The data for this report were gathered from Health registries like New Zealand Health Information Service (NZHIS) for inpatient episodes, The Royal NZ College of General Practitioners (RNZCGP) database for data on GP visits and Pharmac (Health Promotion Authority, NZ) for medication costs. This study aims to address the lack of bottom-up data on the health economic impact and effect on quality of life (QoL) of SSc.

2 | MATERIALS AND METHODS

2.1 | Data collection

This is a retrospective cross-sectional questionnaire-based study. Patients with SSc attending the Rheumatology clinic at Waikato hospital, Hamilton from 2005 to 2019 were invited to complete the survey. Through the survey, the participants were asked to provide data retrospectively from the last 6 months. Basic data were retrieved from the Waikato Hospital SSc clinic database of the patients who have been followed prospectively since 2005.

All patients fulfilled the 2013 American College of Rheumatology/European League against Rheumatism (ACR/EULAR) criteria for SSc.[13] The date of SSc diagnosis is defined from the appearance of the first non-Raynaud symptom that fulfilled the 2013 ACR/EULAR criteria or the very early diagnosis of SSc criteria.[14] This information was gathered from patient's clinical notes. LeRoy's criteria[15] were used to classify lcSSc, dcSSc, and SOS.

Patients were invited to complete questionnaires after signing an informed consent. The questionnaires consisted of demographic information, health-related direct and indirect costs, income and health insurance (Appendix 1). Other questionnaires included the work limitation questionnaire (WLQ),[16] the scleroderma health assessment questionnaire-visual analogue scale (SHAQ-VAS),[17] the short-form survey (SF-36),[18,19] and the New Zealand Index of Deprivation (NZiDep).[20] The questionnaires were either posted out to the participants or given to them when they attended clinic. A covering letter outlining the purpose of the study was enclosed with the questionnaires.

2.2 | Demographics and health economic questionnaire

This questionnaire along with the standard demographic questions, had questions including hourly pay, those about direct health-related expenses in the last 6 months included costs for GP visits, travel to appointments, blood tests, accommodation (if traveling from a different town for appointment), fuel costs, prescription costs; indirect costs included cost of carers, mobility aids.

2.2.1 | SHAQ-VAS

The SHAQ is a targeted tool that has eight categories that can be scored 0 to 3, where 0 equals without difficulty and 3 equals unable to do. The VAS is a part of the questionnaire that measures the impact of pain, intestinal problems, breathing problems, Raynaud, digital ulcers, and disease activity based on pain, discomfort, and

limitations in daily life. This is a 15 cm scale that is converted to a continuous variable between 0 and 3.

2.2.2 | WLQ

The work limitations questionnaire measured the impact of SSc on the individual's ability to work. It has four sub-scales including physical demands, time demands, mental-interpersonal demands, and output demands.[21]

2.2.3 | SF-36

This is a questionnaire consisting of 36 items of self-reported measure of health and well-being measured in eight domains. The results were compared with the SF-36 data of the general adult population of NZ as published by the Ministry of Health.

2.2.4 | NZiDep

The New Zealand index of Deprivation is a simple tool to measure socioeconomic deprivation in New Zealand. It is based on eight simple questions which take only 2–3 min to answer. This tool is designed as a variable in research and is indicative of deprivation in general.[20]

2.3 | Ethics

The study was approved by the Health and Disability Ethics committee. The study was deemed out of scope of the HDEC and that it did not require review. Approval was obtained from local authorities including the Waikato District Health Board (DHB) and Te Puna Oranga Maori Consultation Research Review Committee. Permissions for copyrighted questionnaires including Work Limitations Questionnaires (WLQ) and New Zealand Index of Deprivation (NZiDep) were obtained from the relevant authorities.

2.4 | Cost analyses

A cost estimation was derived from the societal perspective. All costs were valued in 2018/2019 New Zealand dollars (NZ$). Direct costs were calculated by adding up out-of-pocket costs using the bottom-up approach and costs of public healthcare services. Costs of public healthcare services were computed by multiplying the number of medical resources with the unit costs of each medical resource type. The unit costs of pharmaceuticals were obtained from the Pharmac online Pharmaceutical Schedule[22] and from Waikato DHB Pharmacy. The unit costs of inpatient and outpatient services were provided by the Waikato DHB. Costs of productivity loss were calculated from the WLQ index scores.

2.5 | Statistical analyses

The QoL and total costs were compared by gender, ethnicity, age group (18–24, 25–44, 45–64, 65+ years), and disease duration (0–5, 6+ years). Continuous variables are presented as mean (standard deviation) or as median (range) where appropriate. Between groups comparisons used a Student *t* test or analysis of variance where appropriate for continuous variables or χ^2 test for categorical data. Fisher exact test was used to analyze the p value between variables. For all tests, a p value less than 0.05 was taken as the level of significance.

3 | RESULTS

3.1 | Demographics

Our cohort consisted of 131 patients. Eighty-six of 131 patients returned the completed responses (65.6%). The sample was representative of the cohort in all demographic aspects. Patient characteristics including age, type of disease, and age of the whole cohort from the database were analyzed. There were 119 (90.8%) female patients, 12 (9.1%) male patients; 28 (21.3%) has diffuse disease, 79 (60.3%) had limited disease, and 24 (18%) had overlap syndromes. These percentages are similar to those of the participants.

Most participants were from the Waikato DHB region (64; 74%), 19 (22%) were from the Bay of Plenty DHB, one from Lakes DHB, and two from Taranaki DHB. Table 1 shows the patient characteristics. Female to male ratio in our cohort was 9:1 (77 female and 9 male). There were 55 (63%) patients with lcSSc, 19 (22%) with dcSSc, and 12 (13%) with an SOS. Ethnicity data showed 72 (83%) were NZ European, two were dual NZ European/Māori, five patients were Māori, and seven identified themselves as being of other ethnicity. Of these, one was Australian, two were European, one was German, one Indian, one British, and one from the Philippines. Median age of the cohort was 65 years, with a range of 18–88 years. The median duration of the disease was 11 years, range 0–45 years (Table 1). More patients were unemployed and only a few patients had medical insurance (Table 2).

3.2 | Direct costs

The average total direct costs for the 6 months for health as reported by patients was NZ$ 444.50 (NZ$ 0–4700). The average costs for the dcSSc group were NZ$ 598.00, NZ$ 439.00 for the lcSSc group, and NZ$ 204.90 for the SOS group. The breakdown of the expenses related to health care is shown in Figure 1.

TABLE 1 Demographics

Gender	n (%)
Female	77 (90)
Male	9 (10)
Type of systemic sclerosis (SSc)	
Diffuse cutaneous SSc	19 (22)
Limited cutaneous SSc	55 (63)
Scleroderma overlap syndromes	12 (13)
Age (years)	
17–24	1 (1.1)
25–44	4 (4.6)
45–54	12 (13.9)
55–64	24 (27.9)
65 or more	44 (51.1)
Marital status	
Married/Living with partner	50 (58.1)
Single	35 (40.6)
No response	1 (1.1)
Ethnicity	
NZ European	72 (83)
Māori/NZ European	7 (8)
Others	7 (8)
Disease duration (years)	
0–5	23 (26.7)
6–10	29 (33.7)
11–20	20(23.2)
20+	13(15.1)
Education	
Bachelor's degree or above	28(32.5)
Others	58(67.4)

TABLE 2 Economic aspects

Employment (working for pay), n (%)	
Yes	**36 (41.8)**
No	47 (54.6)
Not known	3 (3.4)
Private medical insurance, n (%)	
Yes	21 (24.4)
No	64 (74.4)
Not known	1 (1.1)

Self-funded health practitioner visits constituted visits to allied health-like physiotherapists, podiatrists, and occupational therapists. The others constituted expenditure for any miscellaneous purposes that were not included in the previous questions. Most of these costs were found to be for protective gear and aids for mobility and functioning. Patients spent an average of NZ$ 65 towards GP visits related to SSc. Costs for accommodation (mean NZ$ 6.25) and investigations (mean NZ$ 10.50) were the least, with the next highest costs being for medications. There was no significant difference between the overall direct health costs based on age, gender ($P = 0.28$), and duration of disease ($P = 0.12$) or disease subtype ($P = 0.03$) (Table 3).

The costs incurred by the Waikato DHB for inpatient admissions, outpatient visits, investigations, and medications was NZ$ 1186 per patient for the 2018/2019 fiscal year. The overall costs for SSc for Waikato DHB for the year were estimated at NZ$ 83 290. Patient-reported average costs, upon conversion to annual costs amounted to NZ$ 889 per year. Cost of usual medications at standard doses for SSc was obtained from the Waikato DHB pharmacy. This was NZ$ 88.36 per month, amounting to NZ$ 1060.32 per year. The average total cost per SSc patient is approximately calculated at NZ$ 3091.

3.3 | Indirect costs

3.3.1 | WLQ

The WLQ was completed by 38 patients; 36 of responders reported being employed. Of the remaining two, one was a student, the occupation of the other was unknown. There was a significant difference in scores between the dcSSc and lcSSc groups in time management, and interpersonal and output demands. There was no significant difference observed between physical demands between the disease subtypes. The average WLQ index score was 1.86 (0–5.5), with a calculated average productivity loss score of 46.5%.

3.4 | Quality of life measures

Quality of life measures including SF-36, SHAQ-VAS, and NZiDep were compared between age, gender, ethnicity, type, and duration of disease.

3.4.1 | SF-36

The SF-36 scores were calculated in the eight domains (Figure 2). These were compared with the SF-36 scores of the general adult population of NZ obtained from the Ministry of Health data. These scores were not age- or gender-matched. The average score for each domain was much lower than that in the general population. The mean scores for physical function (48 vs 89), role limitation due to physical health (39.9 vs 80.7), and general health (43.7 vs 73.8) were particularly low compared with those of the general NZ population. The *P* value between mean scores of SSc patients and the general population was less than 0.0001 with all variables. Patients with lcSSc disease performed better than those with dcSSc disease in all domains; *P* value for lcSSc and dcSSc was less than 0.05 for all

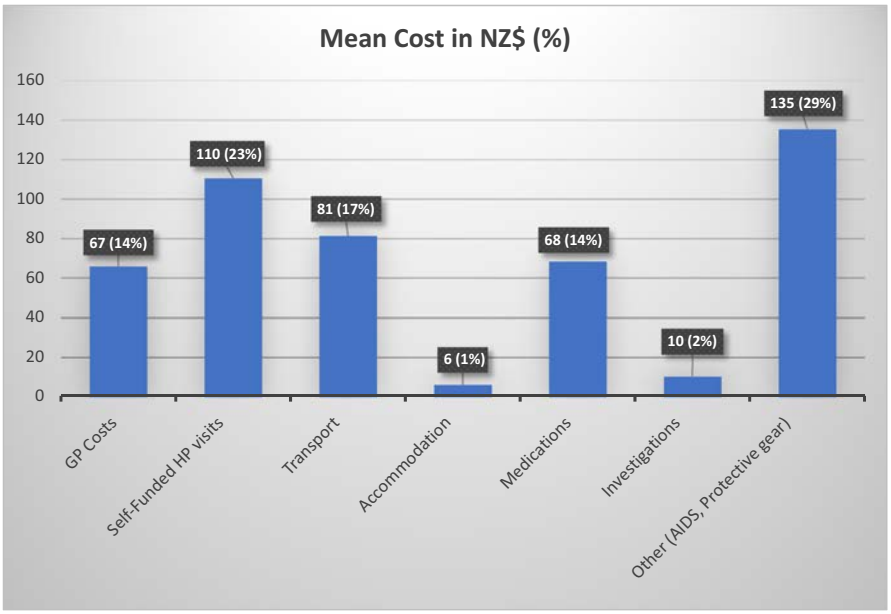

FIGURE 1 Direct out-of-pocket costs per 6 months

variables except for physical function and general health, in which there was no statistically significant difference.

3.4.2 | SHAQ-VAS and NZiDep

Patients completed the SHAQ and VAS scores as a part of their disability assessment. Forty-seven (54.6%) had a mean score of less than 1 suggestive of mild to moderate disability as defined by the EUSTAR group,[9] 28 (32.5%) had a score of 1 or more to less than 2 and 5 (5.8%) scored 2 or more, suggestive of severe disease. VAS scores were highest for overall disease activity. In the subtypes, patients with dcSSc disease scored highest for overall disease activity. There was no significant difference in the VAS scores between the subgroups. On further analysis, there was no significant difference in SHAQ scores based on duration of disease, gender, or age. NZiDep analysis showed higher deprivation in males, dcSSc group, and other ethnic groups, but this was not statistically significant.

4 | DISCUSSION

Although SSc is a rare disease, it is a significant health issue with major disability, adversely affecting general well-being, functional ability, and financial stability of the individual. Consequently, it is a huge burden on society in terms of reduced employment and high health costs. This cross-sectional study is the first in New Zealand to collect bottom-up data on patients with SSc. The data were collected from patients attending Rheumatology clinics at Waikato Hospital. The patient demographics are similar to those seen in studies from overseas.[1,23] Māori represented 7% of the study participants, with the percentage population of Māori in the Waikato and Bay of Plenty regions being 24% and 29.4% respectively.[24]

The Canadian Scleroderma Research Group Study[7] showed that disease duration and diffuse subtype were statistically significant determinants of employment. Similar difference in the disease duration was seen in our cohort, but there was no significant variation in disease sub-types. This was also reflected in our cohort, with higher unemployment rates in patients with longer disease duration. However, patients with dcSSc were more likely to be employed than the lcSSc group and earned higher wages, at an average of NZ$ 19.00 per hour. The income of the dcSSc group was higher than the other groups. This was slightly lower than the average hourly wage in NZ in 2019. The average hourly wage in New Zealand in 2018 was NZ$ 25.00 and in 2019 was NZ$ 25.50.

The calculated productivity loss was 46.5%. Arthritis New Zealand data showed a 15.7% reduction in productivity in arthritis in general.[12] The 2018 Australian Scleroderma cohort study[25] showed a 38.4% reduction in productivity and reduced quality of life. Although the questionnaire used for work productivity assessment was different from the one in our study, significant productivity loss was noted. The calculated average loss in annual income amounted to NZ$ 22 786. This was calculated by multiplying the productivity loss index by the average national annual individual income for 2018 as per NZ statistics, which was NZ$ 49 004.[26] Comparing with other rheumatic conditions, SSc appears to have a higher percentage of productivity loss; the Ankylosing spondylitis study in New Zealand showed a productivity loss index score of 4.8%[27] and an American study of patients with rheumatoid arthritis showed a 4.9% productivity loss compared with the general population.[28]

Overall, the direct healthcare costs were higher in the dcSSc group, but no significant difference was noted between the groups. The highest expenditure was observed for self-funded health practitioner visits and for the Others category. This is reflective of the significant physical disability caused by the condition. Patients admitted to hospital were admitted for longer durations and had multiple problems, increasing the costs incurred.

TABLE 3 Comparison of direct health costs based on gender, disease duration, ethnicity, and disease sub-type

Variable	GP consultations (SD)	Self-funded HP visits (SD)	Transport (SD)	Accommodation (SD)	Investigations (SD)	Medications (SD)	Others (SD)	Total (SD)
Gender								
Male	54.4 (51.7)	44.4 (101.3)	224.4 (393.8)	0 (0)	0 (0)	53.88 (67.16)	213.3 (596.3)	590 (828.3)
Female	67.2 (79.2)	118.7 (329.4)	62.35 (112.2)	7 (48)	11.6 (53.3)	70.1 (81.5)	125.2 (518.8)	427.2 (699.3)
Disease duration (years)								
0–5	60.6 (56.7)	101.9 (343.9)	69.2 (199.5)	0 (0)	5 (24.4)	79.8 (78.6)	125.9 (253.0)	415.6 (431.9)
6+	67.9 (83.5)	114.0 (343.9)	86.4 (199.5)	8.7 (53.8)	12.6 (57.9)	63.7 (78.6)	139.9 (600.5)	450.1 (792.1)
Ethnicity								
NZE	68.8 (79.6)	79.3 (170.1)	67.8 (124.8)	7.39 (49.5)	12.2 (54.4)	70.7 (82.9)	134.8 (553.7)	414.9 (700)
Māori	71.6 (67.5)	193.8 (276)	61.6 (37.1)	0 (0)	0 (0)	60 (48.9)	95 (212.4)	399.7 (509)
Others	31.1 (41.4)	348 (920.7)	282.4 (514.0)	0 (0)	0 (0)	52.4 (75.3)	181.6 (430.4)	789 (962.7)
Disease sub-type								
dcSSc	76.5 (56.2)	226.7 (597.6)	138.7 (300.9)	0 (0)	62.3 (16.0)	62.3 (16.0)	148.1 (287.4)	598 (668.6)
lcSSc	61.8 (76.5)	89.9 (170.1)	70.3 (132.0)	9.9 (57.2)	13.1 (61.3)	78.7 (90.5)	155.4 (631.0)	439.0 (779.1)
SOS	67 (105.5)	26.1 (61.1)	49.3 (49.4)	0 (0)	9.5 (32.9)	33.3 (48.4)	23 (60.0)	204.9 (215.9)

Abbreviations: dcSSc, diffuse cutaneous systemic sclerosis; GP, general practitioner; lcSSc, limited cutaneous systemic sclerosis; NZE, New Zealand European; SD, standard deviation; SOS, scerloderma overlap syndrome.

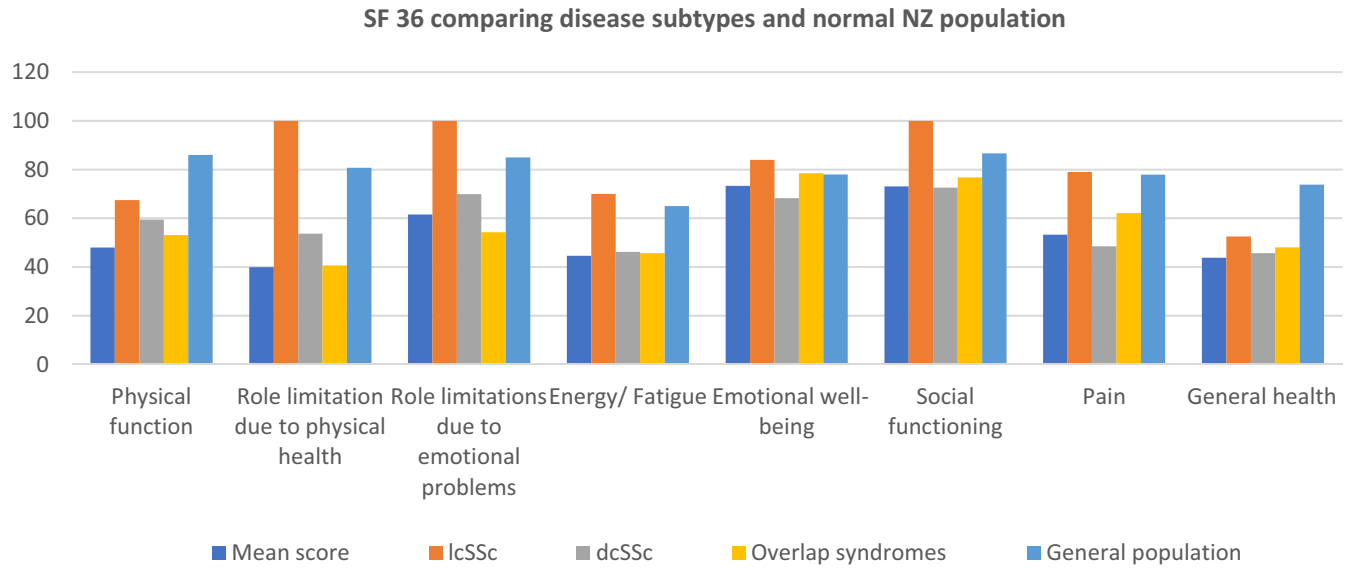

FIGURE 2 Short form-36 comparing disease subtypes and general New Zealand population

TABLE 4 Comparison of direct healthcare costs[32]

Country (year)	Sample size	Currency (year)	Direct costs/ patient/ year
New Zealand (2018)	86	NZD (2018)	3091
Canada, (2009)[33]	457	CDN (2007)	5038
France, (2015)[1]	147	Euros (2012)	1606
Spain, (2014)[34]	147	Euros (2011)	8235
Spain, (2016)[a,35]	589	Euros (2012)	1413–17 300
Hungary, (2010)[8]	80	Euros (2016)	3300

[a]Study included seven European countries, explaining the range of costs.

Table 4 compares the direct health costs reported in various international SSc studies with the results of this study.

The health-related QoL measures reported were significantly lower than those in the general population. SF-36 scores were significantly lower compared with the general NZ population.[29,30] The WLQ summative scores showed significant differences between the dcSSc and lcSSc sub-types in three of the four domains without any such difference between the Physical demands domain. This correlated with the SF-36 results, which also did not show a significant difference in the physical function and general health. A systematic review conducted by the Canadian Scleroderma group showed significantly reduced SF-36 scores in SSc patients compared with the general population and lower scores in the dcSSc group.[7] The SHAQ-VAS scores were high overall compared with the general population.[31]

The QoL impairment can explain the work place limitations and hence reduced employment and increased deprivation as a result of financial instability. The chronic and progressive nature of the condition poses a risk of progressive decline in the functional abilities of patients affected by the condition. This health-related QoL data may be a reflection of interventions to manage the condition and may be useful in implementing early interventions for management of the condition in order to improve QoL and employment abilities, and reduce healthcare costs.

There are a few limitations in this study. The sample size is small. The patients had to provide information from the previous 6 months, which may have resulted in recall bias. The costs incurred by the hospitals might be an underestimate, as this was collected only from the study hospital, which had the majority (74%) of patients. Admissions or clinic visit costs from other regions were not included. Government subsidies for GP visits were not known and the medication costs were an estimate rather than information from individual patient prescriptions. The QoL and work disability outcomes were not compared directly with extent of organ involvement, which could have provided more precise information on factors contributing to the limitations. This could be a subject for further study. The work productivity loss was calculated from index scores and there were no data on presenteeism or absenteeism.

Although SSc is a rare disease, it has a significant impact on quality of life, work abilities, and productivity. As a result, this has a significant societal burden and increased healthcare costs. The higher female prevalence of the condition predisposes them to productivity losses and reduced hours of work, contributing hugely to the gender pay gap. Knowledge regarding these aspects will guide healthcare personnel and patients to review modifiable factors to improve productivity and reduce healthcare costs. Further studies taking into consideration the extent of disease and organ involvement will provide more comprehensive information on health-related costs. There is a lack of specific treatments for SSc, which is a significant contributor to disease-related morbidity unlike other rheumatic conditions like rheumatoid arthritis and ankylosing spondylitis, for which multiple-targeted treatments are available.

Identifying the costs of health care, non-medical costs, and lost productivity may help to identify the burden of this rare condition and areas of deficiency in these patients, health system, and society, which may help the implementation of measures to improve their quality of life and reduce costs of healthcare utilization.

ACKNOWLEDGEMENTS

We would like to thank Mrs. Joanna Schollum, Clinical Nurse Specialist, Rheumatology Department. Waikato Hospital, for assistance with data collection, Dr. Chunhuan Lao, Senior Research fellow at the Waikato Medical Research Centre, University of Waikato, for advice on WLQ data analysis and calculation of income loss, Mr. Roger Williams and Mr. Erwin Davina, costing accountants from the Finance Advisory, Waikato DHB for providing the DHB costing data, and Ms. Marinda van Zyl-Greene, Pharmacist, Waikato DHB pharmacy for providing the unit costs of medications. We would like to thank Dr. Douglas White and Dr. Kamal Solanki for their constant support and mentoring.

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How to cite this article: Padala SD, Lao C, Solanki K, White D. Direct and indirect health-related costs of systemic sclerosis in New Zealand. *Int J Rheum Dis*. 2022;25:1386-1394. doi: 10.1111/1756-185X.14433

APPENDIX 1

Individual Study Number: _____';

Sociodemographics

1. What is your **gender**?
☐ Male
☐ Female
2. **In what year** were you **diagnosed** with systemic sclerosis?
3. What ethnic group do you belong to?
☐ New Zealand European
☐ Māori
☐ Samoan
☐ Cook Island Māori
☐ Tongan
☐ Niuean
☐ Chinese
☐ Indian
☐ Other: _____
4. What is your highest completed education **qualification**?
☐ None
☐ NZ School Certificate
☐ NZ Sixth Form Certificate
☐ University Entrance
☐ Diploma
☐ Post-grad Diploma
☐ Bachelor's Degree
☐ Master's Degree
☐ PhD
☐ Trade Qualification
☐ Professional Qualification
☐ Prefer not to answer
☐ Do not know
☐ Others (please specify in the following box)
5. What is your **occupation**?
6. What is your **marriage status**?
☐ Single
☐ Separated
☐ Partner
☐ Divorced
☐ Married/in civil union
☐ Engaged
☐ Widowed
7. What is your **postcode**?

Systemic sclerosis related out-of-pocket costs

1. Do you have health insurance?
☐ Yes
☐ No
2. How much is your premium per year?

In the following questions, please indicate your out-of-pocket costs related to systemic sclerosis.

Please include the costs that were covered by insurance and not covered by insurance.1. During the **last 6 months** how many **general practitioner consultations** related to systemic sclerosis did you have?
2. During the **last 6 months** how much did you spend on **general practitioner consultations** related to systemic sclerosis?
3. During the **last 6 months** how much did you spend on **self-funded visits to other health professionals** related to systemic sclerosis?

4. During the **last 6 months** how much did you spend on **transportation (taxi, bus, fuel and parking)** for your visits to health professionals (including GPs, physiotherapists, and other health professionals) related to systemic sclerosis?

5. During the **last 6 months** how much did you spend on **accommodation** for your visits to health professionals (including GP, specialist, and other health professionals) related to systemic sclerosis?
6. During the **last 6 months** how much did you spend on systemic sclerosis-related **medications (including unsubsidized medications and the $5 prescription charge forsubsidized medications)**?
7. During the **last 6 months** how much did you spend on systemic sclerosis-related **investigations**?
8. Did you have a **caregiver** (including partner, spouse, children or others) for your systemic sclerosis over the last 6 months?

☐ Yes
☐ No

9. Please indicate the **cost of the caregiver** (including loss of salary) for your systemic sclerosis over the last 6 months.
10. Please indicate **other out-of-pocket costs** related to systemic sclerosis that are not listed above.

11. What is your hourly salary?

Received: 28 March 2022 | Revised: 17 May 2022 | Accepted: 17 August 2022

DOI: 10.1111/1756-185X.14434

International Journal of Rheumatic Diseases WILEY

ORIGINAL ARTICLE

Jian Pi Shen Shi formula alleviates hyperuricemia and related renal fibrosis in uricase-deficient rats via suppression of the collagen-binding pathway

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Funding information
Construction Project of National Traditional Chinese Medicine Clinical Research Base, Grant/Award Number: 2018 No. 131

Abstract

Aim: Jian Pi Shen Shi Formula (JPSSF) is a beneficial treatment for hyperuricemia and related tissue damage in the clinical setting. This study was designed to investigate its therapeutic potential and underlying mechanisms in uricase-deficient rats ($Uox^{-/-}$ rats).

Methods: $Uox^{-/-}$ rats were used to assess the therapeutic potential of JPSSF on hyperuricemia. Protein extracts from renal tissues of a $Uox^{-/-}$ group and a JPSSF group were analyzed using tandem mass tag labeling quantitative proteomic workflow. Collagen deposition in $Uox^{-/-}$ rat kidneys was analyzed by Masson trichromatic staining. The gene expression associated with collagen-binding-related signaling pathways in the kidneys was further explored using quantitative polymerase chain reaction assay. The protein expressions of collagen 1a1 (col1a1), col6a1, and α-smooth muscle actin were measured by Western blotting and immunohistochemistry.

Results: JPSSF significantly decreased renal function indices and alleviated renal injuries. The action of JPSSF was manifested by down-regulation of col6a1 and interleukin-1 receptor-associated kinase-like 2, which blocked the binding sites on collagen and further prevented kidney injury. The anti-renal fibrosis effect of JPSSF was confirmed by reducing the collagen deposition and hydroxyproline concentrations. JPSSF treatment also intensely down-regulated the mRNA and protein expressions of col6a1, col1a1, and α-smooth muscle actin, which inhibited the function of the collagen-binding-related signaling pathway.

Conclusion: Our results indicated that JPSSF notably ameliorated hyperuricemia and related renal fibrosis in $Uox^{-/-}$ rats through lowering uric acid and down-regulating the function of the collagen-binding pathway. This suggested that JPSSF is a potential empirical formula for treating hyperuricemia and accompanying renal fibrosis.

Na Yin and Xiaosi Li contributed equally to this work.

KEYWORDS
collagen deposition, hyperuricemia, hyperuricemic nephropathy, Jian Pi Shen Shi Formula, renal fibrosis, renal injury, uricase-deficient rats

1 | INTRODUCTION

In recent years, epidemiologic and clinical data have indicated that prevalence of hyperuricemia and associated diseases have increased worldwide.[1] Hyperuricemia is a well-known disorder of purine metabolism, and can be diagnosed by serum uric acid (SUA) above 70 μg/mL in men or above 60 μg/mL in women.[2,3] The increase of SUA is caused either by uric acid over-synthesis, or its insufficient excretion. Considering that uric acid and its salts are polar compounds with poor solubility,[4] excessive uric acid is prone to deposit in the joints, kidney, and bones in the form of crystals, usually resulting in renal dysfunction and gout. Normally, uric acid is cleared through the renal and extrarenal pathways, and the renal system discharges more than two-thirds of the uric acid from the body.[5] The kidney is considered to be one of the most distributed uric acid organs, an important regulator of circulating uric acid, and the organ that is usually effected by hyperuricemia.[6] Urate crystals deposited in the kidneys could cause hyperuricemic nephropathy, leading to glomerular hypertrophy and tubulointerstitial fibrosis.[7] Growing evidence has demonstrated that the level of SUA was correlated not only with clinical renal failure indices, but also with renal pathology. Hyperuricemia has been reported to be an independent risk factor for segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis.[8] However, hyperuricemic nephropathy has been a difficult clinical problem with few effective treatments.

According to the theory of traditional Chinese medicine, "Deficiency of liver and kidney, phlegm and dampness stasis" are the main pathogenesis of gout and hyperuricemia. Based on this theory, the Wu School in Yunnan developed an empirical formula, Jian Pi Shen Shi Formula (JPSSF), which is widely applied to treat hyperuricemia and exhibits an excellent curative effect. JPSSF consists of 12 kinds of Chinese herbal medicine, including root of *Codonopsis pilosula* (*Dangshen*), *Atractylodes macrocephala* Koidz. (*Baizhu*), *Poria cocos* (*Fuling*), powder of *Panax notoginseng* (Burk.), F. H. Chen (*Sanqifeng*), *Rhizoma pinelliae* preparatum (*Fabanxia*), *Rhizoma dioscorea* (*Bixie*), *Lysimachia christinae* Hance (*Jinqiancao*), *Smilax glabra* Roxb. (*Tufuling*), *Orthosiphon stamineus* Benth. (*Maoxucao*), Corn silk (*Yumixu*), Plantain (*Cheqiancao*), and Coix seed (*Yiyiren*). Previous reports from preclinical and clinical studies suggests that JPSSF is useful in treating hyperuricemia and renal injury.[9,10] However, its mechanism of action remains unclear.

Urate oxidase (uricase, Uox) is a big obstacle to scientists establishing stable animal models for studying hyperuricemia and associated disorders. Due to the low survival rate of uricase-deficient ($Uox^{-/-}$) mice, $Uox^{-/-}$ rats, named Kunming-DY rats, were generated by Prof. Duan on a background of Sprague-Dawleyrats using the CRISPR/Cas9 technique. The uricase-deficient rats were apparently healthy with more than 95% survival rate. Meanwhile, the male animal's purine metabolism is consistent with that of humans and presents similar SUA. The rat is considered as an alternative model animal to study hyperuricemia and associated diseases.[11] The present study first reported that JPSSF exerted therapeutic potential in treating hyperuricemia in the $Uox^{-/-}$ rats; the possible mechanism was further explored. Tandem mass tag (TMT) -labeled proteomics data have strongly supported that uric acid causes renal injury and further leads to fibrosis and innate inflammatory pathogenesis. The study shows that oral administration of JPSSF can reduce the collagen deposition and intensely block the collagen-related signaling pathway and inflammatory pathway at both mRNA and protein levels. These data suggest that JPSSF is promising for treating hyperuricemia with or without related renal fibrosis.

2 | MATERIALS AND METHODS

2.1 | Experimental animals

Male wild-type Sprague-Dawley rats were obtained from Chengdu Dossy Experimental Animals Co., Ltd, Chengdu, China (Certification No. SCXK [Chuan] 2008–24). Male $Uox^{-/-}$ rats, 45 days old, were presented as a gift from Prof. Weigang Duan (Yunnan University of Traditional Chinese Medicine, Kunming, China). Rats were maintained at 22 ± 1°C, with a humidity of 45%-55% under alternating 12 h light and 12 h dark photoperiods. All animals were given free access to food and water, and allowed 1 week to adapt to the environment before experiments. All experiments were conducted in accordance with the Institutional Ethical Guidelines on Animal Care approved by the Institute Animal Care and Use Committee of the First Affiliated Hospital of Yunnan University of Traditional Chinese Medicine.

2.2 | Reagents and antibodies

Uric Acid ver.2 (UA2), UREAL (UREA/blood urea nitrogen), and Creatinine plus ver.2 (CREP2) assay kits were manufactured by Roche Diagnostics GmbH (Mannheim, Germany). TMT® Mass Tagging kits and reagents were manufactured by Thermo Fisher (Waltham, MA, USA). Dithiothreitol and iodoacetamide were manufactured by Sigma Aldrich (St. Louis, MO, USA). Trypsin was manufactured by Promega (Madison, WI, USA). Bradford protein quantitative kits were manufactured by Beyotime Biotechnology (Shanghai, China). Hydroxyproline (HYP) assay kits were manufactured by Nanjing Jiancheng Bioengineering Institute (Nanjing,

China). RNA extraction kits were manufactured by Qiagen (Valencia, CA, USA). Prime Script RT Master Mix Perfect Real Time kits and SYBR Premix Ex Taq II kits were manufactured by TaKaRa (Dalian, China). Anti-α smooth muscle actin (α-SMA, ab124964), anti-collagen 1a1 (col1a1 [ab260043]), anti-col6a1, (ab182744), and goat antibody to rabbit IgG (horseradish peroxidase) (ab205718) were manufactured by Abcam (Cambridge, UK). GAPDH (D16H11) XP® Rabbit monoclonal antibody (5174) was manufactured by Cell Signaling Technology (Danvers, MA, USA). IRDyeR680LT (C60302-06) were manufactured by Odyssey (Lincoln, NE, USA).

2.3 | Preparation and determination of effective compounds in JPSSF

The composition of JPSSF (60 kg adult dose) is as follows: *Codonopsis pilosula* 15 g, *Atractylodes macrocephala* Koidz.15 g, *Poria cocos* 15 g, powder of *Panax notoginseng* (Burk.) F. H. Chen 15 g, *Rhizoma pinelliae* preparatum 15 g, *Rhizoma dioscorea* 15 g, *Lysinwchia christinae* Hance 15 g, *Smilax glabra* Roxb. 15 g, *Orthosiphon stamineus* Benth. 30 g, *Corn silk* 30 g, *plantain* 30 g, *Coix seed* 15 g. All drugs were provided at the pharmacy of Kunming Municipal Hospital of Traditional Chinese Medicine. The preparation of JPSSF was performed as follows: add 10 times the volume of water, soak for 30 min, boil for 30 min, decoct three times, combine the decocted liquid, filter with gauze, centrifuge, evaporate with a rotary evaporator to concentrate the extract, weigh the mass, and convert 6.01 g of raw medicine into 1 g of extract of the original medicine. This is based on the conventional dose of JPSSF used in the clinical treatment of hyperuricemia.

The standard and sample solutions were filtered through a 0.45 μM PVDF-syringe filter. A reverse-phase C18 column (4.6 × 250 mm, 5 μm) was used for the analysis of the effective components. Ginsenoside-Rg1, ginsenoside-Rb, and notoginsenoside-R1 were found under the conditions of gradient elution of mobile phases A (acetonitrile) and B (water) and at a detection wavelength of 203 nm. Astiloxin was detected in methanol-0.1% glacial acetic acid (32:68) as mobile phase at a detection wavelength of 291 nm.

2.4 | Therapeutic regimens

As shown in the schedule, male 8 week old $Uox^{-/-}$ rats were given JPSSF orally for 8 weeks. Before administration, $Uox^{-/-}$ rats were randomly divided into two groups (n = 8 per group): the treated group (JPSSF, 12.50 g/kg/day) and the control group ($Uox^{-/-}$, saline of the same volume). Eight wild-type rats (WT, saline of the same volume) were selected from the same background and considered as a blank control group. Serum was sampled, and renal function indices (UA, UREA, and CREA) were monitored monthly during the treatment. At the end of the experiment, all animals were anesthetized with urethane (1.0 g/kg), and their kidneys were collected.

2.5 | Biochemical parameters

Blood urea nitrogen and creatinine concentrations in serum were determined by cobasc311 automatic biochemical analyzer (Roche) according to the protocols provided by the manufacturer. Hydroxyproline in renal tissue was assessed using the photometrical method in hydrolysates. Briefly, 40 mg of renal tissue was accurately weighed in the tube and HYP was extracted by alkali hydrolysis. Then, all steps of the HYP assay were carried out according to the protocol of the HYP assay kit. The content of HYP in the sample was determined by its absorption at 550 nm.

2.6 | Renal histopathology

At the end of the experiment, all animals were anesthetized with urethane (1.0 g/kg), and their kidneys were collected immediately. The kidneys were routinely fixed in 4% formalin followed by dehydrating, embedding, sectioning (with a thickness of 4 μm each) and staining with hematoxylin & eosin kits.

For immunohistochemical staining, renal tissues were deparaffinized with xylene, and dehydrated in serial dilutions of alcohol. The antigens in sections were recovered by microwaves, and the endogenous peroxidases were blocked with 3% hydrogen peroxide. The sections were incubated with primary antibody (anti-α-SMA 1:1000 and anti-col6a1 1:250) at 4°C overnight, and immunoreactions were visualized with a horseradish peroxidase anti-rabbit DAB detection kit (Maixin). Images were randomly acquired using a microscope. The integrated optical density of positive expression was counted by Image Pro Plus software.

Renal fibrosis was evaluated by Masson staining. In brief, the slices were dehydrated then sequentially stained with mixed hematoxylin A and B solution, Ponceau acid fuchsin solution, phosphomolybdic acid solution, and aniline solution. After color separation, dehydration, decolorization, and sealing were performed, final images were acquired using a polarized light microscopy. Collagen was stained blue, renal matrix, myofibers, and red cells were red, and the nucleus was blue-brown. Image J Pro Plus software was used to calculate the positive area and analyze the whole microscope field of view, and the proportion chart of positive expression was drawn based on the results.

2.7 | Protein extraction, quality test, TMT labeling, and fraction separation for proteomic study

To obtain total protein, another aliquot of kidney tissues was lyzed with PASP lysis buffer (100 mM NH_4HCO_3, 8 M urea, pH 8), followed by ultrasonication on ice and centrifugation (4°C and 10 000 *g* for 5 min). The supernatant was collected and put in a tube. Dithiothreitol and iodoacetamide were added to the supernatant in turn. Subsequently, samples were incubated with precooled acetone and the precipitated protein was collected and washed. Total protein

concentration of samples was determined by the protocol of the Bradford protein quantitative kit. Fifty micrograms of protein was loaded, separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue R-250. The gel was decolored until the bands were visualized clearly to complete the protein quality test. Proteins were digested by trypsin and desalinated in a C18 column. The peptides were labeled with TMT reagent and the reaction was stopped by ammonia. All labeling samples were desalted and lyophilized. The lyophilized powder was fractionated by a C18 column (Waters BEH C18, 4.6 × 250 mm, 5 μm) on a Rigol L3000 HPLC system, and eluted by gradient with mobile phase A (2% acetonitrile, adjusted to pH 10.0 using ammonium hydroxide) and phase B (98% acetonitrile) to develop 10 fractions.

2.8 | RNA extraction and real-time quantitative PCR assay

The total RNA was extracted by RNeasy Kit in renal tissue as previously described elsewhere.[12] Reverse transcription (RT) of RNA (1 μg) to cDNA was performed using the Prime Script RT Master Mix Perfect Real Time. Synthesized cDNA was used in real-time RT-polymerase chain reaction (PCR) experiments using SYBR Premix Ex Taq II kits. The relative expression of *col6a1*, *col1a2*, *col3a1*, *col4a1*, *col4a2*, *α-SMA*, *Fn1*, *MMP9*, and *TIMP-1* were determined by normalizing the expression level of each gene to that of the GAPDH gene using the $2^{-\Delta\Delta Ct}$ method. The primer sequences of specific genes were as follows:

α-SMA: (forward) 5′-ACTGGGACGACATGGAAAAG-3′.
(reverse) 5′-TACATGGCAGGGACATTGAA-3′.
col1a2: (forward) 5′-TGCTCAGCTTTGTGGATACG-3′.
(reverse) 5′-GGGACCATCAACACCATCTC-3′.
col3a1: (forward) 5′-GCACAGCAGTCCAACGTAGA-3′.
(reverse) 5′-TCTCCAAATGGGATCTCTGG-3′.
col4a1: (forward) 5′-GCCAAGTGTGCATGAGAAGA-3′.
(reverse) 5′-AGCGGGGTGTGTTAGTTACG-3′.
col4a2: (forward) 5′-AAAGGTGTGTCTGGGGACAG-3′.
(reverse) 5′-ATCCAGCCTCTCCCTTGATT-3′.
col6a1: (forward) 5′-ATTAAGAAGGGGCTGGAGGA-3′.
(reverse) 5′-GACCTTGATGCCCAAGTGTT-3′.
Fn1: (forward) 5′-AATGGAAAAGGGGAATGGAC-3′.
(reverse) 5′-TGGTGTCCTGATCATTGCAT-3′.
MMP9: (forward) ′-CCACCGAGCTATCCACTCAT-3′.
(reverse) 5′-GTCCGGTTTCAGCATGTTTT-3′.
TIMP-1: (forward) 5′-TCCCCAGAAATCATCGAGAC-3′.
(reverse) 5′-TCAGATTATGCCAGGGAACC-3′.
GAPDH: (forward) 5′-AGACAGCCGCATCTTCTTGT-3′.
(reverse) 5′-CTTGCCGTGGGTAGAGTCAT-3′.

2.9 | Western blotting assay

Western blotting was performed according to the procedures previously described.[13,14] Briefly, renal tissues were cut and lyzed. After the supernatant of the lysate was obtained, a total of 40 μg of protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The separated protein was transferred to a PVDF membrane. The membrane was blocked with 5% non-fat milk at room temperature. Primary antibody (GAPDH 1:1000, anti-α-SMA 1:5000, anti-col1a1 1:1000, anti-col6a1 1:2000) and membrane were incubated on a shaker at 4°C overnight. After the membrane was incubated with the secondary antibody, IRDyeR680LT (1:5000) was added and incubated at room temperature for 1 h. The bands were visualized by an infrared fluorescence scanning imaging system (Odyssey CLX), and the protein abundances were analyzed by IMAGE J software.

2.10 | Statistics

Values were expressed as mean ± standard error (SE). If a normal distribution of values was verified by the normality test (Shapiro-Wilk test), Student *t* test (for two groups, two-tailed) or one-way analysis of variance (for three or more groups) was performed to compare means between groups. If there was a significance, post-hoc tests between every two groups were performed using the S-N-K method (equal variances assumed) or Tamhane T^2 method (equal variances not assumed). Otherwise, a nonparametric test for two independent samples (Mann-Whitney *U* model, two-tailed) was performed. Pearson correlation was performed to analyze the relationship between two variables. Statistical significance was set at $P < 0.05$.

3 | RESULTS

3.1 | Determination of effective compounds in JPSSF

Among the Chinese medicinal materials constituting JPSSF, the qualitative markers of *Panax notoginseng* and *Smilax glabra* Roxb. were recorded in the Chinese Pharmacopeia. The content of notoginsenoside-R1, ginsenoside-Rg1, ginsenoside-Rb1, and astilbin in JPSSF were determined by high-performance liquid chromatography. The results showed that the four effective compounds were detected in JPSSF, and the concentration of them from high to low was ginsenoside-Rg1, ginsenoside-Rb1, notoginsenoside-R1, and astilbin (Figure 1A).

3.2 | JPSSF alleviated hyperuricemia in uricase-deficient rats

In the present study, the $Uox^{-/-}$ rats were adopted to assess the therapeutic potential of JPSSF on hyperuricemia. As shown in Figure 1B, the levels of UA, UREA, and CREA were obviously higher in the $Uox^{-/-}$ group than in the WT group. It was found that JPSSF significantly lowered UA, UREA, and CREA in $Uox^{-/-}$ rats. Hematoxylin & eosin staining showed that, in the $Uox^{-/-}$ group, the renal structures

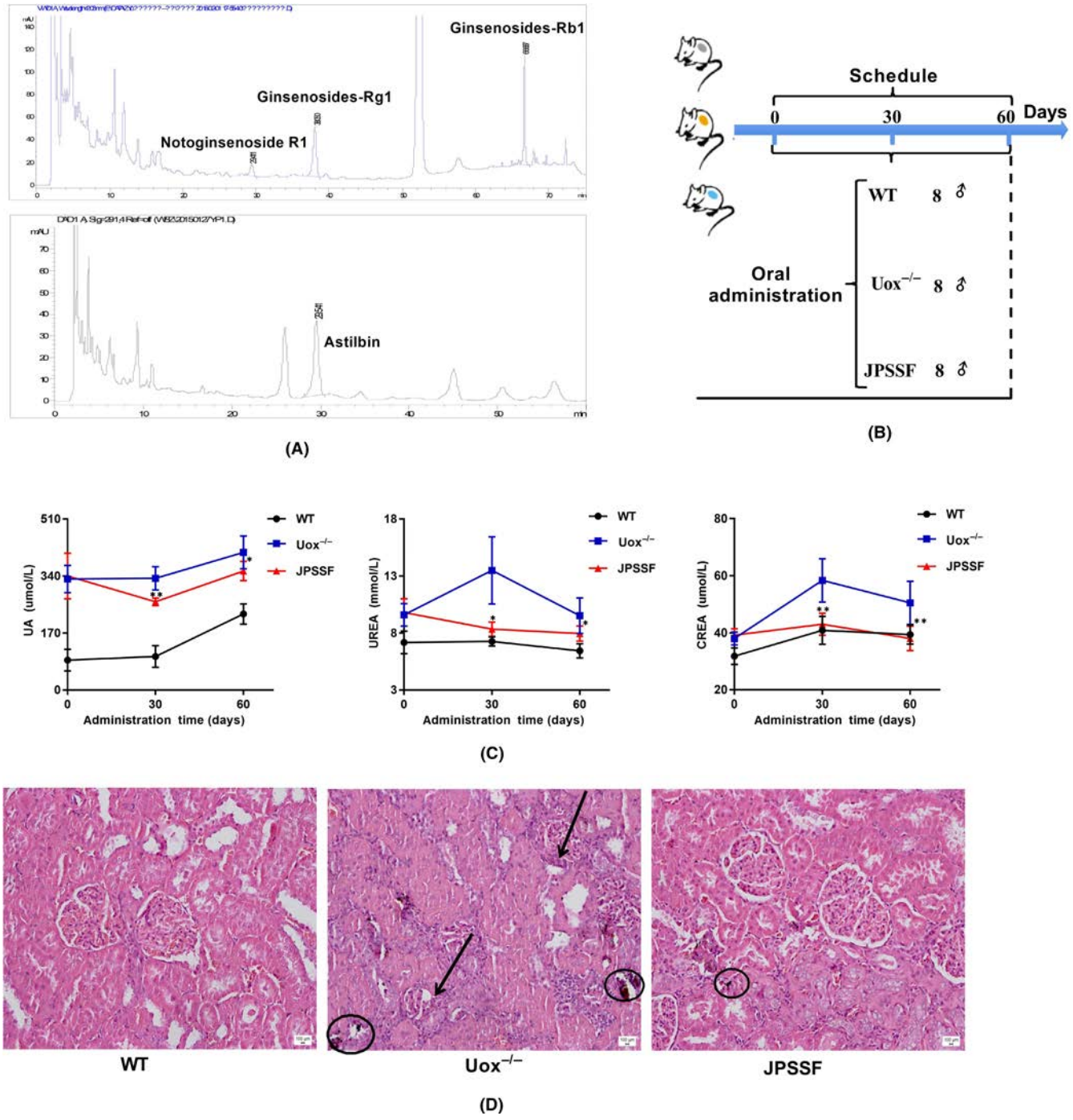


FIGURE 1 Jian Pi Shen Shi Formula (JPSSF) alleviated hyperuricemia in uricase-deficient rats ($Uox^{-/-}$). Chromatogram of effective components of JPSSF (A). Scheme of the $Uox^{-/-}$ model and animal treatments (B). The levels of UA, UREA, CREA in serum were determined by automatic biochemical analysis (mean ± SE, n = 8; $^{*}P < 0.05$, $^{**}P < 0.01$ JPSSF group vs $Uox^{-/-}$ group, analysis of variance) (C). Kidney micrographs were stained with hematoxylin and eosin; black circles indicate crystal deposition and black arrows indicate inflammatory cell infiltration (×20) (D).

were disordered and some structures even disappeared. The interstitiae were infiltrated with inflammatory cells, and the renal tubules were dilated or severely atrophied by the crystal deposition. However, these phenomena were hardly observed in the WT group and JPSSF group (Figure 1C). These results indicated that JPSSF can be served as a promising therapeutic agent for treating hyperuricemia and related renal damage.

3.3 | JPSSF inhibited collagen-binding and inflammatory response signaling pathway in $Uox^{-/-}$ rats

In order to further explore the mechanism of JPSSF in treating hyperuricemia in $Uox^{-/-}$ rats, the renal tissue protein extracts from the $Uox^{-/-}$ group and JPSSF group were analyzed using TMT labeling

quantitative proteomic workflow. The concrete technical roadmap is shown in Figure 2A. TMT proteomic analysis showed that among the 6964 proteins identified, 41 proteins were significantly changed in the JPSSF group compared with the Uox$^{-/-}$ group ($P < 0.05$), including 28 up-regulated proteins and 13 down-regulated proteins in Uox$^{-/-}$ vs JPSSF (Figure 2B,C). Gene ontology functional enrichment analysis revealed that these different proteins were mainly focused on collagen-binding and inflammatory response signaling pathways, including the I-κ kinase/nuclear factor-κB signaling and interleukin-1-mediated signaling pathways (Figure 2D,E). These pathways are highly associated with renal fibrosis, suggesting that the inhibitory effect of JPSSF on the collagen-binding and inflammatory signaling pathways may be responsible for its therapeutic effects against hyperuricemia-related renal fibrosis.

3.4 | JPSSF down-regulated collagen protein expression

Proteomic analysis indicated that these different proteins that are related to renal fibrosis were mainly focused on collagen binding and inflammatory response as described above. We therefore analyzed different proteins in collagen-binding pathways from array data. Surprisingly, compared with the Uox$^{-/-}$ group, most proteins in the collagen-binding pathways were decreased in the JPSSF group, including col6a1 col6a2, col1a1, col1a2, and col3a1 (Figure 3A,B). Among these proteins, the decrease of col6a1 was statistically significant ($P < 0.05$), implying that it could be the main target protein of JPSSF.

3.5 | JPSSF significantly reduced collagen deposition in Uox$^{-/-}$ rat kidneys

Renal fibrosis is a gradual pathophysiologic process of renal function from health to injury, to damage, and to loss of function.[15] Masson trichromatic staining is a classic method to assess the degree of renal fibrosis. The blue area represents the collagen-positive area. As shown in Figure 4A,B, uricase deficiency remarkably caused collagen protein deposition in renal interstitial tissues. However, the collagen-positive area in the JPSSF-treated group was less than that in the Uox$^{-/-}$ group. Hydroxyproline, a typical collagen deposition marker, is unique to collagen fibers. In line with the above results, HYP concentration in the Uox$^{-/-}$ group was markedly higher than that in the WT group ($P < 0.01$). However, oral administration of JPSSF markedly decreased the HYP level (Figure 4C). Furthermore, uric acid was significantly correlated with the levels of UREA and CREA (Figure 4D,E). These data demonstrated that renal fibrosis caused by uricase deficiency is mediated via hyperuricemia, and the effect of JPSSF against the fibrosis could also be associated with uric acid lowering.

3.6 | JPSSF significantly suppressed the gene transcripts associated with collagen-binding-related pathways

In order to explore how JPSSF inhibited the pathways on renal fibrosis, the gene expression associated with collagen-binding-related signaling pathways in the kidneys was further explored by using quantitative PCR. As indicated in Figure 5, in comparison with the WT group, genes participating in collagen-binding-related signaling pathways such as col6a1, col1a2, col3a1, col4a1, col4a2, α-SMA, Fn1, MMP9, and TIMP-1, were up-regulated at mRNA level in the Uox$^{-/-}$ group. In contrast, JPSSF markedly deceased the transcription of col6a1, col1a2, col3a1, col4a2, α-SMA, and Fn1. However, no influences on col4a1, MMP9, and TIMP-1 were observed.

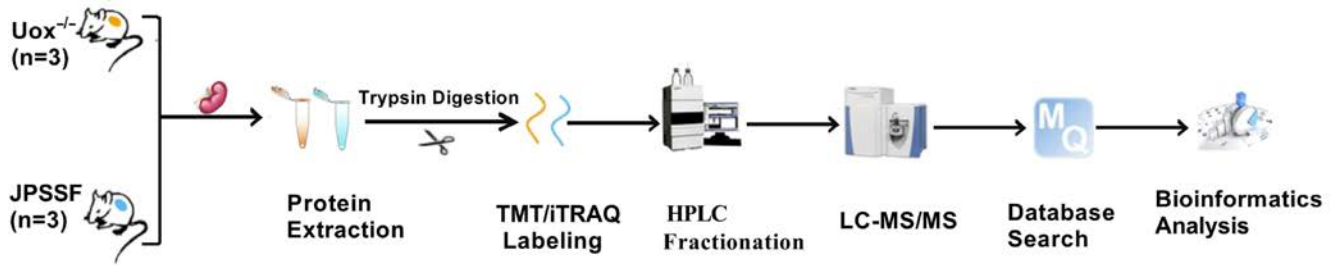
3.7 | JPSSF markedly down-regulated proteins involved in renal fibrosis

Recent evidence has demonstrated that the extracellular matrix is primarily produced by α-SMA-positive fibroblasts.[16] Both col6a1 and col1a1 are closely associated with the pathogenesis of renal fibrosis.[17] In the current study, it was also found that oral administration of JPSSF dramatically blocked transcription expression of col1a1, col6a1, and α-SMA in renal lesions as described above. To analyze the role of collagen-binding-related pathways in JPSSF against fibrosis induced by hyperuricemia, protein levels of col1a1, col6a1, and α-SMA were measured by Western blotting and immunohistochemistry. Surprisingly, as indicated in Figure 6, in comparison with the Uox$^{-/-}$ group, the protein expressions of col6a1 and α-SMA were significantly down-regulated in JPSSF group.

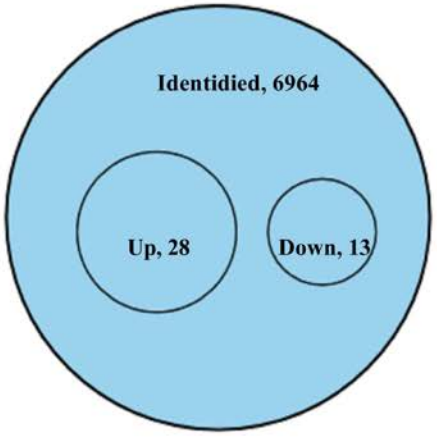
4 | DISCUSSION

Model animals that closely mimic the human purine metabolism are necessary for biomedical scientists who study hyperuricemia and associated diseases and for pharmacologists who develop uric acid-lowering drugs. Uricase, encoded by the Uox gene, is a crucial

FIGURE 2 Tandem mass tag proteomic analysis revealed proteins from renal tissues. Proteomic analysis revealed proteins from renal tissues in the Jian Pi Shen Shi Formula (JPSSF) group by comparing with the uricase-deficient (Uox$^{-/-}$) group. The workflow for quantitative proteomic analysis of renal tissues (A). Summary of identified and quantified proteins. Forty-one proteins differently expressed in the JPSSF group compared with the Uox$^{-/-}$ group (Fold Change (FC) ≥1.2 or ≤0.83, $P \leq 0.05$) (B). Volcano map of differentially expressed proteins (DEPs) (C), red dots represent up-regulated proteins, green dots represent down-regulated proteins in Uox$^{-/-}$ vs JPSSF. Histogram of differentially expressed protein in gene ontology functional enrichment analysis of Biological Process (BP) and molecular function class (D, E).

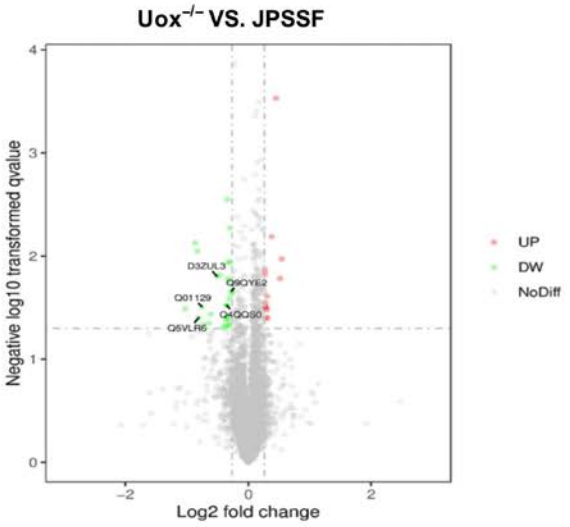


(A)

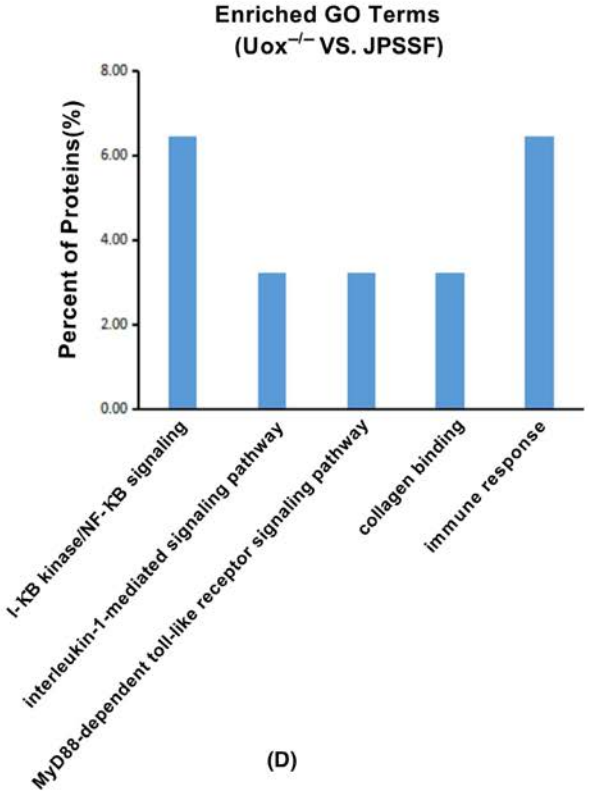


Differentially expressed proteins (FC ≥ 1.2 or ≤ 0.83 & $P \leq 0.05$) in Uox$^{-/-}$ vs. JPSSF

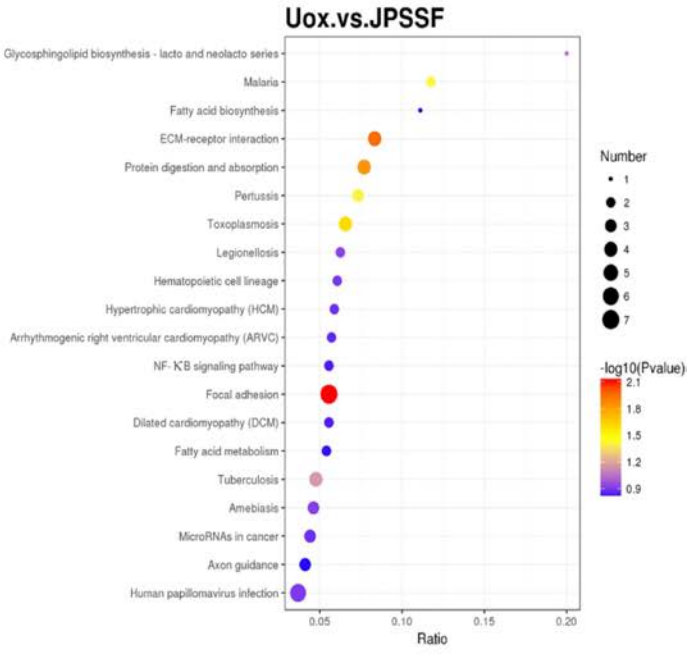
(B)



(C)



(D)



(E)

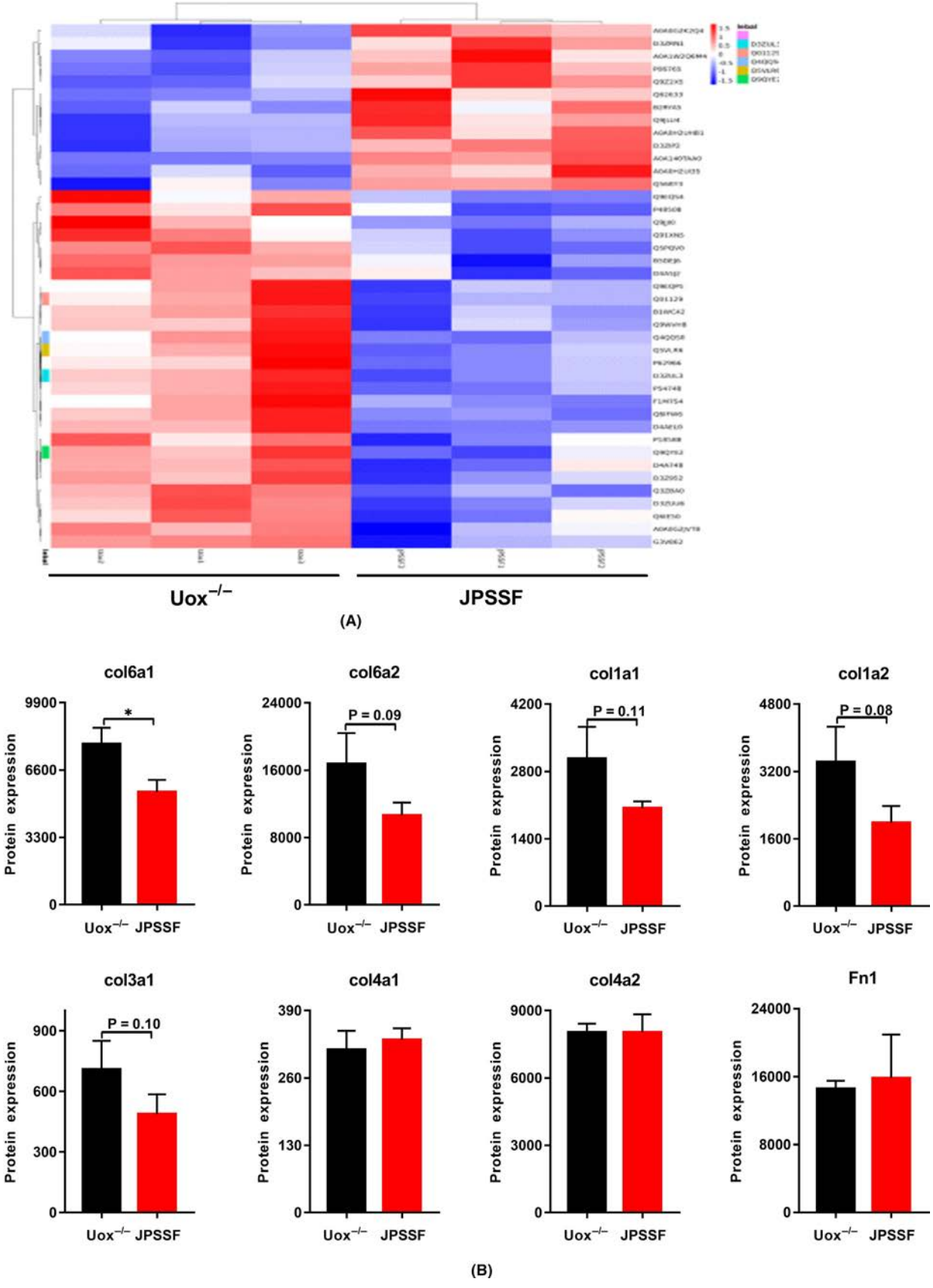


FIGURE 3 Visualization of differentially expressed proteins (DEPs). Cluster analysis of DEPs (A), red represents strong enrichment and blue represents weak enrichment. Fold Change (FC) ≥1.2 or ≤0.83, $P < 0.05$ were considered statistically significant. Statistical expression of DEPs associated with kidney fibrosis (mean ± standard error, n = 8. *$P < 0.05$, JPSSF group vs Uox$^{-/-}$ group, Student *t* test) (B).

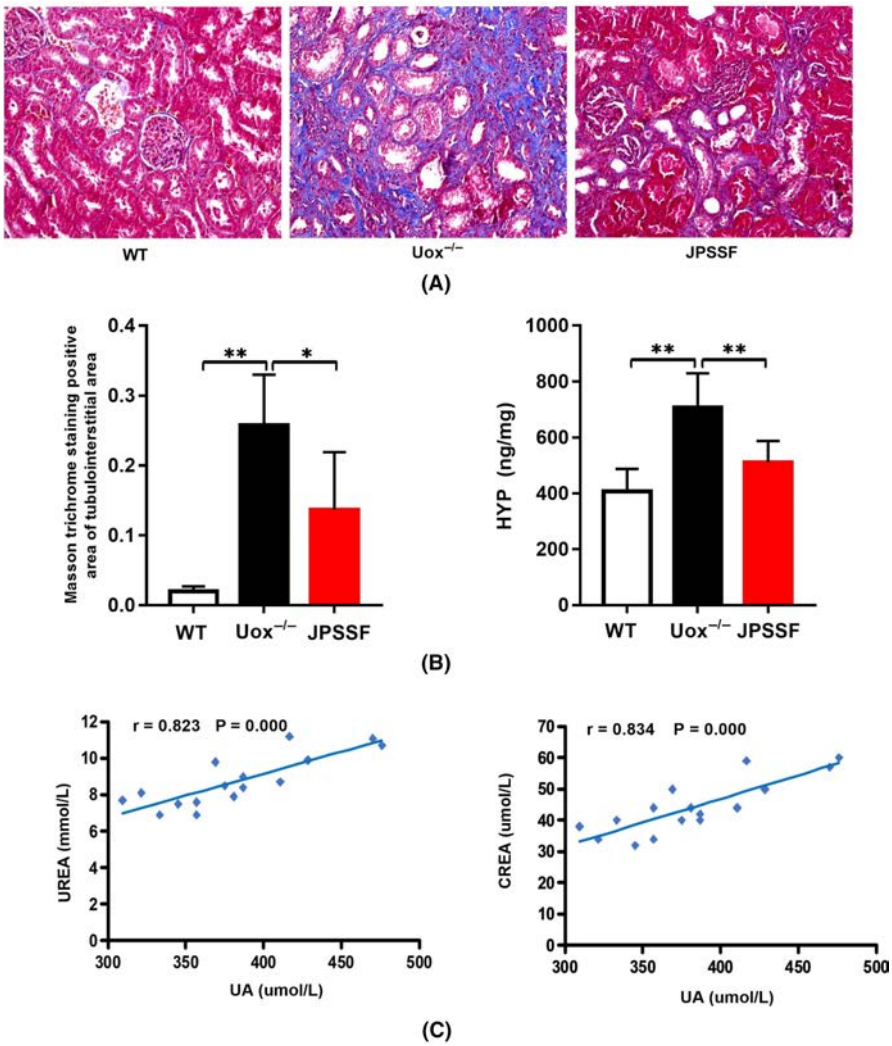


FIGURE 4 Jian Pi Shen Shi Formula (JPSSF) significantly reduced collagen deposition in renal lesions in uricase-deficient ($Uox^{-/-}$) rats. The renal lesions were subjected to Masson staining (200×) (A) and quantified by IMAGE PRO PLUS 6 (mean ± standard error [SE], n = 8; $^{*}P < 0.05$, $^{**}P < 0.01$, JPSSF vs $Uox^{-/-}$, Student *t* test) (B). The level of hydroxyproline (HYP) in kidney tissue were determined by HYP assay kit (mean ± SE, n = 8; $^{*}P < 0.05$, $^{**}P < 0.01$, JPSSF vs $Uox^{-/-}$, Student *t* test) (C). Correlation between UA and UREA (D) and CREA (E) in $Uox^{-/-}$ rats.

enzyme in transforming uric acid into more soluble chemicals during the process of purine metabolism.[18] Unfortunately, Uox is a pseudogene in humans and cannot be translated into uricase, making humans vulnerable to hyperuricemia and related diseases.[19] Naturally uricase-deficient mammals could be the ideal experimental animals for studying hyperuricemia-associated disorders; however, they are not commonly used in laboratories.

Scientists are trying to knock out or modify the murine *Uox* gene to obtain uricase-deficient rodents, but the homozygous mice die 11 weeks after birth as the result of renal failure, or display a low survival rate even when kept in sterile environments.[20] Recently, Duan and colleagues demonstrated a uricase-deficient model animal based on Sprague-Dawley rats using the CRISPR/Cas9 technique.[11] Their $Uox^{-/-}$ rats are apparently healthy and can survive for more than 1 year. In the current study, it was first found that uricase deficiency can cause obvious renal fibrosis that is characterized by collagen deposition in renal lesions, suggesting that $Uox^{-/-}$ rats are suitable for evaluating drugs against renal fibrosis induced by hyperuricemia. The rats can be used as model animals for studying hyperuricemia and associated diseases.

Gout is an inflammatory disease caused by monosodium urate deposition in the joints, kidneys, and other tissues, which always leads to serious complications. Patients with gout have a higher risk of cardiovascular disease, such as coronary artery disease, stroke, and heart failure, as well as metabolic syndrome, diabetes mellitus, and kidney disease compared with healthy individuals.[21] In a recent study, gout increased the risks of dementia (about 17%-20%),[22] so the 2021 Asia-Pacific League of Associations for Rheumatology clinical practice guideline recommended that treatment of gout should be holistic including urate-lowering therapy, appropriate lifestyle choices, and treatment of comorbidities. At present, evidence shows that uric acid-lowering therapy is effective in improving comorbidities. However, there is still insufficient evidence to support the therapy of gout with moderate to severe.[23] Thus, an increasing number of scholars have conducted relevant studies on Chinese Herbal Medicine therapy for gouty nephropathy. The results reveal that Chinese Herbal Medicine therapy may delay disease progression in gout patients with chronic kidney disease, and might be a promising safe therapy in treating gout.[24]

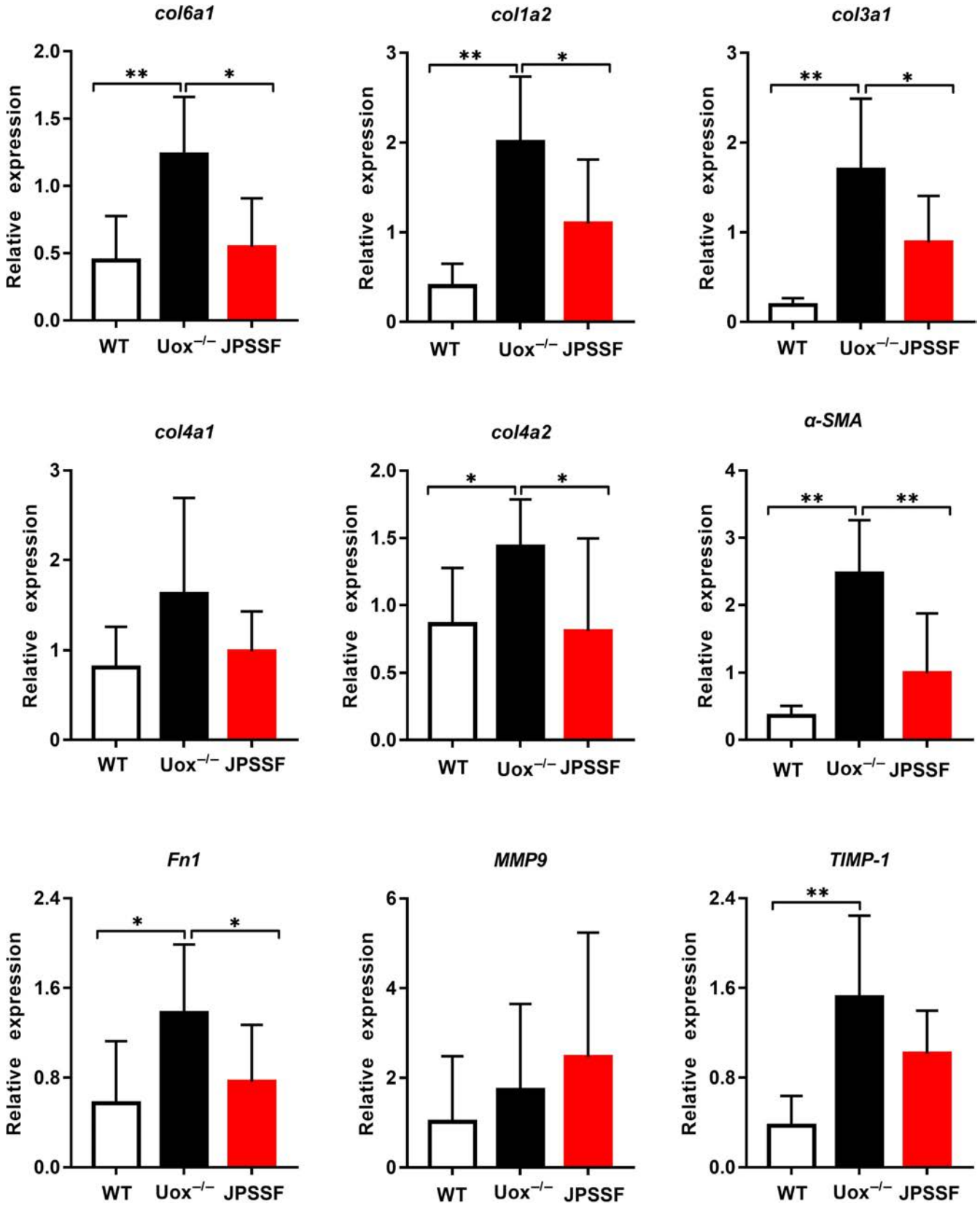


FIGURE 5 Jian Pi Shen Shi Formula (JPSSF) significantly suppressed the transcripts participating in collagen-binding-related pathways. The total RNA in the kidneys was isolated using RNeasy kits. Then, 1 μg of total RNA was used to synthesize its cDNA. The transcripts participating in collagen-binding-related pathways were detected using SYBR Premix Ex Taq II kits, and relative abundance of mRNA expression was calculated using the ΔΔ Ct value. GAPDH was a housekeeping gene, and was used as an internal reference. Results presented are mean ± standard error, n = 8; $^{*}P < 0.05$, $^{**}P < 0.01$, JPSSF vs uricase-deficient ($Uox^{-/-}$), analysis of variance.

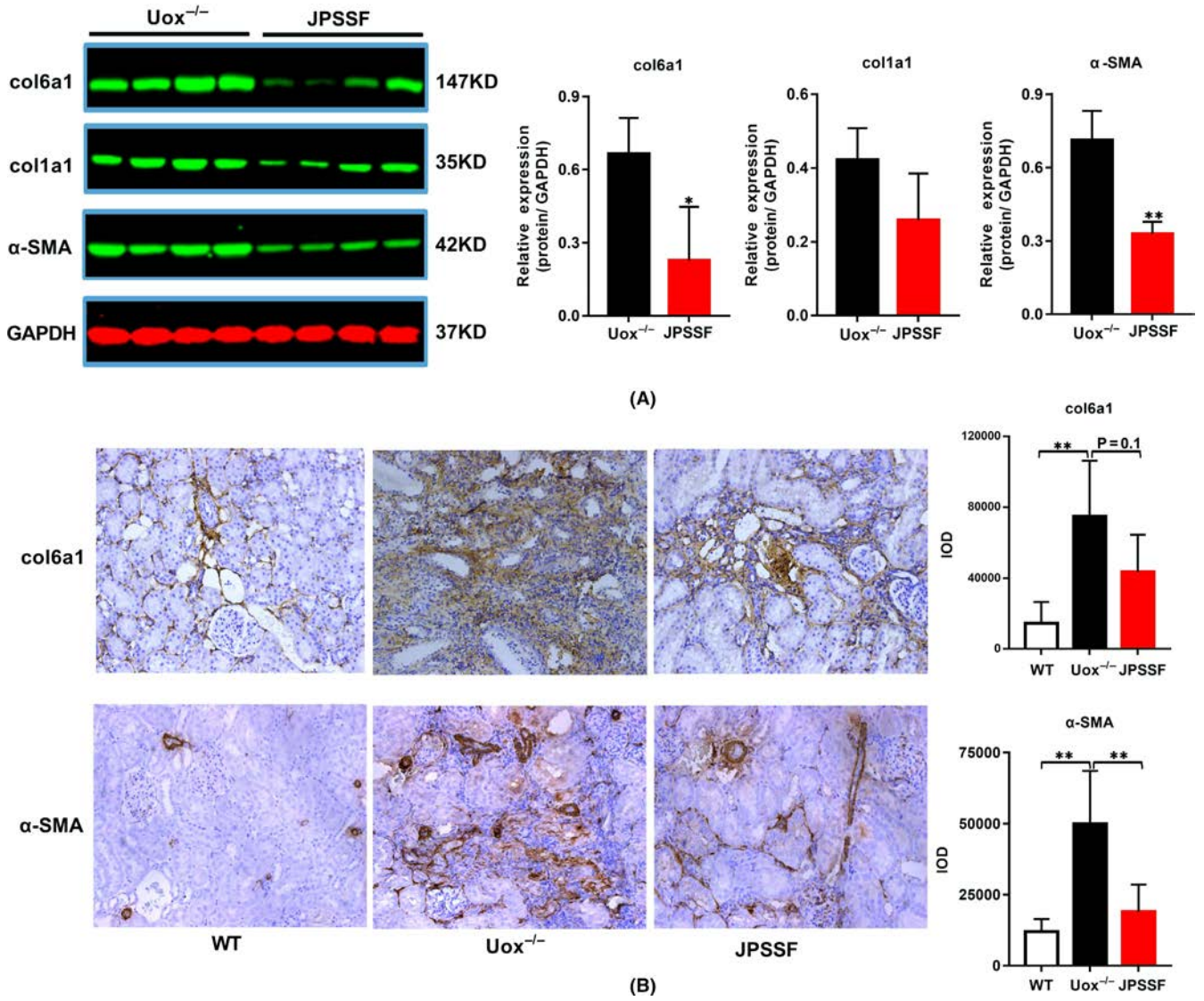


FIGURE 6 Jian Pi Shen Shi Formula (JPSSF) markedly down-regulated the proteins involved in renal fibrosis. Proteins in tissue extract were measured by Western blot, and the proteins of interest were detected using the indicated antibody for assessing collagen-binding-related pathways (A). Sections of renal tissue were subjected to immunohistochemical staining with specific antibodies for col6a1 and α-SMA, and quantified by IMAGE PRO PLUS 6 (B). Results presented are mean ± standard error, n = 8; $^{*}P < 0.05$, $^{**}P < 0.01$, JPSSF vs uricase-deficient ($Uox^{-/-}$), analysis of variance.

JPSSF is an empirical formula that was developed to treat hyperuricemia by the Wu School in Yunnan based on the theory of "Invigorating spleen and removing dampness, eliminating phlegm and freeing channels". Previous clinical and experimental studies have shown that JPSSF has displayed some promising therapeutic effects on hyperuricemia. In the present study, its therapeutic effects were confirmed in $Uox^{-/-}$ rats, in agreement with previous reports. Oral administration of JPSSF markedly improved the renal failure indices, including UA, UREA, and CREA, and alleviated hyperuricemic nephropathy, implying that JPSSF presents a promising therapy in treating hyperuricemia with renal dysfunction. However, the underlying mechanism has not been fully elucidated.

Based on previous reports, we further analyzed the differential expression in proteomics from the kidney using a TMT labeling quantitative proteomic approach. Proteomic analysis indicated that among the 6964 proteins identified, 41 were significantly changed in the JPSSF group compared with the $Uox^{-/-}$ group. In the molecular function class, these different proteins were mainly focused on collagen binding and inflammatory response, and these biologic processes are highly associated with renal fibrosis. These clues lead us to speculate the underlying mechanism of JPSSF in treating hyperuricemia-related renal fibrosis.

Renal fibrosis is characterized by massive activation and proliferation of renal fibroblasts, followed by an excessive accumulation and deposition of extracellular matrix components, resulting in structural damage and impairment of renal function. In a meta-analysis, gout was proven to be an independent risk factor for chronic kidney disease and nephrolithiasis. The hyperuricemia damages the

kidney mainly through urate crystal deposition and the soluble uric acid pathway, thereby accelerating the progression of chronic kidney disease.[25] Increasing preclinical and clinical evidence indicates that hyperuricemic nephropathy leads to glomerular hypertrophy and tubulointerstitial fibrosis.[7] Uric acid is not only correlated with clinical renal injury indices but also with renal pathology. Whereas, hyperuricemia has been reported to be an independent risk factor for segmental glomerulosclerosis, tubular atrophy, and interstitial fibrosis.[26]

In the present study, $Uox^{-/-}$ rats displayed typical pathophysiologic characteristics of renal fibrosis, including renal dysfunction and collagen protein deposition in renal interstitial tissues. Furthermore, the present study found that UA was positively correlated with UREA and CREA, suggesting that renal fibrosis caused by uricase deficiency was mediated via hyperuricemia. However, oral administration of JPSSF markedly attenuated renal fibrosis caused by hyperuricemia; the collagen-positive areas were decreased and the reduced HYP was the concrete proof.

Renal fibrosis may be induced by either overproduction of matrix components or defects in their degradation.[27] Therefore, the mRNA expression of antifibrotic cytokines and activation of profibrotic cytokines were further investigated. Interestingly, JPSSF blocked the transcriptional expression involved in collagen-binding-related signaling pathways, including col6a1, col1a2, col3a1, col4a2, α-SMA, and Fn1.

The extracellular matrix is thought to be produced primarily by α-SMA positive fibroblasts, and the fibroblasts can also produce collagen. Its isotype types, including collagens I, III, and VI, are closely associated with organ fibrosis.[16] The present study revealed that oral administration of JPSSF dramatically down-regulated the protein expression of col6a1 and α-SMA in $Uox^{-/-}$ rats. Furthermore, immunohistochemistry results demonstrated the down-regulation of col6a1 and α-SMA in renal tissues at protein level, suggesting that JPSSF ameliorates renal fibrosis caused by hyperuricemia by inhibiting collagen-binding signaling pathways.

In summary, this study demonstrated that JPSSF attenuated hyperuricemic nephropathy in $Uox^{-/-}$ rats, the mechanisms of which are associated with uric acid lowering and down-regulation of collagen-binding signaling pathways. All the results implied that JPSSF is suitable for treating hyperuricemia with renal fibrosis.

ACKNOWLEDGEMENTS

Funding was provided by: Yunnan Provincial Department of Education Teacher Science and Technology Project under Grant Agreement (2021Y463), Construction Project of National Traditional Chinese Medicine Clinical Research Base (2018 No. 131), The "Chinese Medicine Modernization Research" key project of China's National Key R&D Programmes (2017YFC1704005), and Yunnan Provincial Fund for Medical Research Center: Clinical Evaluation and Basic Research on the Treatment of rheumatoid arthritis and gout by Traditional Chinese medicine (202102AA310006).

CONFLICTS OF INTEREST

The authors disclose no conflict of interests regarding this study.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author on reasonable request.

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How to cite this article: Yin N, Li X, Liu W, et al. Jian Pi Shen Shi formula alleviates hyperuricemia and related renal fibrosis in uricase-deficient rats via suppression of the collagen-binding pathway. *Int J Rheum Dis*. 2022;25:1395-1407. doi: 10.1111/1756-185X.14434

Received: 21 June 2022 | Revised: 12 August 2022 | Accepted: 25 August 2022

DOI: 10.1111/1756-185X.14435

International Journal of Rheumatic Diseases WILEY

ORIGINAL ARTICLE

Determinants of quality of life and hand function among people with hand osteoarthritis

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Funding information
Lincoln Centre for Research Into Bone and Joint Diseases; NHMRC Program Grant (APP1091302)

Abstract

Objective: The objectives of this study are to ascertain the determinants of quality of life (QoL) and hand function among persons with hand osteoarthritis (OA) and to assess the influence of hand function on QoL among persons with OA.

Methodology: Two hundred and four participants in a clinical trial completed the baseline assessment. Demographic, socioeconomic, QoL (AqoL-4D), hand function (Functional Index for Hand Osteoarthritis, FIHOA), pain assessment, radiographic and clinical characteristics of participants were measured using standard methods. Univariate and multivariate analyses were performed to evaluate potential associations.

Results: We studied 204 participants (76% female, age 65.63 ± 8.13 years, body mass index 28.7 ± 6.5 kg/m^2) with hand OA. The mean pain score of the participants on a visual analog scale was 57.8 (*SD* ±13.6). There was a significant, negative moderate correlation between hand function and QoL scores except for the sense domain score. Global assessment, household income and serious illness were associated with QoL ($P < .001$) and explained 18% of the variance of the QoL. Pain scale, Patient Global Assessment, Mental Health Score, grip strength and cyst index were associated with hand function score and explained 26% of the variance of hand function.

Conclusion: The results indicate increasing impairment in hand function decreases the QoL of persons with hand OA. Some determinants were significantly associated with hand function and QoL. Determinants related to hand functions may be modifiable. In future, appropriate intervention strategies should be implemented, and further studies should be conducted to identify the effectiveness of those interventions.

KEYWORDS
determinants, hand function, hand osteoarthritis, quality of life

1 | INTRODUCTION

Osteoarthritis (OA) is a chronic condition which leads to pain and disability.[1] It is one of the most common joint disorders[2] and affects the hips, knees, hands and spine.[3] It is characterized by progressive loss of cartilage in association with remodeling and sclerosis of the subchondral bone,[4] inflammation, progressive joint failure, and loss of mobility and function.[5] Adults of all ages with OA have poorer quality of life (QoL) compared to non-OA adults.[6,7] OA in the hand joint is one of the most prevalent joints compared to other joints.[8] Pain, loss of range of motion (ROM), joint stiffness, reduced grip strength, impaired hand function, and difficulty performing activities

of daily living are commonly seen among people with hand OA.[8,9] Studies reported that people with OA have a higher level of hand pain, tenderness, number of nodes, lower grip and pinch strength.[10]

QoL is defined as an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns.[11] OA has a significant impact on patients' daily activities[12] and it is reported that people with hand OA have a clinically relevant lower physical health-related QoL[13] and significant impact on hand function.[14]

Previous studies have evaluated the QoL in patients with OA in different joints[11,13] and how hand OA affects their hand functions.[14] Studies have reported that pain, body weight, mental health education,[15] and functional independence affect QoL. One study's results indicated that psychological factors are strongly related to pain level.[16] Pain contributes to functional limitations and reduced QoL.[17] Physical function, bodily pain, and mental health are strongly associated with functional independence.[18]

Also, it is reported that women and older people, people with less than 12 years of education, those out of work, and those overweight have potentially higher prevalence of OA.[8] However, the determinants of QoL and hand function among people with hand OA are not well represented in the literature.[19,20] Many factors influence QoL and hand function, such as pain, joint stiffness and joint cracking during movement.[17] In this study, we assessed most of the factors such as weight, patient global assessment, pain level, grip and pinch strength, presence of joint swelling and tenderness, ROM, and duration of joint stiffness, reported in the literature to determine the factors that influence hand function and QoL of patients with hand OA. This study aims to reveal the determinants of QoL and hand function among persons with hand OA and how hand function influences QoL among persons with hand OA.

These factors should be identified to better manage patients with hand OA. The findings will be helpful in designing and initiating an effective method to improve QoL and hand function of patients with hand OA. This will help healthcare professionals implement the most effective care for patients with hand OA.

Practitioner points

- Modifiable determinants have been shown to contribute to hand function in persons with hand OA.
- Interventions should be implemented to enhance mental health scores and pain levels among persons with hand OA.

2 | MATERIALS AND METHODS

This is a cross-sectional study. This analysis draws from baseline data from a published clinical trial. The COMBO trial was a randomized, controlled, assessor and statistician blinded, parallel, 2-arm superiority trial with a 1:1 allocation ratio.[21,22] The trial was conducted at a single center in Australia. The protocol was designed in accordance with the principles of the Declaration of Helsinki. Ethics approval was obtained from the local ethics committee of Royal North Shore Hospital (HREC/15/HAWKE/479). The study procedures, response rates, and measures are explained in a previous article.[22] Baseline assessments were completed by 204 individuals with thumb OA, who also fulfilled the following eligibility criteria: age ≥40 years; pain at the base of the thumb at least half of the days in the past month; average pain ≥40 on a 100 mm visual analog scale (VAS), over the past 30 days and in the 48 hours before the screening visit; scores ≥6 on the Functional Index for Hand Osteoarthritis (FIHOA, range 0–30) and radiographic evidence of thumb base OA read by a trained rheumatologist (Kellgren-Lawrence grade [KLG] ≥2). Participants were excluded if they: were diagnosed with crystal-related, autoimmune arthritis, hemochromatosis or fibromyalgia; had hand surgery in the last 6 months or planning to undergo surgery in the next 6 months; used concomitant medications potentially directed at OA, unless at a stable dosage for at least 1 month for analgesics and nonsteroidal anti-inflammatory drugs or 3 months for slow-acting symptomatic or structure-modifying drugs; had intra-articular hyaluronic acid injection in the affected joint in the past 6 months; had intra-articular steroid injection in the affected joint in the past month; had significant injury to the affected joint in the past 6 months; had any other self-reported hand condition that is likely to be contributing to the pain at the base of the thumb; had poor general health likely to interfere with compliance or assessments, judged by the investigator; had known hypersensitivity to diclofenac; had current history of advanced renal failure; had past or current history of gastrointestinal ulceration, bleeding and/or perforation; were women who were pregnant or breastfeeding.[21,22] The data from the baseline assessment were used for this study.

2.1 | Measures

Demographic, socioeconomic, pain assessment, radiographic and clinical characteristics of participants were also measured. Demographic data such as age, date of birth, gender, ethnicity, financial status, marital status, symptom duration, height, weight and OA at other joints (such as knee or hip), and the duration of stiffness were collected during the screening visit. Presence of synovitis and other structural features, including osteophytes, cartilage damage, erosions and stability of the first carpometacarpal joint, assessment of tenderness and swelling were assessed during the baseline assessment using radiographs.[22]

2.2 | Dependent variables

2.2.1 | Health-related QoL

Health-related QoL was assessed using the Assessment of Quality of Life—4D instrument (AqoL-4D), a 12-item tool, with 3 items

per dimension, good validity and reliability, including questions related to independent living, mental health, relationship and senses. It was used as an interviewer-administrated questionnaire. Scores for each dimension and overall utility score can be obtained. The scores across the scales are combined using a multiplication scoring procedure. Algorithms were used to calculate scores. It is scored from −0.04 to 1.00, with 1.00 indicating full health.[23,24]

2.2.2 | Hand function

Hand function was assessed using the FIHOA tool, composed of 10 items scored using a semi-quantitative 4-point scale.[25] It is a self-reported questionnaire evaluating the functional performance of 10 distinct activities involving the hand that has demonstrated good measurement properties, including reliability, feasibility and sensitivity to change.[25] The 0 score indicates no functional impairment and the score of 30 points indicates maximal impairment.

2.2.3 | Independent variables

The independent variables were identified such as age, gender, financial status, current or past activities involving intensive use of the hands, weight, primary occupation, pain level (VAS, 0–100 mm), grip and pinch strength (kg), presence of joint swelling and tenderness (present/absent), duration of joint stiffness (minutes), patient's global assessment (5-point Likert scale) and use of rescue medication (yes/no), ROM of the first metacarpophalangeal (MCP) joint (degree), thumb base OA severity (0–IV), presence of synovitis (present/absent) and other structural features (present/absent). The previously published protocol outlines data collection for the abovementioned variables.[22]

2.3 | Statistical analysis

Data were analyzed using SPSS 26.0 version (Statistical Package for the Social Sciences), and a descriptive analysis was performed to understand the characteristics of the study sample. Data were summarized using means, standard deviations (*SD*) and range; frequency tables and percentages were provided for categorical variables. Univariate analyses were performed to test the significance of the association of each predictor using Pearson's correlation and linear regression (continuous), *t*-test (2 groups) and analysis of variance (categorical with 3 or more levels) using Bonferroni's test for post-hoc pairwise comparisons. The significant predictors identified in the analysis were used in multivariate regression analysis. The forward selection method was used to predict QoL and hand function determinants. $P < .05$ was considered as a level of significance.

3 | RESULTS

3.1 | Participants' characteristics

Overall, 204 participants with hand OA were included in the study. The participants' characteristics are described in Table 1. Participants had a mean age of 65.6 years (*SD* ±8.1, range 44–86). The cohort included 76% females and 35% of the participants were never married, divorced, widowed or separated. The mean body mass index (BMI) was 28.7 (*SD* ±6.5) kg/m^2. About 40% of the participants had swelling, and most had tenderness on palpation, assessed by joint examination. Half of the participants (49%) indicated serious illness in the past 5 years. The mean pain score of the participants on VAS was 57.8 (*SD* ±13.6). There were 84.3% who reported OA in other joints. According to the KLG, all participants had score ≥2 for index.

3.2 | Dependent variables

Scores of the dependent variables, QoL (AqoL-4D) and hand function (FIHOA), are presented in Table 2. Regarding the assessment of QoL, the mean total score was 84.3 (*SD* ±9.5). The independent living score was 89.4 (*SD* ±12.4). The hand function assessment mean score was 10.7 (*SD* ±4.0).

3.3 | Relationship between hand function and QoL

There was a significant correlation between hand function and QoL score except for the senses domain of Aqol-4D (Table 3).

3.4 | Independent variables

Statistical analysis was performed to identify significant independent variables that influenced the QoL (AqoL-4D) and hand function (FIHOA) among patients with hand OA (Figure S1). According to the statistical analysis, the QoL showed a significant difference between marital status, financial status, weight, BMI, pain level, patient global assessment (PGA), swelling, grip strength, and extension ROM. Hand function showed a significant difference between gender, marital status, financial status, height, BMI, pain level, PGA, swelling, tenderness, grip strength, tip strength, duration of stiffness, extension ROM, KLG index, cyst, erosion and sclerosis. These results were used for multivariate analysis.

3.5 | Multivariate regression analysis

Results of multivariate analysis on the QoL and hand function are presented in Tables 4 and 5. The forward selection algorithm was used to develop a parsimonious form of the predictive model of QoL.

TABLE 1 Demographic details and characteristics of the participants with hand OA

	n	(%)	Mean	(*SD*)
Demographic details				
Gender				
Female	155	(76.0)		
Age, y			65.6	(8.1)
BMI, kg/m^2			28.7	(6.5)
Household income per wk and per mo				
Negative or nil income	9	(4.4)		
$1–$3999 ($1–$207 999)	176	(86.3)		
>$4000 (>$208 000)	12	(5.9)		
Marital status				
Divorced, widowed, separated, never married	71	(34.8)		
Married	133	(65.2)		
Cause OA				
No trauma	174	(85.3)		
Trauma	30	(14.7)		
OA in other joints				
Yes	172	(84.3)		
No	32	(15.7)		
Clinical characteristics				
Swelling				
No	122	(59.8)		
Yes	82	(40.2)		
Tenderness				
No	42	(20.6)		
Yes	162	(79.4)		
Good health				
No	15	(7.4)		
Yes	189	(92.7)		
Health change past y				
No	151	(74.0)		
Yes	53	(26.0)		
Serious illness past 5 y				
No	104	(51.0)		
Yes	100	(49.0)		
Kellgren-Lawrence system				
Index				
Score ≥2	204	(100.0)		
Non-index				
Score ≥2	147	(72.0)		
1st MCP index				
Score ≥2	14	(6.8)		
1st IP index				
Score ≥2	78	(38.3)		
Pain scale (0–100)			57.8	(13.6)
Global assessment			39.5	(21.8)

(Continues)

TABLE 1 (Continued)

	n	(%)	Mean	(*SD*)
Grip strength			23.55	(9.3)
Kellgren-Lawrence grading system for CMC joint (index)			2.8	(0.7)
Flexion of the 1st MCP			50.2	(13.3)
Extension of the 1st MCP			−23.6	(17.9)
Osteoarthritis index			1.7	(0.9)
Joint space narrowing			1.3	(1.1)
Erosion index			0.01	(0.1)
Sclerosis index			0.7	(0.5)
Cyst index			0.4	(0.5)

Note: Mean and *SD* are presented for continuous variables, frequency, percentage are shown for categorical variables.
Abbreviations: BMI, body mass index; CMC, carpometacarpal; IP, interphalangeal; MCP, metacarpophalangeal; OA, osteoarthritis.

TABLE 2 Quality of life scores (AqoL-4D) and hand function scores (FIHOA) of participants

Dependent variables	Mean	(*SD*)	Range
FIHOA	10.7	(4.0)	5–26
Aqol-4D	89.4	(12.4)	33–100
Independent living score			
Relationship score	88.8	(14.3)	33–100
Senses	89.7	(10.5)	44–100
Mental health score	69.2	(16.0)	33–100
Total score	84.3	(9.5)	47–100

Abbreviations: Aqol-4D, Assessment of Quality of Life-4D; a higher score indicates better quality of life; FIHOA, Functional Index for Hand Osteoarthritis, 0 (no functional impairment) to 30 points (maximal impairment).

3.6 | QoL and hand function

Global assessment, household income and serious illness were associated with QoL ($P < .001$) and explained 18% of the variation in the QoL ($R = 0.445$). It indicates decreasing PGA score (0.274), and serious illness (0.186) increasing the QoL.

Pain scale, global assessment, Mental Health Score, grip symptoms and cysts were associated with hand function score and explained 26.3% of the variation of hand function ($R = 0.538$). It indicates increasing the global assessment score (0.195), and pain score (0.209) increases the hand function impairment score; whereas decreasing grip strength (0.197) and mental health score (0.197) increases the hand function impairment score.

4 | DISCUSSION

4.1 | Summary of the study findings

The study was conducted to identify the determinants of QoL and hand function among patients with hand OA. PGA, household income and serious illness were associated with QoL and pain scale, PGA, Mental Health Score, grip symptoms and cysts were associated with hand function score.

In this study, PGA, household income and serious illness were associated with QoL. A study conducted to determine the determinants of health-related QoL among patients with knee OA indicated that Higher Fear Avoidance Belief Questionnaire Physical Activity Scale (FABQ-PA), higher psychological distress and greater comorbidities on the Charlson Comorbidity Index (CCI) were associated with worse health-related QoL.[2] Another study conducted in Japan indicates that knee extension muscle strength on the unaffected side and hip flexion ROM on the affected side were associated with health-related QoL of patients with hip OA.[26] Some of the findings of this study are similar to other studies conducted on OA. According to a prior research on QoL among patients with knee OA, most demographic factors and BMI were not associated with QoL.[2] However, in this study, although BMI was not associated with QoL of patients with hand OA, household income impacts QoL of patients with hand OA. These indicate that the QoL of different joints depends on a range of different factors.

Determinants of hand function in this study indicate that increasing pain level and score in the PGA were associated with increased impairment in hand function. However, the decreasing mental health score increases hand function impairments. PGA is associated with psychological distress, comorbidities, and other health aspects.[27] The study on QoL among knee OA patients is similar to the hand function findings of this study, especially on psychological distress, fear avoidance belief,[2] which indicated that psychological distress is associated with worse physical performance. In this study, decreasing mental health score increases hand function impairment. However, according to a study conducted in 2005, anxiety and depression did not associate with physical performance,[28] which contradicts the present study's findings.

According to this study, grip strength is associated with the hand function of a patient with OA. A similar finding was reported among patients with rheumatoid arthritis, where grip and pinch strength were significantly associated with hand function[18] and a decrease in grip strength is a strong indicator of functional disability in patients with rheumatoid arthritis.[19] Another study reported that weak grip

TABLE 3 Correlation between hand function and quality of life

	Quality of life (AqoL-4D)				
Hand function score (FIHOA)	**Independent living score**	**Relationship score**	**Senses**	**Mental health score**	**Total score**
Pearson correlation	−0.269	−0.208	0.066	−0.367	−0.304
Sig. (*P*)	*P* < .001	.003	.352	*P* < .001	*P* < .001

Note: Correlation is significant at *P* < .05.

Hand function score: FIHOA, FIHOA, Functional Index for Hand Osteoarthritis, 0 (no functional impairment) to 30 points (maximal impairment); Aqol-4D, Assessment of Quality of Life-4D; a higher score indicates better quality of life.

TABLE 4 Association between participants' characteristics and quality of life

	Ɓ[a]	*SE*	Ɓ[b]	*t*	*P*	95% CI
Independent variables	91.818	1.271				
(Constant)				72.259	<.0001	
PGA	−0.098	0.027	−0.274	−3.592	<.0001	−0.153 to −0.044
Household income	−5.565	1.615	−0.262	−3.447	.001	−8.757 to −2.372
Serious illness	−2.894	1.191	−0.186	−2.43	.016	−5.248 to −0.539

Note: Dependent variable: total score.

$R = 0.445$, $R^2 = 0.198$, Adjusted R^2 0.18.

Visual analog scale 0–100 mm, where 0 is very well and 100 is very poor.

Abbreviations: PGA, patient global assessment.

[a]Unstandardized coefficients.

[b]Standardized coefficients.

TABLE 5 Association between participants' characteristics and hand function

Independent variables	Ɓ[a]	*SE*	Ɓ[b]	*t*	*P*	95% CI
(Constant)	10.197	2.246		4.541	<.001	5.756 to 14.637
Pain scale	0.062	0.023	0.209	2.671	.008	0.016 to 0.108
PGA	0.035	0.014	0.195	2.551	.012	0.008 to 0.062
Grip strength	−0.083	0.031	−0.197	−2.642	.009	−0.145 to −0.021
Mental health score	−0.051	0.02	−0.197	−2.548	.012	−0.091 to −0.011
Cyst index	1.377	0.578	0.175	2.383	.019	0.234 to 2.520

Note: Dependent variable: hand function score.

$R = 0.538$, $R^2 = 0.289$, adjusted $R^2 = 0.26.3$ Hand function score, FIHOA, Functional Index for Hand Osteoarthritis, 0 (no functional impairment) to 30 points (maximal impairment).

Visual analog scale 0–100 mm, where 0 is very well and 100 is very poor.

Abbreviations: PGA, patient global assessment.

[a]Unstandardized coefficients.

[b]Standardized coefficients.

strength is related to impaired hand dimensions of mobility, self-care, and usual activity in a patient with arthritis.[20] These findings indicate that an exercise protocol focusing on grip strength would benefit patients with OA.

Pain, PGA, grip strength, mental health score and cyst index explain only 53.8% of factors that influence hand function in a patient with hand OA. Other factors influencing hand function need to be analyzed (46%). According to this study results, pain, mental health, and PGA, which have a psychological component, influence hand function. A study has mentioned that pain level is associated with psychological factors.[16] Remarkable functional limitations are associated with pain among patients with knee OA. Similarly, the pain scale explains a higher percentage (Ɓ = .209) of hand function than other identified factors among patients with hand OA.

PGA, household income and serious illness explain only 19% of factors that influence QoL in a patient with hand OA. However, other factors which explain 81% of factors should be studied. In this study, it is noted that household income has an impact on QoL. Household income can be related to the workplace environment. Another study conducted among people with OA revealed that individuals

employed in workplaces offering better policies had significantly fewer symptoms.[29] This should be focused on in future studies.

5 | CLINICAL IMPLICATIONS

In this study, determinants of QoL among patients with hand OA are non-modifiable except for household income. However, determinants of hand function are modifiable such as pain scale and mental health score. The results suggest that the hand function of individuals with hand OA could be enhanced by some interventions such as pain coping skills and mental health-related interventions. Cognitive and behavioral techniques can improve pain coping skills.[30] Previous studies have shown a positive impact of pain coping skills in movement/performance-related outcomes among people with different conditions causing pain.[31,32]

This study indicated that having strategies to improve the pain and mental health score would improve the hand function of patients with OA. It also suggests that improving hand function will positively impact the QoL of patients with hand OA.

This is conducted among people with a mean age of 65.6 (±8.1) years. QoL and hand function of patients with hand OA were assessed. Further, in this study, BMI is considered an independent variable to determine QoL and hand function. This study's results indicate that BMI does not influence the QoL of patients with hand OA. However, BMI could influence the QoL of people with other joint OA. Most of the study participants had reported OA in other joints as well. Therefore, it is difficult to clearly differentiate the impact of hand OA on QoL. There is a potential for the influence of other joints on QoL.

The analysis to determine the association between hand function and QoL indicates decreasing impairment in hand function will improve the independent living score, relationship score, mental health score and total score of QoL of the participants. This is a cross-sectional study. The association could be clearly explained using longitudinal data. Therefore, future studies should determine the association between hand function and QoL of patients with hand OA.

5.1 | Strengths and limitations

The determinants of QoL and hand function among people with hand OA were analyzed in this study. Validated methods were used in the data collection. There were 204 participants included in this study. Different potential independent variables were assessed to ascertain the determinants of the hand function and QoL of patients with hand OA.

The FIHOA questionnaire was used for the hand function assessment. Although it was a recommended tool during the data collection period, it is outdated and does not assess the impact of the disease on function when the non-dominant hand is the index hand. In future studies, tools with greater sensitivity should be used for data collection. These results have been demonstrated in a well-educated, predominantly Caucasian study population and our findings may not necessarily be generalizable to other communities.

6 | CONCLUSIONS

The results show that PGA, household income and serious illness were associated with QoL; pain scale, PGA, mental health score, grip and cysts were associated with hand function score. The hand function findings suggest that some of the identified determinants may be modifiable, more research is needed to evaluate strategies such as pain coping skills, behavioral techniques to improve pain and mental health scores to improve the hand function of patients with hand OA.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Deveza, Robbins and Cinthuja had access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design: Deveza, Robbins, Duong, Cinthuja, Venkat, Hunter. Acquisition of data: Deveza, Robbins, Duong, Hunter. Analysis and interpretation of data: Cinthuja, Venkat, Deveza, Robbins, Duong, Hunter.

FUNDING INFORMATION

This work was supported by an National Health and Medical Research Council (NHMRC) Program Grant (APP1091302) and the Lincoln Centre for Research Into Bone and Joint Diseases. Dr Hunter is supported by an NHMRC Investigator Grant. Role of the funder/sponsor: the funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

CONFLICT OF INTEREST

Dr. Hunter provides consulting advice on scientific advisory boards for Pfizer, Lilly, TLCBio, Novartis, Tissuegene, Biobone. Other authors declare no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Pathmanathan C, Deveza LA, Robbins SR, Duong V, Venkatesha V, Hunter DJ. Determinants of quality of life and hand function among people with hand osteoarthritis. *Int J Rheum Dis*. 2022;25:1408-1415. doi: 10.1111/1756-185X.14435

Received: 14 June 2022 | Revised: 2 August 2022 | Accepted: 30 August 2022

DOI: 10.1111/1756-185X.14436

International Journal of Rheumatic Diseases WILEY

ORIGINAL ARTICLE

Lin28A alleviates ovariectomy-induced osteoporosis through activation of the AMP-activated protein kinase pathway in rats

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Abstract

Aim: To investigate the role of Lin28A in ovariectomy-induced osteoporosis and to elucidate the underlying molecular mechanism.

Methods: Bilateral ovariectomy was conducted to generate an ovariectomy (OVX) rat model. Western blotting was performed to assess the relative expression levels of Lin28A, osteocalcin (OCN), runt-related transcription factor 2 (RUNX2), adenosine monophosphate-activated protein kinase (AMPK) and phosphorylated AMPK (p-AMPK) proteins. Enzyme-linked immunosorbent assays were performed to detect the serum levels of calcium, E2, alkaline phosphatase (ALP) and interleukin (IL)-1β. Three-point bending test was used to assess biomechanical parameters of left femoral diaphysis. Hematoxylin and eosin (HE) staining was conducted to detect the trabecular structure of bone tissue. Dihydroethidium assay kit was used to measure the intracellular reactive oxygen species (ROS) level in osteoclasts. Alizarin red staining revealed the calcium deposit in bone marrow stromal cells (BMSC).

Results: The expression levels of Lin28A, OCN, RUNX2, AMPK and p-AMPK proteins were significantly decreased in OVX rats. The serum levels of calcium, E2, ALP and IL-1β were significantly declined in OVX rats. Biomechanical parameters of left femoral diaphysis were significantly decreased in OVX rats. OVX-induced trabecular abnormalities. ROS level was dramatically increased in the bone tissue of OVX rats, and calcium deposit was dramatically decreased in BMSC cells of OVX rats. These effects induced by OVX could be prevented by overexpression of Lin28A.

Conclusion: Lin28A alleviates ovariectomy-induced osteoporosis through activation of AMPK pathway in rats.

KEYWORDS
AMPK pathway, Lin28A, osteoporosis, ovariectomy

1 | INTRODUCTION

Osteoporosis is a bone disease characterized by decline of bone mass and deterioration of bone structure, eventually resulting in fragile bones and increased fracture risk.[1-6] Osteoporosis causes an imbalance of bone homeostasis, leading to abnormal functions of osteoblasts, which is the primary cause of osteoporosis.[3] Differentiation and mineralization of osteoblasts are critical for bone formation, meanwhile, differentiation of osteoclasts participates in bone resorption.[3] Among postmenopausal women, osteoporosis is easily induced by estrogen deficiency because of a decline of bone mass caused by excessive activity of osteoclasts.[1-5]

Therefore, prevention of bone resorption induced by osteoclasts is one of the key strategies for osteoporosis, and to elucidate the molecular mechanisms of osteoporosis is important.

Autophagy regulates bone differentiation and contributes to osteogenesis. Activation of the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway attenuates mitochondrial dysfunction and sepsis-induced injury to promote mitochondrial autophagy.[7] AMPK favors metabolic reprogramming under cellular oxidative stress. Upregulation of AMPK inhibits generation of reactive oxygen species (ROS), thereby promoting osteogenic differentiation.[2,8,9] The AMPK signaling pathway has been verified to mediate differentiation and mineralization of osteoblastic cells stimulated by autophagy.[2]

Lin28 was first discovered in *Caenorhabditis elegans*, and mammalian Lin28 exists as 2 conserved paralogs, Lin28A and Lin28B. Lin28A encodes a conserved RNA-binding protein, which is involved in regulating stem cell activity including self-renewal and differentiation.[10-12] Lin28 regulates messenger RNAs (mRNAs) and controls the progression of cell fate.[10] Lin28A enhances osteoblast differentiation and mitochondrial activity in human periosteum-derived cells.[11] Taurine upregulated gene 1 (TUG1) is upregulated in osteogenically induced periodontal ligament stem cells, and Lin28A is downregulated in TUG1 knockdown cells.[12] Lin28A increases the expression levels of several osteogenic genes and facilitates osteogenic differentiation.[12] Lin28A contains multiple binding sites for TUG1, and TUG1 promotes osteogenic differentiation by interaction with Lin28A.[12] Upregulation of Lin28A ameliorates ischemia–reperfusion induced injury and promotes nerve cell autophagy.[13] Lin28A promotes autophagy by upregulation of SIRT3 expression level, and activation of AMPK-mammalian target of rapamycin (mTOR) signaling pathway.[13] However, the role of Lin28A in osteoporosis is poorly understood. This present work revealed that Lin28A could alleviate osteoporosis by activation of the AMPK signaling pathway, providing a therapeutic target for osteoporosis.

2 | MATERIALS AND METHODS

2.1 | Rats and antibodies

Adult male Sprague Dawley (SD) rats were supplied by Beijing HFK Bioscience Co. Ltd. The protocol for animal care and use of laboratory animals was conducted in accordance with National Institutes of Health Guide for Care and Use of Laboratory Animals. Ethical approval was obtained from the Ethics Committee of Jiu Jiang NO.1 People's Hospital. Primary antibodies against osteocalcin (ab93876), runt-related transcription factor 2 (RUNX2) (ab76956), AMPK (ab207442), phosphorylated AMPK (p-AMPK) (ab133448) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (ab8245) were purchased from Abcam. The working solution of these antibodies was diluted by phosphate-buffered saline (PBS) with Tween 20 at a ratio of 1:1000.

2.2 | Ovariectomy (OVX) rat model

SD rats were randomly divided into 4 groups: Sham group, OVX group, OVX+vector group and OVX+OE (overexpression)-Lin28A group. There were 6 rats in each group. The rats were anesthetized, and the OVX rat model underwent bilateral ovariectomy while the sham control rats underwent sham operation.[14,15] Empty vector or a Lin28A complementary DNA (cDNA) with the cytomegalovirus promoter was microinjected into SD rat fertilized eggs, which were then transferred to pseudopregnant females. Those offspring were used for OVX+vector group and OVX+OE-Lin28A group. All rats were finally sacrificed by cervical dislocation.

2.3 | Three-point bending

Three-point bending test was performed as described.[16] Briefly, a phosphate buffer was used to soak the bones for 24 hours. Force was applied to the fixed bones perpendicular to the midpoint using a flat-tipped wedge. A transbridge amplifier was used to amplify the force. WinDaq data acquisition software was used to record the force.

2.4 | Histological analysis

The femur tissue was embedded by paraffin, and sliced for hematoxylin–eosin (HE) staining, then photographed with a microscope.

2.5 | Measurement of intracellular ROS level

DHE (dihydroethidium) assay kit (ab236206) was used to detect the cellular ROS level. Briefly, the osteoclasts were seeded into a 96-well black plate and cultured overnight. The culture medium was carefully removed and about 150 μL cell-based assay buffer was added to each well. About 130 μL cell-based assay buffer was aspirated from each well and 130 μL ROS staining buffer was added. The plate was incubated at 37°C for 1.5 hours, then the staining buffer and 100 μL cell-based assay buffer were aspirated. The fluorescence (ex 480–520 nm/em 570–600 nm) was measured with a fluorescence microscopy.

2.6 | Alizarin red staining

The cell culture medium was removed and washed with PBS 3 times. After fixation with 4% formaldehyde for 15 minutes, the cells were washed with water 3 times. Then, 40 mmol/L alizarin red staining solution was added (TMS-008; Sigma-Aldrich) and incubated for 30 minutes with gentle shaking at room temperature. The dye was removed and the cells washed with water 5 times. The water was

completely removed and the dye photographed with a microscope. The absorbance at 405 nm was detected with a plate reader.

2.7 | Western blotting

Cells or rat bone tissue lysate were subjected to sodium dodecyl sulfate – polyacrylamide gel electrophoresis, followed by immunoblotting. The blots were visualized with enhanced chemiluminescence.

2.8 | Statistical analysis

Data are presented as mean ± *SD*. Statistical significance was evaluated by GraphPad Prism software. $P < .05$ was considered as statistically significant.

3 | RESULTS

3.1 | Effects of Lin28A on blood indexes related to bone metabolism

The relative expression level of Lin28A protein decreased in the OVX rat model, while overexpression of Lin28A dramatically increased the protein level of Lin28A (Figure 1A). The serum calcium was significantly declined in the OVX rat model, which was rescued by overexpression of Lin28A (Figure 1B). The serum estradiol (E_2) significantly declined in the OVX rat model, as well (Figure 1B). The amount of alkaline phosphatase and interleukin (IL)-1β markedly increased in the serum of OVX rat, which was prevented by overexpression of Lin28A (Figure 1B). These results indicated bone disorders in the OVX rat model, which was mediated by Lin28A.

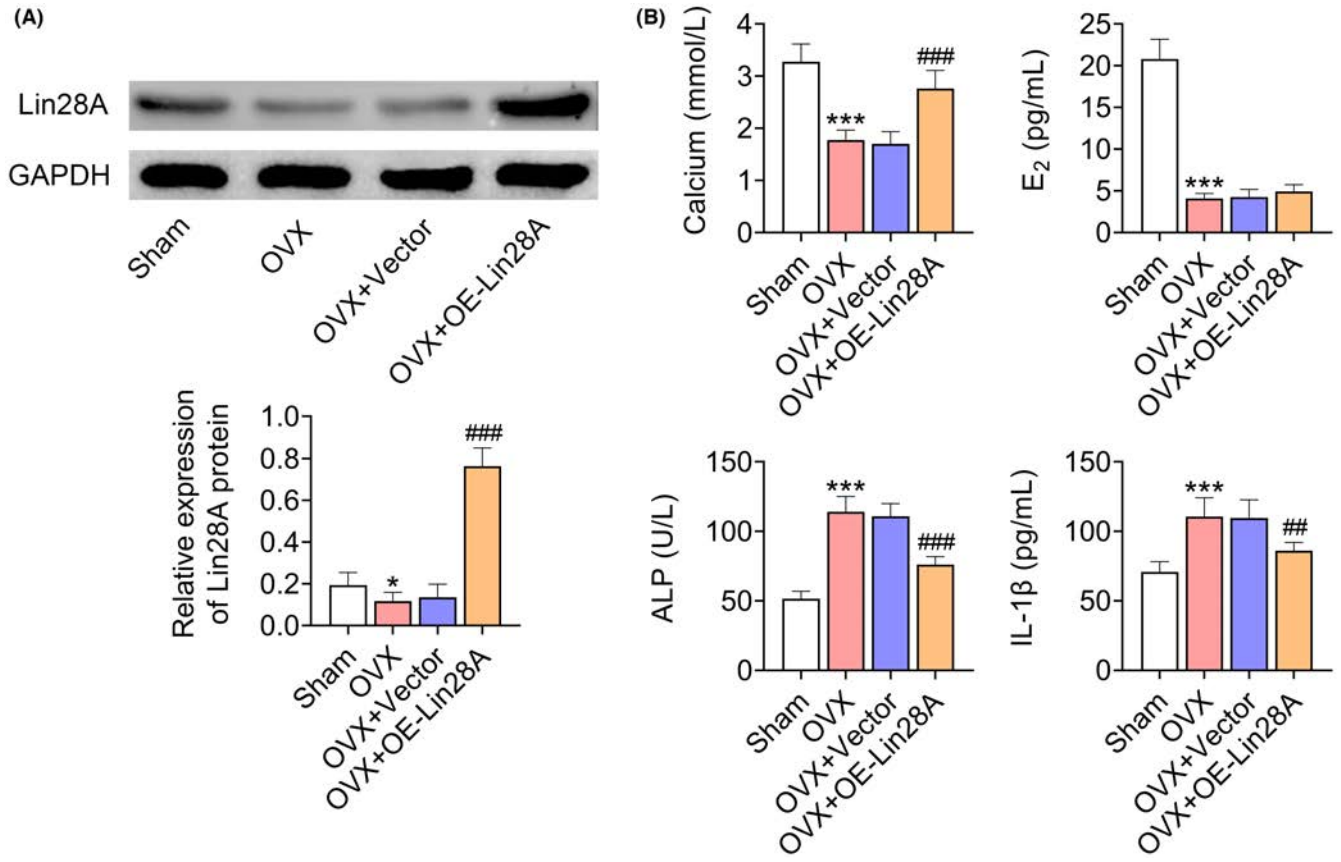

FIGURE 1 Effects of Lin28A on blood indexes related to bone metabolism. Bilateral ovariectomy was conducted to generate an ovariectomy (OVX) rat model, $n = 6$. (A) Western blotting was performed to assess the relative expression of Lin28A protein. *Upper*, representative blots of Lin28A and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the bone tissue of sham rat group (Sham), OVX rat group (OVX), OVX rat expressing the empty vector (OVX + Vector) and OVX rat transfected with a plasmid carrying Lin28A gene (OVX + OE-Lin28A). *Lower*, the relative expression of Lin28A protein normalized to GAPDH. $^{*}P < .05$ vs Sham, $^{###}P < .001$ vs OVX + Vector. Data are mean ± *SD* of 6 independent experiments. (B) Enzyme-linked immunosorbent assay kits were used to detect the serum levels of calcium, prostaglandin E_2 (E_2), alkaline phosphatase (ALP) and interleukin (IL)-1β in Sham, OVX, OVX + Vector and OVX + OE-Lin28A. $^{***}P < .001$ vs Sham, $^{##}P < .01$ vs OVX + Vector, $^{###}P < .001$ vs OVX + Vector. Data are mean ± *SD* of 6 independent experiments.

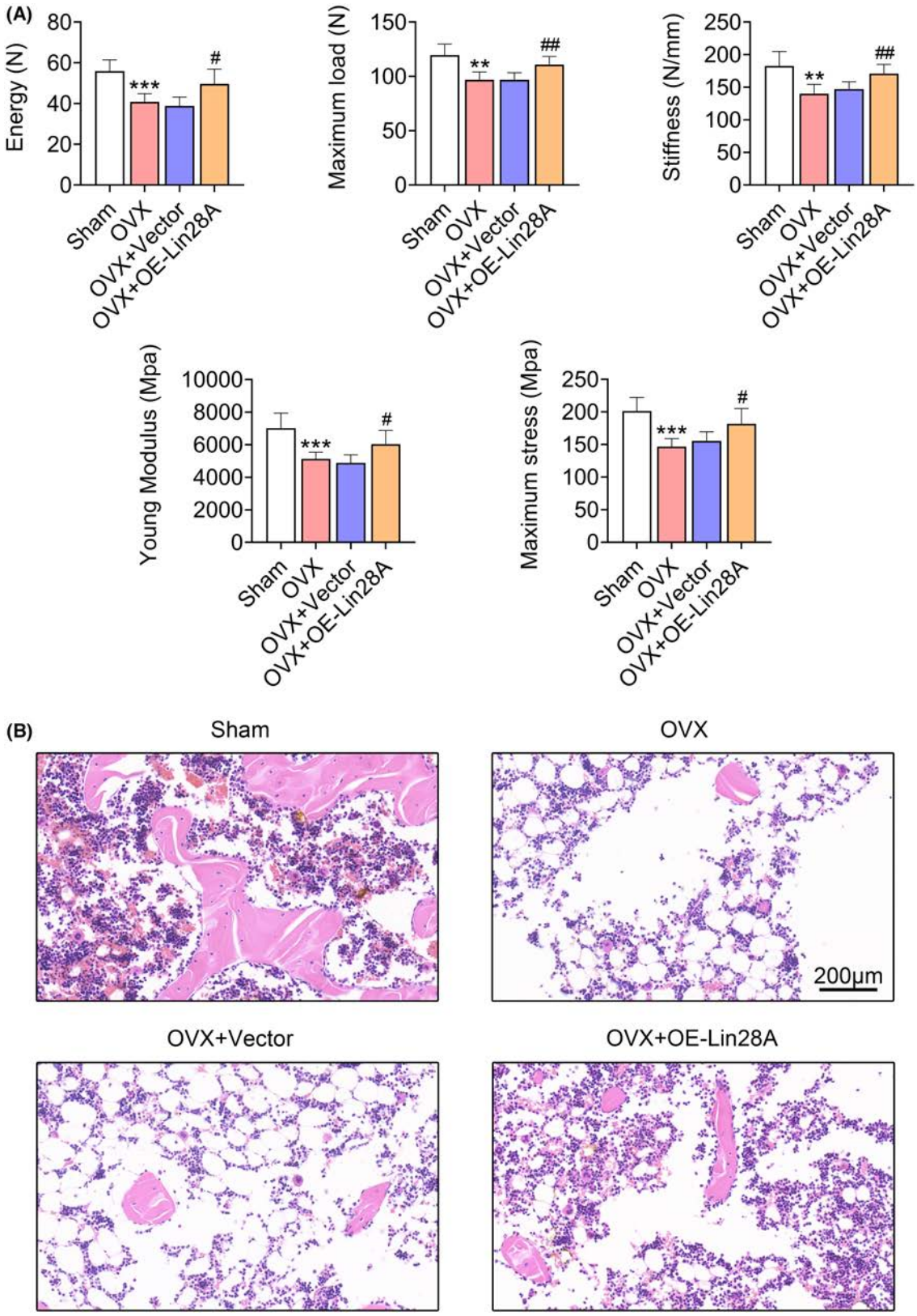

FIGURE 2 Lin28A improves the biomechanical parameters and pathological changes of bone tissue in ovariectomy (OVX) rats. (A) Three-point bending test was used to assess biomechanical parameters of left femoral diaphysis including energy, maximum load, stiffness, young modulus and maximum stress of the left shaft of femur in Sham, OVX, OVX + Vector and OVX + OE-Lin28A. $^{**}P < .01$ vs Sham, $^{***}P < .001$ vs Sham, $^{\#}P < .05$ vs OVX + Vector, $^{\#\#}P < .01$ vs OVX + Vector. Data are mean ± *SD* of 6 independent experiments. (B) Representative hematoxylin and eosin (HE) staining images of femur tissue of Sham, OVX, OVX + Vector and OVX + OE-Lin28A.

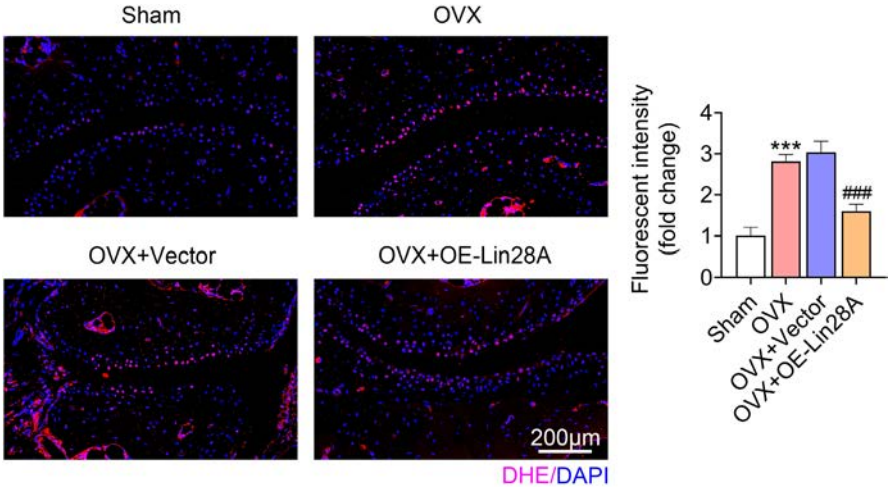

FIGURE 3 Lin28A reduces the reactive oxygen species (ROS) level in bone tissue of ovariectomy (OVX) rats. DHE (dihydroethidium) assay kit was used to measure the intracellular ROS level in osteoclasts of Sham, OVX, OVX + Vector and OVX + OE-Lin28A. *Left*, representative images of osteoclasts stained with DHE and 4′, 6-diamidino-2-phenylindole (DAPI). *Right*, the fluorescence intensity of DHE was normalized to DAPI, and the fluorescence ratio of DHE/DAPI in Sham was set as 1 unit. $^{***}P < .001$ vs Sham, $^{###}P < .001$ vs OVX + Vector. Data are mean ± *SD* of 6 independent experiments.

3.2 | Lin28A improves the biomechanical parameters and pathological changes of bone tissue in OVX rats

Biomechanical parameters of left femoral diaphysis including energy, maximum load, stiffness, young modulus and maximum stress significantly decreased in OVX rats, which was prevented by overexpression of Lin28A (Figure 2A). HE staining revealed that the trabecular structure of the sham rats was intact and arranged in order (Figure 2B). In OVX rats, the trabecular thickness, number and density were reduced, which was ameliorated by overexpression of Lin28A (Figure 2B). These results demonstrated that Lin28A could improve biomechanical parameters of bone tissue and ameliorated trabecular abnormalities in OVX rats.

3.3 | Lin28A reduces the ROS level in bone tissue of OVX rats

The DHE fluorescent dye was used to detect superoxide and hydrogen peroxide. The intracellular ROS level dramatically increased in the bone tissue of OVX rats, which was suppressed by overexpression of Lin28A (Figure 3). This observation indicated that oxidative stress was enhanced in the OVX rat model, and further induced osteoporosis, which could be ameliorated by Lin28A.

3.4 | Lin28A promotes osteogenic differentiation of bone marrow stromal cells

Alizarin red staining was performed to evaluate calcium deposit in bone marrow stromal cells (BMSC). The calcium deposit dramatically decreased in BMSC of OVX rats, which was alleviated by overexpression of Lin28A (Figure 4A). The relative expression of osteocalcin (OCN) and runt-related transcription factor 2 (RUNX2) proteins markedly declined in BMSC cells of OVX rats, which could be prevented by overexpression of Lin28A (Figure 4B). These results demonstrated that Lin28A favored osteogenic differentiation of BMSC.

3.5 | Lin28A activates the AMPK signaling pathway

Next the molecular mechanism through which Lin28A favored osteogenic differentiation was investigated. AMPK signaling pathway was inhibited in the bone tissue of OVX rats, which could be alleviated by overexpression of Lin28A (Figure 5A). Overexpression of Lin28A could improve the calcium deposit in BMSC of OVX rats, which was prevented by inhibition of the AMPK pathway (Figure 5B). Overexpression of Lin28A dramatically increased OCN and RUNX2 expressions in BMSC of OVX rats, which was prevented by inhibition of the AMPK pathway (Figure 5C). These observations indicated that Lin28A alleviated OVX-induced osteoporosis through activation of the AMPK signaling pathway.

4 | DISCUSSION

Osteoporosis is a progressive bone disease, which is characterized by decreased bone mass and bone structural deterioration.[1-4] Differentiation and mineralization of osteoblasts are crucial for bone formation, and abnormal functions of osteoblasts cause osteoporosis.[3] The incidence of osteoporosis is high in postmenopausal women because of estrogen deficiency. Thus, to elucidate the molecular mechanisms of osteoporosis will provide therapeutic targets for osteoporosis.

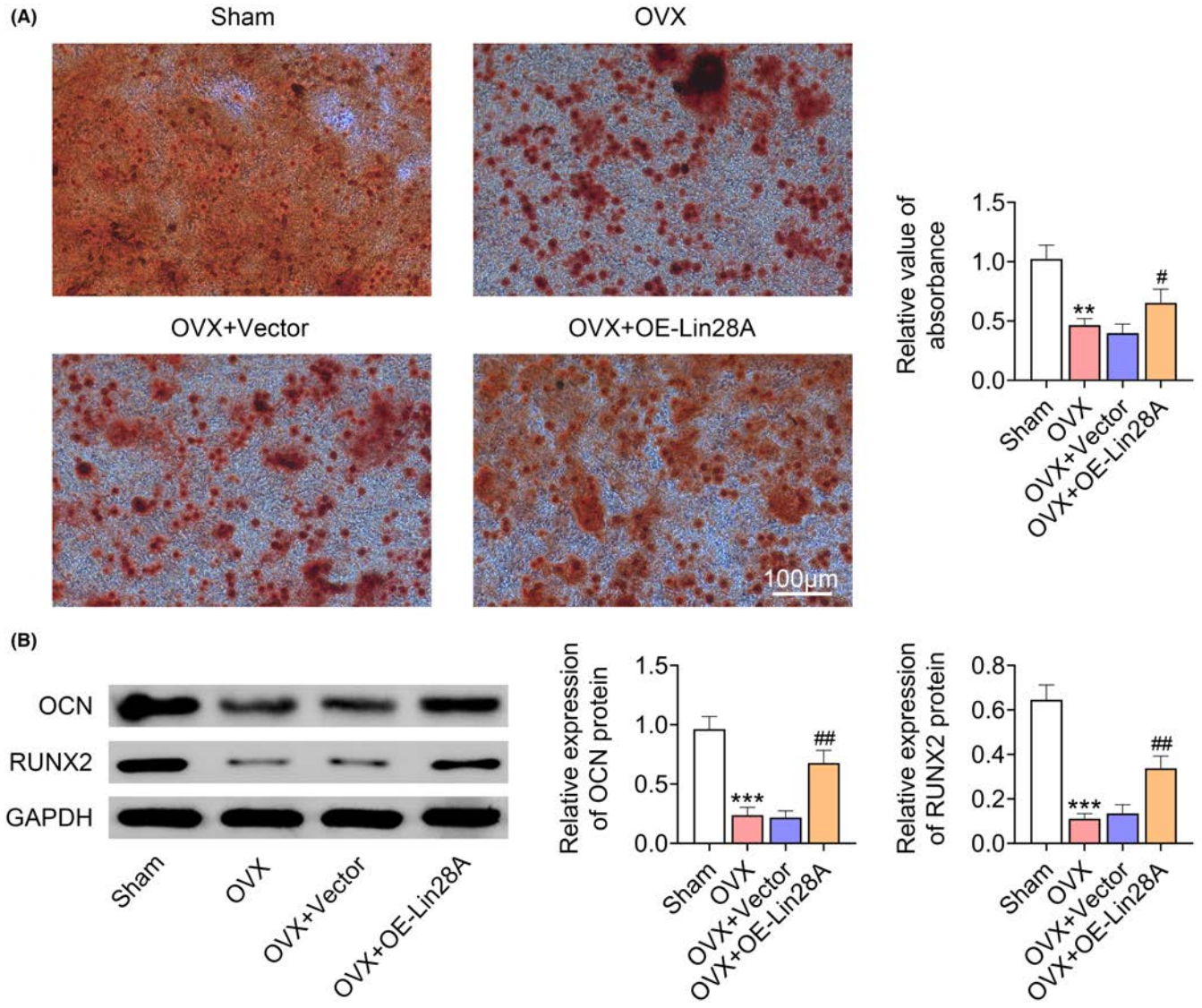

FIGURE 4 Lin28A promotes osteogenic differentiation of bone marrow stromal cells (BMSC). (A) Alizarin red staining revealed the calcium deposit in BMSC cells isolated from Sham, ovariectomy (OVX), OVX + Vector and OVX + OE-Lin28A rats. $^{**}P < .01$ vs Sham, $^{\#}P < .05$ vs OVX + Vector. Data are mean ± *SD* of at least 3 independent experiments. (B) Western blotting was performed to assess the relative expression of osteocalcin (OCN) and runt-related transcription factor 2 (RUNX2) proteins in BMSC cells isolated from Sham, OVX, OVX + Vector and OVX + OE-Lin28A rats. $^{***}P < .001$ vs Sham, $^{\#\#}P < .01$ vs OVX + Vector. Data are mean ± *SD* of at least 3 independent experiments.

Oxidative stress may cause excessive oxidation of cellular lipids, proteins and DNA, contributing to the development of aging and many pathophysiological processes. Oxidative stress is also critical for osteoporosis development. Cellular redox state impaired by ROS enhances bone resorption of osteoclasts and osteoclastogenesis.[1,17] Based on clinical studies, estrogen deficiency will reduce the ability to ameliorate oxidative stress in postmenopausal women. Oxidative damage of bone tissue may cause cell apoptosis of osteoblasts and exacerbation of osteoporosis, thus, to reduce oxidative stress is important to alleviate osteoporosis in postmenopausal women.[1] Upregulation of AMPK inhibits ROS formation, which further promotes osteogenic differentiation.[2,8,9] Lin28A favors the activation of the AMPK-mTOR signaling pathway.[13] However, the role of Lin28A in osteoporosis remains unclear.

This present work demonstrated that Lin28A alleviated OVX-induced osteoporosis via activating the AMPK signaling pathway. Ovariectomy was performed to generate an osteoporosis rat model.[14,15] Here it was shown that the expression of Lin28A was decreased in the OVX rat model, accompanied with declined levels of serum calcium, E_2, alkaline phosphatase and IL-1β. This bone disorder induced by OVX could be rescued by overexpression of Lin28A. Biomechanical parameters such as energy, maximum load, stiffness, young modulus and maximum stress were markedly decreased in OVX rats. The bone tissue of OVX rats exhibited trabecular abnormalities with reduced trabecular thickness,

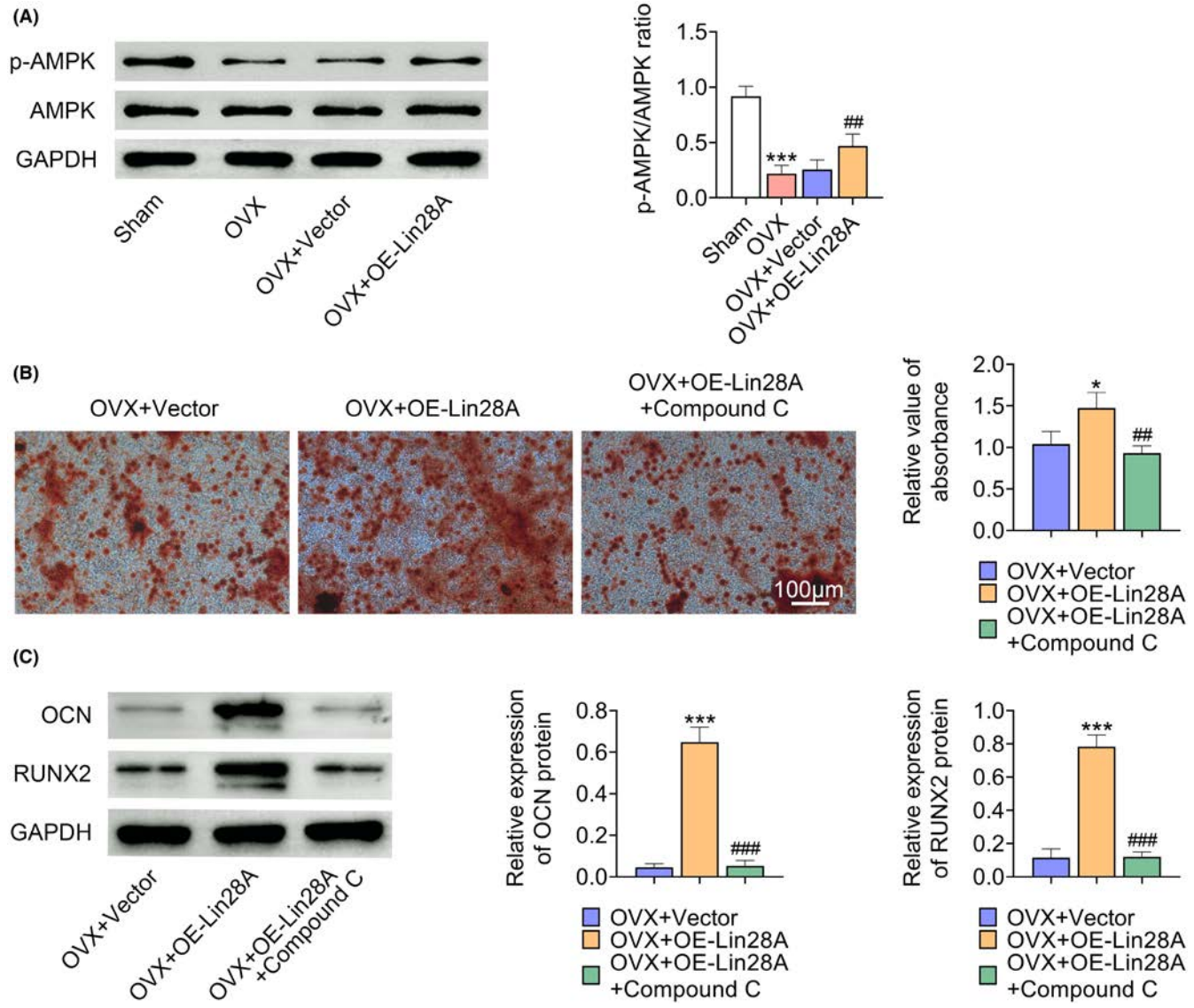

FIGURE 5 Lin28A activates adenosine monophosphate-activated protein kinase (AMPK) signaling pathway. (A) Western blotting was performed to assess the relative expression of AMPK and phosphorylated AMPK (p-AMPK) proteins in the bone tissue of Sham, ovariectomy (OVX), OVX + Vector and OVX + OE-Lin28A rats. *Left*, representative blots of AMPK, p-AMPK and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). *Right*, ratio of relative expression of p-AMPK/AMPK normalized to GAPDH. $^{***}P < .001$ vs Sham, $^{\#\#}P < .01$ vs OVX + Vector. Data are mean ± *SD* of 6 independent experiments. (B) Alizarin red staining revealed the calcium deposit in bone marrow stromal cells (BMSC) isolated from OVX + Vector, OVX + OE-Lin28A and OVX + OE-Lin28A rats treated with AMPK inhibitor Compound C (OVX + OE-Lin28A + Compound C). $^{*}P < .05$ vs OVX + Vector, $^{\#\#}P < .01$ vs OVX + OE-Lin28A. Data are mean ± *SD* of 6 independent experiments. (C) Western blotting was performed to assess the relative expression of osteocalcin (OCN) and runt-related transcription factor 2 (RUNX2) proteins in BMSC isolated from OVX + Vector, OVX + OE-Lin28A and OVX + OE-Lin28A + Compound C. $^{***}P < .001$ vs OVX + Vector, $^{\#\#\#}P < .001$ vs OVX + OE-Lin28A. Data are mean ± *SD* of 6 independent experiments.

number and density. Overexpression of Lin28A could improve the pathological changes of bone tissue of OVX rats. The intracellular ROS level was enhanced in the bone tissue of OVX rats, which was prevented by overexpression of Lin28A. The expression levels of OCN and RUNX2 proteins was decreased, accompanied with declined calcium deposit in BMSC cells of OVX rats, which could be alleviated by Lin28A, suggesting that Lin28A favored osteogenic differentiation. OVX-induced osteoporosis by inhibition of the AMPK signaling pathway could be alleviated by overexpression of Lin28A.

Taken together, ovariectomy impaired bone homeostasis and caused bone disorder, accompanied with declined expression level of Lin28A and inhibited AMPK signaling pathway. Overexpression of Lin28A could rescue OVX-induced osteoporosis by activation of the AMPK pathway *in vivo*. This present study will provide a therapeutic target for osteoporosis.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation and the experiments were performed by Liang Cai. Data

collection and analysis were performed by Zhanwang Gao. The first draft of the manuscript was written by Zhiping Gu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors state there are no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

ETHICS APPROVAL

Ethical approval was obtained from the Ethics Committee of Jiu Jiang No. 1 People's Hospital.

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How to cite this article: Cai L, Gao Z, Gu Z. Lin28A alleviates ovariectomy-induced osteoporosis through activation of the AMP-activated protein kinase pathway in rats. *Int J Rheum Dis.* 2022;25:1416-1423. doi: 10.1111/1756-185X.14436

Received: 19 July 2022 | Revised: 26 August 2022 | Accepted: 31 August 2022

DOI: 10.1111/1756-185X.14437

International Journal of Rheumatic Diseases WILEY

ORIGINAL ARTICLE

The association between hydroxychloroquine use and future development of systemic lupus erythematosus in patients with primary Sjögren's syndrome

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Funding information
Taichung Veterans General Hospital, Grant/Award Number: TCVGH-NHRI10603, TCVGH-1067310C, TCVGH-FCU1068205 and TCVGH-YM1060201,TCVGH-VTA106PREM1

Abstract

Aim: Hydroxychloroquine (HCQ), commonly used to treat patients with primary Sjögren's syndrome (pSS), has been shown to delay the development of systemic lupus erythematosus (SLE). This study aimed to explore the association between HCQ use and future development of SLE in pSS patients based on a nationwide nested case–control study.

Method: Based on the National Health Insurance Research Database of Taiwan, those patients who were diagnosed with SLE at least 1 year after the diagnosis of pSS were identified as cases. Matched controls were randomly selected from pSS patients without a later diagnosis of SLE in a 1:10 ratio. The odds ratios (ORs) of HCQ exposure between cases and controls were analyzed by unconditional logistic regression after adjustment for age.

Results: A cohort of 11 772 pSS patients were extracted from the database during the period from January 1, 2000 to December 31, 2010. A total of 111 (0.9%) pSS patients developed SLE during the follow-up period. Most (79%) of them developed SLE within 5 years after the diagnosis of pSS. There was no significant difference in the odds of HCQ exposure between cases and controls, with an adjusted OR of 2.43 (95% CI: 0.73–8.05). Neither did we observe a significant difference in the odds of exposure to a higher average dose of HCQ (≥100 mg/d vs non-exposed) between cases and controls in the sensitivity analysis.

Conclusion: Nearly 1% of pSS patients may develop SLE. HCQ use in pSS patients was not associated with a lower possibility of the future development of SLE.

KEYWORDS
epidemiology, hydroxychloroquine, prevention, Sjögren's syndrome, systemic lupus erythematosus

1 | INTRODUCTION

Systemic lupus erythematosus (SLE) is a devastating autoimmune disease which leads to significant damage in multiple organ systems. Despite improved survival in the past few decades, the standardized mortality ratio in patients with SLE remains high and a curative treatment is still lacking.[1–3] In recent years, early intervention prior to the onset of SLE has been considered as a potential strategy to reduce disease burden.[4,5]

Hydroxychloroquine (HCQ), an effective therapy in reducing mild relapses and the risk of damage accrual in SLE patients, has

been suggested as being preventive against SLE in the early phase of its development.[6–8] In 2007, a retrospective study of SLE patients conducted among US military personnel demonstrates that an early use of HCQ was associated with delayed clinical symptoms and diagnosis of SLE.[9] However, to our best knowledge, this is hitherto the only report in support of the preventive role of HCQ in the development of SLE.

Given that primary Sjögren's syndrome (pSS) and SLE share common clinical and laboratory features, the proneness of pSS patients to developing SLE has been noted for years.[10–14] Two retrospective studies found that approximately 1% of pSS patients subsequently developed SLE.[15,16] Nevertheless, development of SLE has not been observed in other studies of pSS patients.[17,18] Despite the fact that HCQ is commonly used in pSS patients, the evidence related to the benefit of HCQ in reducing future development of SLE in pSS patients is limited. We therefore conducted a nationwide, population-based, nested case–control study, of pSS patients in Taiwan in order to investigate the association between HCQ use and future development of SLE in pSS patients.

2 | MATERIALS AND METHODS

2.1 | Data source

The National Health Insurance Research Database (NHIRD) comprises comprehensive claims data from >98% of the population in Taiwan and provides de-identified healthcare information.[19,20] Diagnoses are coded by the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). Patients who fulfill the diagnostic/classification criteria of major illnesses are registered in the Catastrophic Illness Patient Database (CIPD) for the exemption of copayment after a critical scrutiny by at least 2 specialists.[21,22] Patients' written informed consent was waived since the data retrieved from the NHIRD was de-identified. This study was approved by the ethics review board of Taichung Veterans General Hospital (IRB TCVGH No: CE13151).

2.2 | Study population

Patients who were newly registered in the CIPD with the diagnosis of pSS (ICD-9-CM code: 710.2) from January 1, 2000 to December 31, 2010, were identified. The diagnosis of pSS was made in accordance with the preliminary European Community criteria[23] before 2002 and the American-European Consensus Group (AECG) criteria[24] from 2002 to 2010. The date that an individual was registered in the CIPD with the diagnosis of pSS was defined as the "index date". Each pSS patient was followed until the diagnosis of SLE was made in the CIPD, the withdrawal from the NHIRD or December 31, 2013, whichever came first. The diagnosis of SLE in the CIPD was established based on the 1997 American College of Rheumatology (ACR) revised criteria.[25]

2.3 | Exclusion criteria

Exclusion criteria included: (1) patients with diagnoses of SLE (ICD-9-CM code: 710.0), rheumatoid arthritis (ICD-9-CM code: 714.0), systemic sclerosis (ICD-9-CM code: 710.1), dermatomyositis (ICD-9-CM code: 710.3), or polymyositis (ICD-9-CM code: 710.4) in the CIPD before the index date; (2) patients diagnosed with SLE within 1 year after the index date; and (3) patients withdrawn from the NHIRD within 1 year after the index date.

2.4 | Cases and controls

Patients with pSS who were diagnosed with SLE at least 1 year after the index date were identified as cases. Eligible controls were randomly selected from pSS patients without a later diagnosis of SLE, and matched to cases in a 1:10 ratio based on gender, the duration between the index date and the end of follow-up (the follow-up time), and exposure to immunosuppressants except for HCQ (defined as a prescription of corticosteroids, methotrexate, leflunomide, sulfasalazine, azathioprine, mercaptopurine, cyclosporine, tacrolimus, mycophenolate, and/or cyclophosphamide ≥180 cumulative days). The exposure to HCQ was defined as any prescription from the index date to 180 days before the end of follow-up due to the fact that the diagnosis of SLE sometimes lagged behind the clinical symptoms, for which HCQ might be prescribed.

2.5 | Statistical analyses

Numerical variables were compared by Student's *t* test. Categorical variables were compared by Chi-squared test. We used unconditional logistic regression to analyze the odds ratios (ORs) of HCQ exposure between cases and controls after adjustment for age. Statistical analyses were conducted using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

2.6 | Sensitivity analyses

Unconditional logistic regression was performed after stratifications by age, gender, the follow-up time, and the use of immunosuppressants. We also categorized HCQ exposure into <100 mg and ≥100 mg per day based on the average daily dose, and re-analyzed the data to investigate the effect of a higher dose of HCQ.

3 | RESULTS

3.1 | Baseline characteristics

From 2000 to 2010, we identified a cohort of 11 772 eligible pSS patients (Figure 1). In the follow-up, a total of 78 (0.7%) SLE cases were identified. These cases were matched to 780 controls. The

baseline characteristics of the cases and controls are shown in Table 1. Among the 78 cases, the mean duration between the diagnosis of pSS and SLE was 4.3 ± 2.6 years. The mean age of the cases (44.9 ± 14.9 years) was significantly younger than that in matched controls (56.5 ± 14.1 years). The number of patients with newly diagnosed SLE each year after the diagnosis of pSS is presented in Figure 2. Most (79%) of the patients developed SLE within 5 years.

3.2 | Unconditional logistic regression and sensitivity analyses

There was no significant difference in the odds of HCQ exposure between cases and controls (crude OR 2.82 [95% CI: 0.87–9.13]; adjusted OR 2.43 [95% CI: 0.73–8.05]) (Table 2). In subgroup analyses as stratified by age, gender, the follow-up time, and the use of immunosuppressants, we found no significant difference in the odds of HCQ exposure between cases and controls after adjustment (Table 3). Furthermore, we did not find a significant difference in the odds of an exposure to an even higher average dose of HCQ (≥100 mg/d vs non-exposed) between cases and controls (OR 2.15 [95% CI: 0.64–7.20] after adjustment, Table 4).

4 | DISCUSSION

HCQ is frequently used to treat pSS patients and its use is associated with suppression of SLE-related cytokines. We hypothesized that the exposure to HCQ is associated with a reduction in the future development of SLE among pSS patients. However, our nested case–control study in a cohort of 11 772 pSS patients did not find such an association.

The evolution from pSS to SLE is uncommon and controversy remains. Anti-Ro/SSA and anti-La/SSB antibodies are considered as

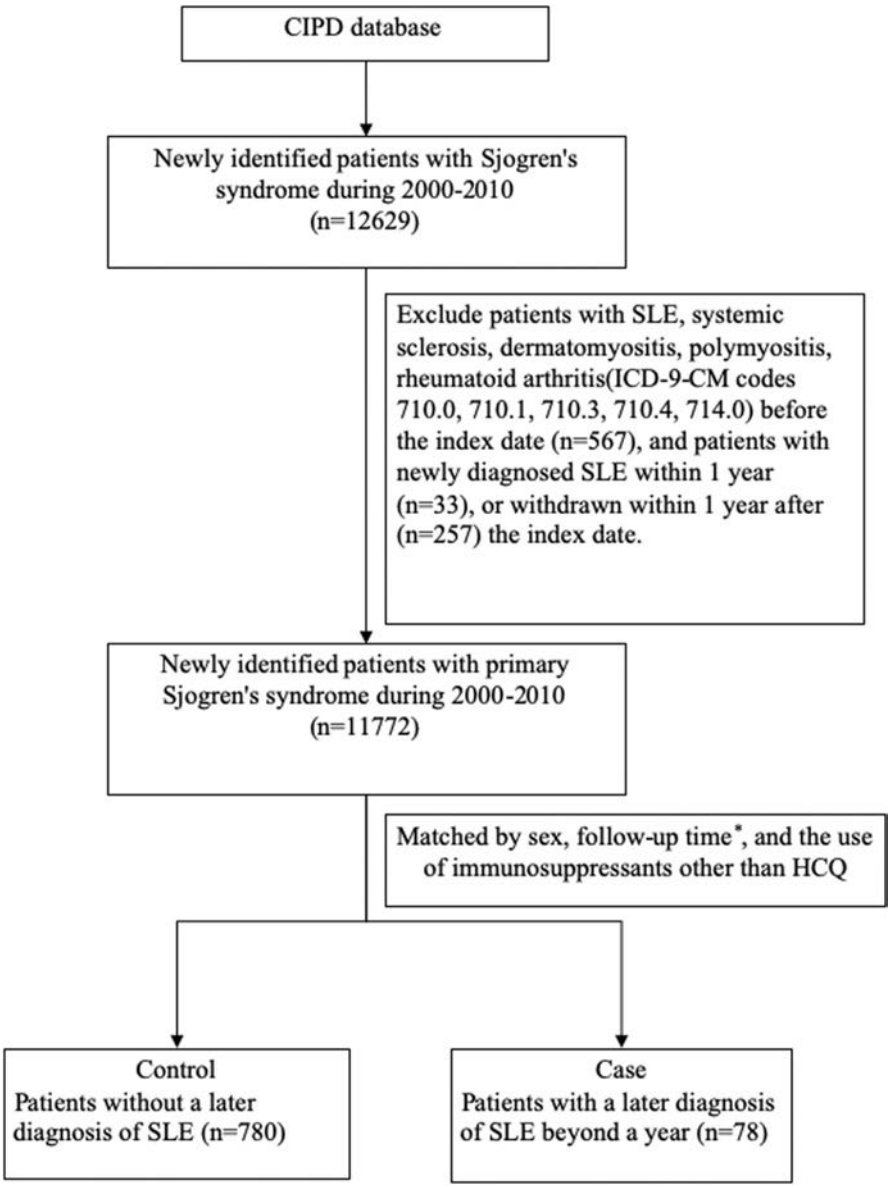

FIGURE 1 Flow chart for identifying the study population. *The duration between the index date and the end of follow-up. CIPD, Catastrophic Illness Patient Database; HCQ, hydroxychloroquine; ICD-9-CM, International Classification of Diseases, Ninth Revision, Clinical Modification; SLE, systemic lupus erythematosus

precursors in the development of SLE.[10–14] The autoimmunity underlying SLE has been speculated to evolve from anti-60 kD Ro antibody through epitode spreading.[12,13] Furthermore, in patients who developed SLE, the presence of anti-La/SSB antibody is suggested to be related to the disease progression.[14] In pre-symptomatic individuals who developed SLE later, the presence of anti-Ro/SSA and anti-La/SSB antibodies correlates with the increase of interferon-γ inducible protein-10 and interferon-α respectively, which were both important factors in the pathogenesis of SLE.[11] Of note, the overexpression of type I and II interferon signatures was observed in both pSS and SLE patients and may predict the evolution of SLE in antinuclear antibody-positive individuals.[26–29] Lazarus et al and Ter Borg et al had reported that none of their pSS patients were later diagnosed as having SLE after a follow-up of 7–10 years.[17,18] Fauchais et al showed that 6 (1.3%) out of 445 pSS patients were identified as having SLE during a follow-up of 77 months.[15] A retrospective study in the United Kingdom found that 2 (1.3%) patients evolved into SLE in 152 pSS patients after a follow-up of 11 years.[16] In the present study, we observed 111 (0.9%) newly diagnosed SLE in a cohort of 11 777 pSS patients within a mean follow-up of 4.3 years. In line with previous findings, our finding supported that around 1% of pSS patients may develop SLE later. To be noted, the risk for development of SLE was the highest within 5 years after pSS had been diagnosed.

TABLE 1 Baseline characteristics of SLE cases and matched controls in the cohort of 11 772 pSS patients

Variables	No. (%)	
	Cases (n = 78)	Controls (n = 780)
Gender		
Female	74 (94.9)	740 (94.9)
Male	4 (5.13)	40 (5.13)
Age, y, mean (SD)*	44.9 (14.9)	56.5 (14.1)
<50 y	54 (69.2)	217 (27.8)
≥50 y	24 (30.8)	563 (72.2)
HCQ		
Non-exposed	3 (3.9)	79 (10.1)
Exposed	75 (96.2)	701 (89.9)
Immunosuppressants other than HCQ		
Non-exposed	34 (43.6)	340 (43.6)
Exposed	44 (56.4)	440 (56.4)
Follow-up time, y (SD)	4.30 (2.6)	4.29 (2.6)
<4 y	46 (59.0)	474 (60.1)
≥4 y	32 (41.0)	306 (39.9)

Abbreviations: HCQ, hydroxychloroquine; pSS, primary Sjögren's syndrome; SLE, systemic lupus erythematosus.
*$P < .0001$.

The LUMINA cohort demonstrated that HCQ use was associated with a lower blood level of interferon-α, together with the clinical improvements in SLE patients.[30] Another study also showed that in lupus patients, the production of interferon-α by plasmacytoid dendritic cells upon stimulation of Toll-like receptor 7/9 was suppressed by HCQ.[31] Interestingly, for individuals with possible/probable SLE (fulfilling 1 to 3 of the 1997 ACR revised criteria), HCQ treatment was associated with a reduced expression level of type I interferon-inducible genes.[32] A retrospective study among US military personnel suggested that HCQ use prior to the diagnosis of SLE was associated with a delayed disease onset in these patients.[9] These results suggested that HCQ was a candidate for preventing the development of SLE. Nevertheless, our results did not demonstrate the protective effect of HCQ treatment on the future development of SLE in pSS patients, even for those who used a higher dose.

The neutral relationship between the prescription of HCQ and the development of SLE in pSS patients in our study does not refute the possibility of HCQ being an early intervention in SLE development. Conversely, it raises questions about an optimized

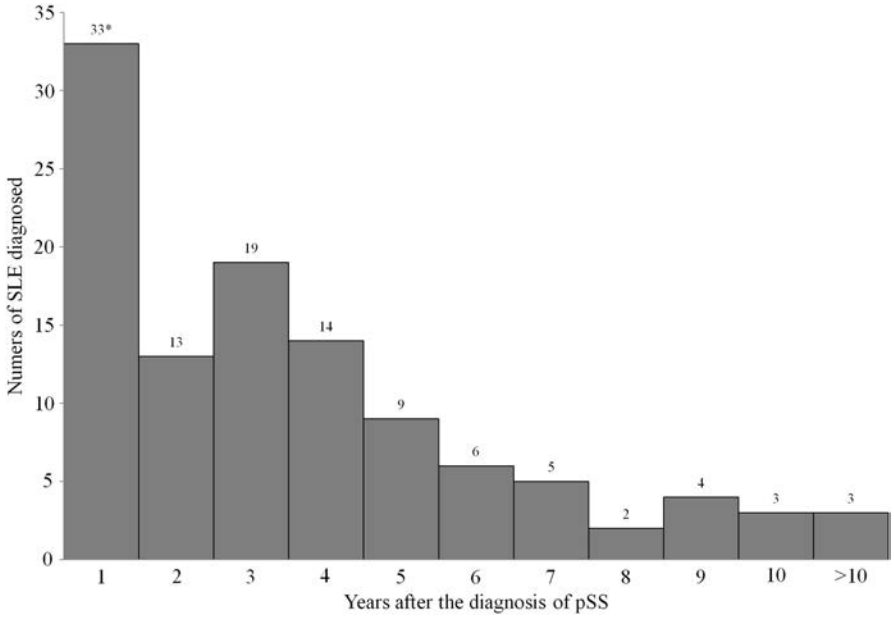

FIGURE 2 The distribution of SLE diagnosed in the cohort of pSS patients. *A total of 111 pSS patients were diagnosed with SLE after the index date. Thirty-three patients with SLE diagnosed within 1 year after the index date were not identified as cases. pSS, primary Sjögren's syndrome; SLE, systemic lupus erythematosus

preventive strategy in the most vulnerable population in the appropriate time window. A universal HCQ prophylaxis for SLE development among pSS patients might not be efficacious based on our results. Nonetheless, it is crucial to identify a subgroup of pSS patients at high risk for development of SLE before implementing preventive measures. Whether it is too late to halt the progression to SLE while specific autoantibodies or clinical manifestations have already emerged is an issue requiring more investigations. The effectiveness of early HCQ prescription in a population at high risk for SLE development mandates more evidence, including the ongoing SMILE trial (ClinicalTrials.gov number, NCT03030118).

There are some limitations in our study. Assuming the proportion difference in HCQ exposure between cases and controls equals 6% (as it is in our data), power of 0.8, and the type I error of 0.05, 171 case patients and 1710 control patients are required to be able to reject the null hypothesis. Therefore, our study may have insufficient power to detect the difference regarding HCQ exposure. Nevertheless, this study includes the largest number of pSS patients by far to investigate the evolution from pSS to SLE. Second, patients were less motivated to register for SLE if they already had beneficence in payment for pSS in the CIPD. The number of newly diagnosed SLE in our cohort was possibly underestimated. Nevertheless, the incidence of SLE in our pSS patients was similar to previous reports. Third, pSS patients with more severe disease were probably more likely to receive HCQ, and such confounding by indication could not be completely avoided in a real-world cohort despite our efforts to eliminate it (matching cases and controls by the use of immunosuppressants). Fourth, the misclassification bias about HCQ use is possible since medication adherence cannot be guaranteed in a claims database. Finally, some studies reported the possibility of misdiagnosis between pSS and SLE.[33,34] Although the clinical manifestation and laboratory data

TABLE 2 Unconditional logistic regression for odds ratios (ORs) of HCQ exposure between cases and controls

Variables	Crude OR (95% CI)	Adjusted odds ratio (95% CI)[a]
HCQ exposure	2.82 (0.87–9.13)	2.43 (0.73–8.05)

Abbreviation: HCQ, hydroxychloroquine.

[a]Adjusted by age.

TABLE 3 Unconditional logistic regression for odds ratios (ORs) of HCQ use after stratification by gender, age, follow-up time and exposure to immunosuppressants

	HCQ exposure		
	Cases (n = 75)	Controls (n = 701)	Adjusted OR relative to controls (95% CI)
Stratified by gender			
Female	71	669	1.94 (0.59–6.43)[a]
Male	4	32	NA
Stratified by age			
Age < 50	51	201	1.40 (0.38–5.11)[b]
Age ≥ 50	24	500	NA
Stratified by follow-up time			
Follow-up <4 y	43	424	1.26 (0.37–4.34)[c]
Follow-up ≥4 y	32	277	NA
Stratified by exposure to immunosuppressants other than HCQ			
Non-exposure	32	289	2.44 (0.55–10.9)[c]
Exposure	43	412	2.44 (0.31–19.2)[c]

Abbreviation: HCQ, hydroxychloroquine; NA, not available.

[a]Adjusted by age.

[b]Adjusted by gender.

[c]Adjusted by age and gender.

TABLE 4 Unconditional logistic regression for odds ratios (ORs) of hydroxychloroquine (HCQ) exposure between cases and controls with respect to average HCQ daily dose

HCQ dose (mg/d)	Cases, n = 78 (%)	Controls, n = 780 (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]	Adjusted OR (95% CI)[b]
Non-exposed	3 (3.85)	79 (10.1)	–	–	–
<100	36 (46.2)	311 (39.9)	3.15 (0.94–10.6)	2.52 (0.74–8.60)	–
≥100	39 (50.0)	390 (50.0)	2.61 (0.79–8.61)	2.15 (0.64–7.20)	0.92 (0.55–1.56)

[a]Adjusted by age.

[b]Adjusted by age, gender, and the exposure to immunosuppressants.

are not retrievable in the NHIRD, the registration of SLE in the CIPD mandates a scrutiny by at least 2 experienced rheumatologists based on a comprehensive review of medical records, including symptoms, signs, laboratory data, and pathology reports. In addition, we excluded those patients who developed SLE within a year since the diagnosis of pSS. Furthermore, we randomly selected 50 pSS patients registered in the CIPD from the Puli branch of Taichung Veterans General Hospital and 50 SLE patients registered in the CIPD from Taichung Veterans General Hospital. Forty-six (92%) pSS patients fulfilled the AECG criteria for pSS and 48 (96%) of 50 patients fulfilled the 1997 ACR revised criteria for SLE (data not shown). The diagnostic accuracy is ensured.

In conclusion, our results highlight that HCQ use is not associated with a lower possibility of later development of SLE in pSS patients. However, interventional studies are needed to further explore the possible preventive role of HCQ in patients more vulnerable to the development of SLE.

ACKNOWLEDGEMENT

This study is based in part on data from the NHIRD provided by the National Health Insurance Administration, Ministry of Health and Welfare and managed by National Health Research Institutes (Registered number 101095, 102148). The interpretation and conclusions contained herein do not represent those of National Health Insurance Administration, Ministry of Health and Welfare or National Health Research Institutes.

FUNDING INFORMATION

This study was supported in part by grants from Taichung Veterans General Hospital, Taiwan (TCVGH-NHRI10603, TCVGH-1067310C, TCVGH-FCU1068205, TCVGH-YM1060201, TCVGH-VTA106PREM1). The authors would like to thank the Healthcare Service Research Center of Taichung Veterans General Hospital for statistical support.

CONFLICT OF INTEREST

None.

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How to cite this article: Hsu B-C, Chen Y-H, Lin C-H, Tang K-T. The association between hydroxychloroquine use and future development of systemic lupus erythematosus in patients with primary Sjögren's syndrome. *Int J Rheum Dis*. 2022;25:1424-1430. doi: 10.1111/1756-185X.14437

Received: 18 April 2022 | Revised: 5 June 2022 | Accepted: 30 August 2022

DOI: 10.1111/1756-185X.14440

International Journal of Rheumatic Diseases | WILEY

ORIGINAL ARTICLE

Detection of Epstein–Barr virus in systemic sclerosis patients: A molecular and serological based study

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Funding information
Indian Council of Medical Research

Abstract

Objective: This study aimed to evaluate an association between Epstein–Barr virus (EBV) and systemic sclerosis (SSc).

Methodology: One hundred and fifty (138 female, 12 male) consecutive adult SSc patients fulfilling the American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) criteria were included in this cross-sectional study. Serological analysis by line blot for class immunoglobulin G (IgG) and IgM antibodies against EBV antigen (EBV capsid antigen [VCA] gp125, VCA p19, EBNA-1, p22, EA-D) and quantification of EBV DNA in whole blood by real-time polymerase chain reaction was performed.

Results: Class IgM antibodies against VCA gp125 (22.8% vs 0%, $P < .0002$), VCA p19 (55.7% vs 4.4%, $P < .0001$), EBNA1 (35.7% vs 0%, $P < .0001$), p22 (24.2% vs 0%, $P < .0001$), EA-D (14.2% vs 2.2%, $P < .04$), and class IgG antibodies against p22 (95.7% vs 82.2%, $P < .02$) and EA-D (54.2% vs 0%, $P < .0001$) reactivities were significantly higher in SSc patients than in controls. The past infection was significantly associated with the control group (42.8% vs 91%, $P < .0001$); and the viral reactivation was significantly associated with the SSc group (55.7% vs 4.4%, $P < .0001$). Only three (2%) out of 150 SSc patients were positive for EBV DNA, similar to the control group (2%) ($P > .9$).

Conclusion: The study shows a strong serological association of EBV (reactivation stage) with SSc patients in the absence of viral DNA in the circulation, indicating the EBV reservoir or tropism presence elsewhere.

KEYWORDS
antigen, DNA, EBV, SSc

1 | INTRODUCTION

Systemic sclerosis (SSc) is a rare chronic connective tissue disease of unknown etiology, characterized by microvasculopathy, immunological abnormalities, and fibrosis of the skin and visceral organs.[1,2] SSc is more common in women than men (ratio between 4:1 and 14:1), usually affecting middle-aged adults.[3,4] It has a higher mortality rate than any other rheumatic disease.[5,6] Epstein–Barr virus (EBV), a lymphotropic virus that chronically affects 90% of the human population, has been proposed as a possible environmental trigger of SSc.[7–12] Topoisomerase autoantibodies, a specific marker for SSc, are shown to be produced by EBV-transformed B cells from healthy

donors,[13] which supports the idea that EBV infection is directly linked to the development of autoantibodies in SSc. Also, studies have demonstrated the presence of EBV viral lytic proteins, genes in the skin, and high titers of class immunoglobulin G (IgG) antibodies against EBV capsid antigen (VCA).[13,14] The lytic form of EBV was found to activate the innate immune system via Toll-like receptor (TLR)-8 and TLR-7/9-MyD88 pathways in vitro.[8,14] However, compared to other SADs (systemic autoimmune diseases) like RA (rheumatoid arthritis), SLE (systemic lupus erythematosus), SS (Sjögren's syndrome), and MS (multiple sclerosis), the role of EBV in SSc pathogenesis is poorly investigated.[15] This study aimed to examine the antibody response of SSc patients to a more extensive set of EBV antigens encoding the viral envelope and representing the latent and lytic cycles than is routinely utilized for normal diagnostic purposes.[16] Also, the EBV viral load (DNA) was accessed in circulation. The findings of this study can substantiate the possible association between EBV infection and SSc.

2 | MATERIALS AND METHODS

2.1 | Study design

This is a cross-sectional study was conducted between the years 2015–2019 in the Department of Immunopathology, Postgraduate Institute of Medical Research and Education, Chandigarh. The study included 150 adult patients with SSc who fulfilled American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) criteria. These patients were not treatment-naive. The study comprised 45 healthy age and gender-matched controls. This study was approved by the Institutional Ethical Committee (NK/3163/Ph.D./362), and written consent from patients for participation in the study was obtained.

2.2 | Methodology

2.2.1 | Real-time polymerase chain reaction (PCR) for EBV viral load quantification

Whole blood DNA was extracted using QIAamp DNA Blood Kits following manufacturer instructions (QIAamp DNA, Qiagen). The quantity and purity of genomic DNA were measured by spectrophotometer (Genova Nano). Real-time PCR (Light Cycler LC480, Roche Applied Science) was used to detect EBV DNA, following the same protocol in a previously published research article from our lab.[17]

2.2.2 | Detection of antibodies against EBV (IgM/IgG) by line immunoblot test

About 2 mL of peripheral blood was collected in a plain vial from each patient and allowed to clot for 20–30 minutes at room temperature; serum samples were obtained by centrifuging at 1500 × *g* for 10 minutes. Antibodies of class IgG and IgM against EBV antigen (VCA gp125, VCA p19, EBNA-1, p22, EA-D) were detected by immunoblot assay (EUROIMMUN, Anti-EBV profile 2, [IgG/IgM], EUROLINE). The results were interpreted electronically by using the EUROLINE Scan.

Subjects were categorized into 4 stages of infection (no previous infection, acute, late phase, and reactivation) based on the serological patterns of 5 antibodies (VCA-IgG, IgM, EBNA/p22-IgG, and EA [D]-IgG)[18,19] as per the following criteria: (a) the presence of VCA-IgG and VCA-IgM, and EA (D)-IgG in the absence of EBNA/p22-IgG indicates acute primary infection; (b) the presence of VCA-IgG and EBNA/p22-IgG with or without VCA-IgM in the absence of EA (D)-IgG indicates past infection; and (c) the presence of VCA-IgG, EBNA/p22-IgG, and EA (D)-IgG in the absence/presence of VCA-IgM indicates possible viral reactivation.

2.3 | Statistical analysis

The statistical software SPSS (version 22.0) was used for the data analysis. Chi-square or Fisher's exact test were used, as appropriate. Results with P values $< .05$ were considered statistically significant.

3 | RESULTS

3.1 | Demographic details of SSc patients

Table 1 depicts the demographic details of 150 SSc patients enrolled in this study. The antinuclear antibody (ANA) and autoantibody profiles of these patients have been recently published by our group.[3]

Hydroxychloroquine was given to all SSc patients. Ninety-five percent of patients received cyclophosphamide pulses monthly for 6 months as induction therapy. Fifty percent of the patients received azathioprine, and 24% received mycophenolate mofetil as maintenance therapy. Twenty percent of patients received rituximab as re-induction therapy for disease worsening or new organ-threatening manifestations of the disease.

TABLE 1 Demographic details of systemic sclerosis (SSc) patients

Demography characteristics	Number (%)
Total SSc patient	150
Gender (female/male)	138/12
Age (mean ± SD) in y	40.3 ± 10.9
Disease duration (mean ± SD) in y	7.1 ± 5.2
Diffuse cutaneous SSc	75 (50%)
Limited cutaneous SSc	75 (50%)

TABLE 2 Epstein–Barr virus seroprofile

	Immunoglobulin M					Immunoglobulin G				
Study group	VCA gp125	VCA p19	EBNA1	P22	EA-D	VCA gp125	VCA p19	EBNA1	P22	EA-D
Controls (n = 45)	0	2 (4.4%)	0	0	1 (2.2%)	42 (93.3%)	42 (93.3%)	39 (86.6%)	37 (82.2%)	0
Patients (n = 70)	16 (22.8%)	39 (55.7%)	25 (35.7%)	17 (24.2%)	10 (14.2%)	59 (84.2%)	69 (98.5%)	63 (90%)	67 (95.7%)	38 (54.2%)
$P < .05$	.0002***	.0001***	.0001***	.0001***	.04*	n.s	n.s	n.s	.02*	.0001***

Abbreviation: n.s, not significant.
*$p < .05$, **$p < .01$, ***$p < .001$.

3.2 | Estimation of EBV humoral response (antibody profile) and viral load

3.2.1 | Viral load estimation by real-time PCR

The EBV DNA was detectable in 2% (3/150) of patients with a mean EBV viral load of 118.64 copies/mL. Among the control, 2% (1/45) were positive for EBV DNA with a mean EBV viral load of 278.6 copies/mL. The difference between the study group for EBV DNA positivity was not statistically significant ($P > .9$).

3.2.2 | Prevalence of EBV IgM and IgG antibodies in SSc patients and controls

Table 2 and Figure 1 show the comparison of EBV seroprofiles of SSc patients and healthy controls for the individual antigens and the corresponding *P* values. Percentages of antibodies of class IgM against VCA gp125, VCA p19, EBNA1, p22 and EA-D reactivities were significantly higher in SSc patients compared to healthy controls (VCA gp125: 22.8% vs 0%, $P < .0002$; VCA p19: 55.7% vs 4.4%, $P < .0001$; EBNA-1: 35.7% vs 0%, $P < .0001$; p22: 24.2% vs 0%, $P < .0001$; EA-D: 14.2% vs 2.2%, $P < .04$). Percentages of class IgG antibodies against VCA gp125, VCA p19, and EBNA1 were comparable between SSc and healthy groups. Anti-p22 (95.7% vs 82.2%, $P < .02$) and anti-EA-D (54.2% vs 0%, $P < .0001$) were present more frequently in SSc patients than healthy controls.

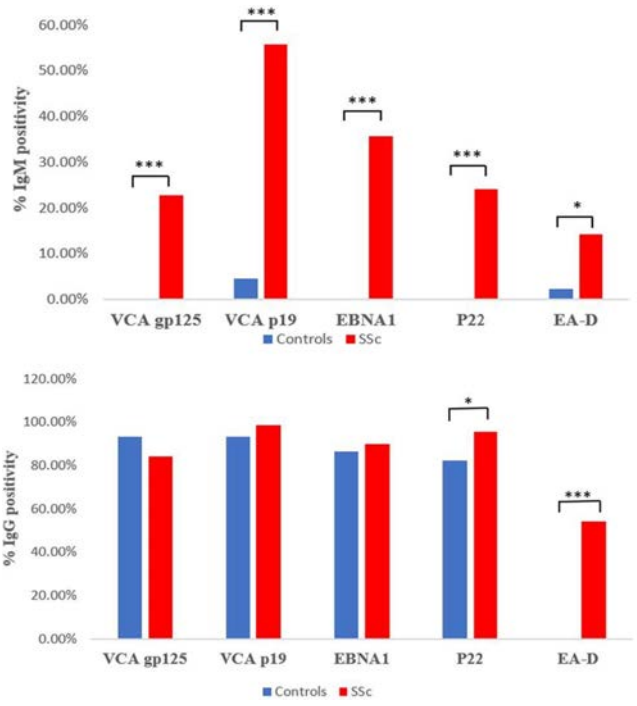

FIGURE 1 Epstein–Barr virus (EBV) seroprofile. Top: % of anti-EBV immunoglobulin M (IgM) antibodies; bottom: anti-EBV IgG antibodies in study groups (Fischer's exact/Chi-square test used for positivity analysis, *P* value was calculated at .05). SSc, systemic sclerosis

TABLE 3 Elucidation of Epstein–Barr virus serological pattern in systemic sclerosis patients[a]

Infection status	Associated antibodies					Patient n = 70	Control n = 45	$P < .05$
	Anti-VCA (IgG)	Anti-VCA (IgM)	Anti-EBNA-1 (IgG)	Anti-p22 (IgG)	Anti-EA-D (IgG)			
No previous infection	-	-	-	-	-	1 (1.4%)	2 (4.4%)	n.s
Acute infection	+	+	-	-	+	0	0	n.s
Late phase infection	+	-	+	+	-	14 (20%)	40 (88.8%)	
	+	+	+	+	-	16 (22.8%)	1 (2.2%)	
Total						30 (42.8%)	41 (91%)	0.0001***
Reactivation	+	+	+	+	+	25 (35.7%)	0	
	+	-	+	+	+	14 (20%)	2 (4.4%)	
Total						39 (55.7%)	2 (4.4%)	0.0001***

Abbreviations: Ig, immunoglobulin; n.s, not significant.

[a]Summarized from references[18,19] and EUROLINE Anti-EBV profile 2.

*$p < .05$, **$p < .01$, ***$p < .001$.

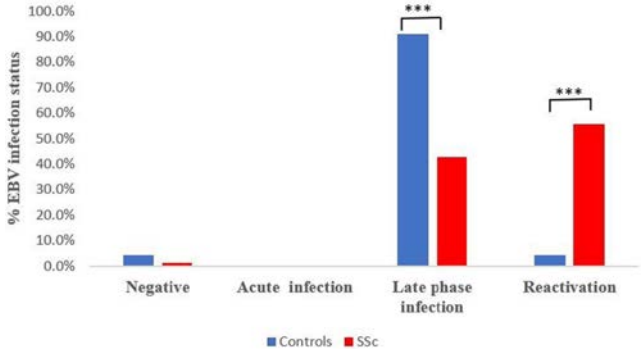

FIGURE 2 Epstein–Barr virus (EBV) infection status in study groups. Fischer's exact used for positivity analysis, *P* value was calculated at .05. SSc, systemic sclerosis

Table 3 and figure 2 summarize the analysis of EBV serological patterns with the corresponding *P* values. The acute primary EBV infection was absent in both the study groups, past infection was found to be significantly associated with the control group (42.8% vs 91%, $P < .0001$) and the viral reactivation was significantly associated with the SSc group (55.7% vs 4.4%, $P < .0001$).

4 | DISCUSSION

To validate the hypothesis of linkage between EBV infection and SSc, we investigated humoral response to different EBV antigens and viral DNA in the whole blood of SSc patients. Antibodies to 5 antigen complexes, namely VCA-IgM, VCA-IgG, EA/D-IgG, EBNA1-IgG, and p22-IgG, were evaluated to see whether the patient is susceptible to EBV, has a primary or past infection, or is experiencing a reactivated EBV infection. Table 4 shows EBV antigen-encoded proteins' functions. Our study showed a higher frequency of class IgM antibodies against VCA gp125, VCA p19, EBNA1, p22, EA-D, and class IgG antibodies against p22 and EA-D reactivities in SSc patients compared to controls, implying a strong humoral response against EBV antigens and a significant association of viral reactivation with SSc patients. However, whether this strong humoral response in SSc patients against EBV antigen in our study is accompanied by disturbed systemic EBV control, we screened a significant number of samples and found no viral DNA in SSc patients' whole blood; only 3 out of 150 patients tested positive for EBV DNA. We found no correlation between viral DNA and antibodies, indicating that the latter is not caused by an elevated viral load in the circulation or the inability to control systemic EBV infection. The mechanisms that could answer these findings are presently unclear. To our knowledge, no prior research in SSc patients has explored a link between antibodies against EBV antigen and viral DNA in the circulation. Previously EBV DNA has been detected in SSc lungs,[16] and one recent study has reported upregulation of viral load in the blood as a possible biomarker of vascular damage in SSc patients.[20] A discrepancy in the anti-EA antibody (increased) and DNA (unchanged) level in the

TABLE 4 Epstein–Barr virus (EBV) antigens and their role in the EBV life cycle[a]

Protein	Function
VCAgp125	Capsid antigen
VCAp19	Capsid antigen
EBNA-1	Latent state nuclear antigen
P22	Latent protein (capsid antigen)
EA-D	DNA polymerase accessory protein (early lytic cycle) (activation/reactivation)

[a]Summarized from references[15,28] and EUROLINE Anti-EBV profile 2.

blood was also observed in primary Sjögren's syndrome (pSS).[21] Patients with SLE and RA, on the other hand, have elevated levels of anti-EBV antibodies and DNA in their blood.[22]

The oropharynx and nasopharynx epithelial cells are the primary sites of infection for EBV. Following replication in the epithelial cells, EBV spreads in the underlying tissue and infects the B cells, mainly acting as a reservoir for the EBV virus.[23] EBV can cause persistent relapsing/reactivating infections, creating a constant challenge to the host due to its ability to transition between latent and lytic life cycles.[23] Studies show that other cell types can also become infected apart from epithelial cells and B cells.[14,24]

Farina et al found EBV viral messenger RNA and lytic proteins in the SSc patients' skin fibroblasts, myofibroblasts, and endothelial cells, implying EBV replication in the skin, and these cells may be a target of EBV infection.[14] In addition, EBV induces the conversion of fibroblasts to myofibroblasts through the TLR7/9 activation pathway.[14] EBV control deficits in SSc may be more localized and restricted to infected cells or tissues.

The dynamic interaction between EBV reactivation and an individual immune response possibly marks the development of a particular disease and its associated clinical symptoms depending upon the reactivation site and infected cells or tissues. For example, EBV reactivation in epithelial cells has been linked to SLE and SS, and reactivation in B cells with RA and MS.[15] Furthermore, some patients often develop multiple autoimmune diseases (overlap syndrome), possibly due to the shuttling of the EBV virus between epithelial cells, B cells, and other atypical cellular targets.

Thus, the study indicates a serological activation of EBV (reactivation stage) in SSc patients in the absence of EBV DNA in blood, suggesting the EBV reservoir is present somewhere else. We suggest reactivation of EBV in their potential cellular target/reservoir (epithelial/endothelial/fibroblast/mesenchymal cells), where it triggers a pro-fibrogenic environment, and impairment of EBV control may be compartmentalized and confined to affected cells. Hence, it is important to perform both serological and molecular tests to detect and monitor EBV infection in SSc patients. New treatments against EBV infection are the subject of ongoing research. Small interfering RNAs targeting EBV genes may be useful in downregulating expression and inducing apoptosis in infected cells, thereby preventing EBV reactivation or cell-based immunotherapies targeting EBV-infected transformed cells.[25] The source of the blood's anti-EBV humoral response should be examined, as the cellular source is unknown, which is a limitation of our study. Another limitation of the study is that we could not recruit any treatment-naive patients; being a tertiary referral center, all patients had received some immunosuppression treatment. Although previous studies on SLE[22,26] and RA[27] patients have reported that the frequency of EBV-infected cells is independent of treatment with immunosuppressive medications, we cannot exclude the possible role of immunosuppression on EBV reactivation in this study.

AUTHOR CONTRIBUTIONS

SM: executed the study, was involved in sample collection and processing, analyzed the data, and wrote the manuscript as part of her PhD thesis. SKS: provided the clinical samples and assisted in the clinical classification of patients, and reviewed the manuscript. YK: helped analyze data and reviewed the manuscript. SS: critically evaluated the manuscript. SA: contributed to experimental work. SH: helped in patient recruitment and relevant clinical information and reviewed the manuscript. RWM: responsible for study conception, design, drafting of the manuscript, and data analysis.

ACKNOWLEDGEMENT

The manpower for this work was supported by the Indian Council of Medical Research, New Delhi (3/2/February 22, 2018/Online Onco Fship/NCD-III).

CONFLICT OF INTEREST

Prof. Surjit Singh is an Editorial Board member of the journal and co-author of this article. He was excluded from the peer-review process and all editorial decisions related to the acceptance and publication of this article. Peer review was handled independently by members of the Editorial Board to minimize bias.

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How to cite this article: Machhua S, Sharma SK, Kumar Y, et al. Detection of Epstein–Barr virus in systemic sclerosis patients: A molecular and serological based study. *Int J Rheum Dis*. 2022;25:1431-1436. doi: 10.1111/1756-185X.14440

Received: 14 May 2022 | Revised: 1 August 2022 | Accepted: 18 August 2022

DOI: 10.1111/1756-185X.14430

International Journal of Rheumatic Diseases WILEY

CASE REPORT

Anti-MDA5 and anti-SSA/Ro52 antibodies double-positive dermatomyositis overlapping with rheumatoid arthritis-associated interstitial lung disease: A case report

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Abstract

Dermatomyositis (DM) is a poorly prognostic autoimmune disease the pathogenesis of which is multifactorial and not clearly defined. DM may be influenced by genes, environment, and immunity. The typical manifestations of DM are Gottron rash, heliotrope rash, rash on the shoulders and buttocks, erythema around fingernails, excessive keratosis of the epidermis, mechanic's hands, and interstitial lung disease (ILD), among others. Anti-melanoma differentiation-associated 5 gene (MDA5) antibody has been strongly associated with DM. Furthermore, anti-SSA/Ro52 antibody has been reportedly associated with DM. A 49-year-old woman presented with cough, expectoration, and dyspnea. Relevant examinations revealed elevated levels of muscle enzyme, double-positive anti-MDA5 and anti-SSA/Ro52 antibodies, positive rheumatoid factor, and a high titer of anti-citrullinated protein antibody. DM overlapping rheumatoid arthritis with ILD was confirmed. We suggest the use of glucocorticoids combined with immunosuppressant therapy, supplemented with gastric and liver protection, and recommend the use of intravenous immunoglobulins and rituximab.

KEYWORDS
anti-melanoma differentiation-associated 5 gene antibody, anti-SSA/Ro52 antibody, dermatomyositis, interstitial lung disease, rheumatoid arthritis

1 | INTRODUCTION

Dermatomyositis (DM) is a heterogeneous autoimmune inflammatory disease affecting the skeletal muscles and skin. Clinical manifestations include symmetrical proximal muscle weakness and typical skin lesions.[1] Anti-SSA/Ro52 and anti-melanoma differentiation-associated gene 5 (MDA5) antibodies appear to be associated with myositis.[2] Interstitial lung disease (ILD) is a relatively common complication of DM, often involving the alveolar wall and perialveolar tissues. ILD is characterized by interstitial inflammation and fibrosis of the lungs.[3] More than 50% of all cases of ILD associated with DM are positive for anti-MDA5 antibody.[4] There are few studies and cases of anti-MDA5 and anti-SSA/Ro52 antibodies. Here, we report a rare case of anti-MDA5 and

Hong Xiong and QianRen Tan are first authors.

anti-SSA/Ro52 antibodies double-positive DM with rheumatoid arthritis (RA)-associated ILD.

2 | CASE REPORT

A 49-year-old woman presented with a 5-year history of pain in both shoulders, knees, ankles, and especially in both palmar knuckles, with positive rheumatoid factor and high titer of anti-citrullinated protein antibody, consistent with a diagnosis of RA. The patient presented with cough and expectoration for half a month, with shortness of breath and dyspnea for 4 days. Physical examination revealed purple erythema around both orbits with edema (Figure 1A), large dark red plaques on the back, and scattered rash on the shoulder and left anterior chest, without ulceration or pruritus (Figure 1B). Additional observations were scatter in the skin of both hands, crusting, weakness in the proximal interphalangeal joints of both hands, joint deformation, and gooseneck deformity (Figure 1C). Her lips were slightly cyanotic, the thorax was symmetrical without deformity, respiratory mobility of both lungs was similar, respiratory sounds of both lungs were thick, and dry or wet rales were not heard in either lung. There was no deformity in the spine, redness and tenderness in multiple joints throughout the body, and high local skin temperature. Blood tests showed elevated muscle enzyme (Figure 1D) and positive anti-MDA5 and anti-SSA/Ro52 double antibodies (Figure 1E). Chest computed tomography revealed changes consistent with ILD (Figure 1F). Based on these results, we diagnosed double-positive DM with anti-MDA5 and anti-SSA/Ro52 antibodies with RA and ILD.

Treatments included methylprednisolone sodium succinate pulse therapy and oral hydroxychloroquine sulfate tablets to improve the symptoms of DM, oral pirfenidone to improve ILD, intravenous injection of pantoprazole sodium to inhibit acid and protect the stomach, and glycyrrhizin to protect the liver. After treatment with this regimen, the muscle enzyme index of the patient was still high. However, clinical symptoms significantly improved, no new rash developed, and joint pain, weakness, and other symptoms were significantly reduced. It was recommended that the patient receive intravenous immunoglobulins (IVIG) and rituximab (RTX), and that she and her family be discharged due to financial problems. One month later, the patient's family reported the patient's death.

3 | DISCUSSION

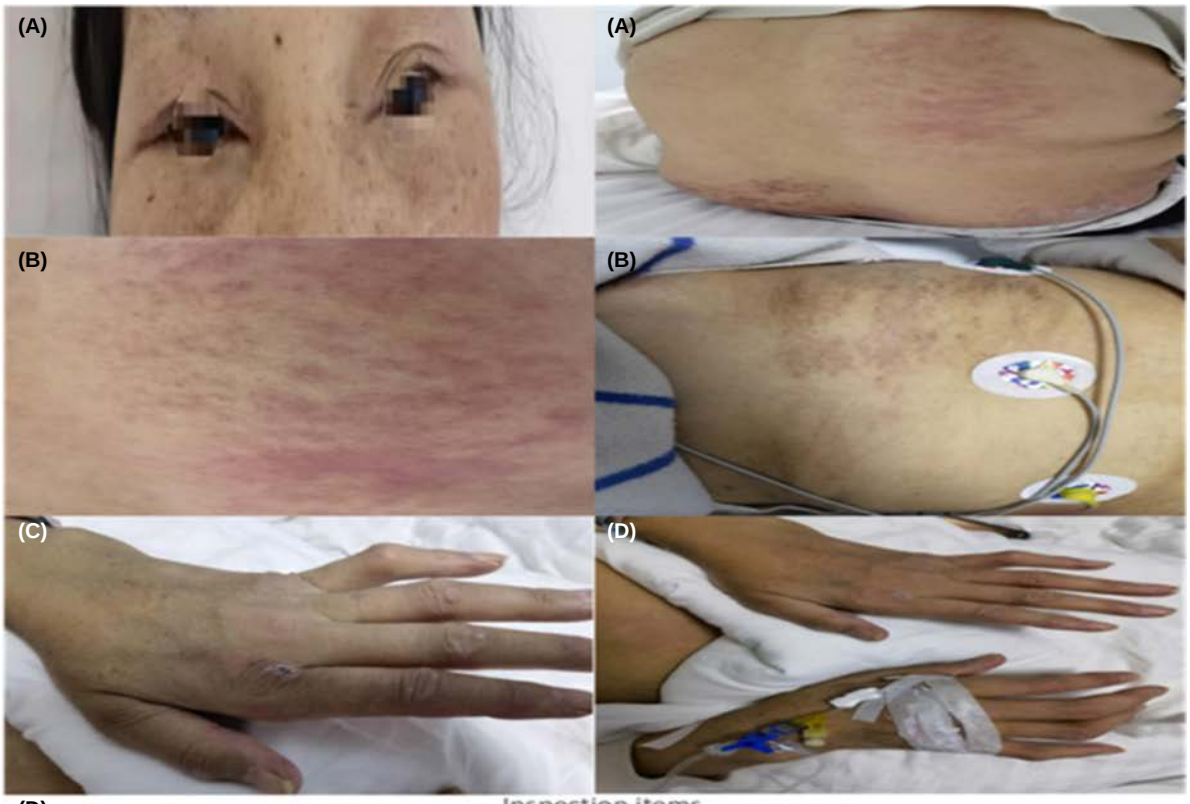
DM is a rare autoimmune disease that mainly affects the skin, muscles, and lung.[5] Myositis-specific antibodies are a new class of biomarkers that are valuable in the diagnosis, treatment, and prognosis of diseases. Anti-MDA5 antibody, formerly known as anti-CADM140 antibody, was initially identified in patients with clinically amyopathic DM (CADM) and are strongly associated with rapidly progressive ILD.[6] A strong association has been reported between anti-Ro52 and anti-MDA5 antibodies in DM, with a significantly lower survival rate of patients with double-positive anti-MDA5 and anti-Ro52 antibodies than that of patients with only positive anti-MDA5 antibody.[7] A North American adolescent myositis cohort study found that 70% of patients with double-positive DM with anti-MDA5 and anti-SSA/RO52 antibodies were diagnosed with ILD, suggesting that anti-SSA/Ro52 antibody was an independent predictor of ILD.[8] Compared to DM, RA is a common autoimmune disease. ILD is a serious pulmonary complication of RA, accounting for 10%–20% of the mortality rate, with an average survival time of 5–8 years.[9] An active marker of anti-MDA5 antibody-positive DM–ILD is a concern, and methioninemia is a predictor of poor prognosis.[10,11] A study in China showed that IVIG treatment reduced ferritin concentration, anti-MDA5 titer, and lung ground-glass opacity score, with a significantly lower 6-mortality rate in the IVIG group than in the non-IVIG group. However, treatment of ILD related to connective tissue disease involves case reports of patients receiving pirfenidone and nidanib as a definitive treatment. While the treatment was described as effective in improving symptoms, there was a lack of evidence-based detail.[12,13] Moreover, DM has a strong association with malignancy, and patients with DM have an increased risk of malignancy at the time of DM diagnosis and 10 years later.

A literature search was performed in PubMed for the last 5 years, using the terms "anti-MDA5 antibody," "anti-SSA/RO52 antibody," "DM," "RA," "ILD," and "case report". The search revealed 7 cases of anti-MDA5 antibody-positive DM with ILD, 1 case of RA with anti-MDA5 antibody-positive ILD, and 2 cases of anti-MDA5 antibody-positive DM with systemic lupus erythematosus. Anti-MDA5 antibody-positive DM overlapping with other rheumatic diseases is relatively rare, and there are currently no guidelines for the treatment of such diseases. The ideal drug for the treatment of DM is not clear. The treatment plan is mainly based on the results of clinical observation. The current cornerstone of DM therapy is glucocorticoids alone or in combination with an immunosuppressant,[14,15] among which cyclophosphamide, azathioprine, mycophenolate mofetil, calcineurin inhibitors, RTX combined with corticosteroids are also the choice of most clinicians.[15–17]

4 | CONCLUSION

In this case, 2 autoimmune diseases overlapped, and both led to ILD. The clinical characteristics, laboratory examination data, and immunological characteristics of the patient met both the European Neuro Muscular Centre 2018 DM diagnostic criteria and the

FIGURE 1 Patient clinical presentation, muscle zymogram, myositis antibody profile and imaging. (A) Typical positivity rash. (B) Typical shawl sign. (C) Gottron sign and joint deformities in rheumatoid arthritis. (D) Changes in muscle zymogram before and after treatment. (E) Positive example of antibody detection by double fluorescent cell transfection (MDA3784-MDA5-G, MDA3784-MDA5-M, MDA3784-MDA5-R) (titer 1:300). (F) Typical cellular shadow in interstitial pneumonia.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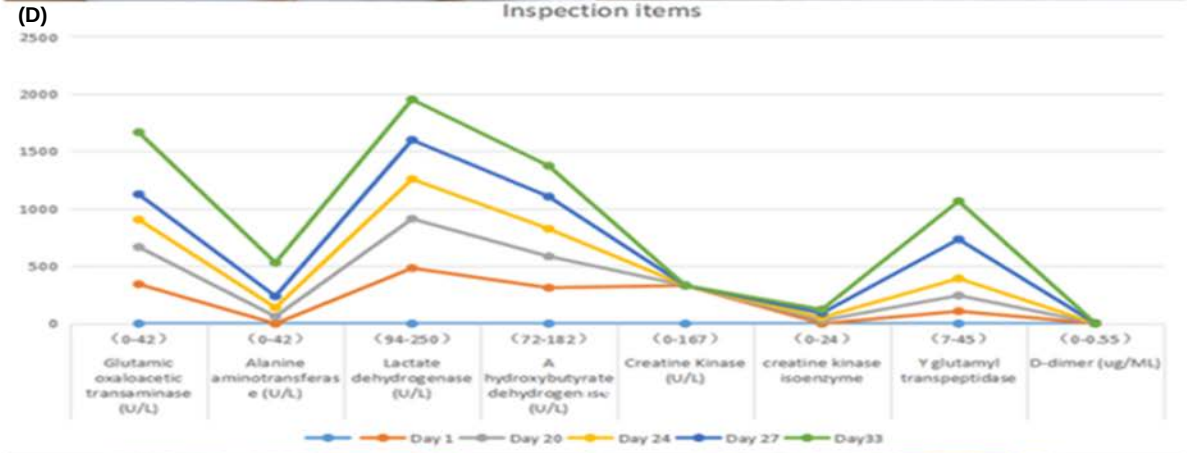
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A hydroxybutyrate dehydrogen ase (U/L)
(0-167)
Creatine Kinase (U/L)
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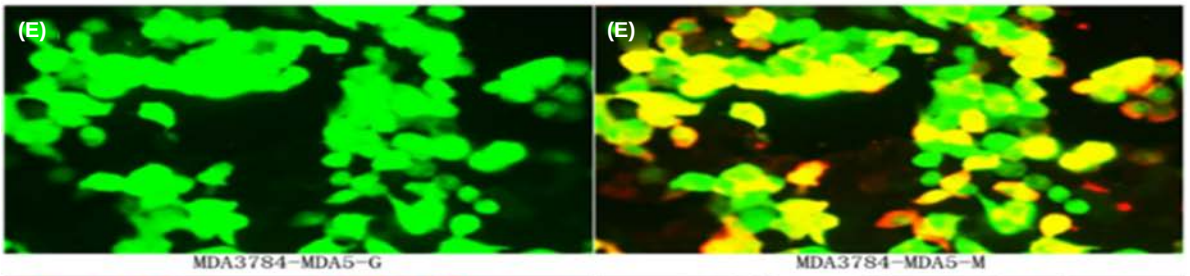
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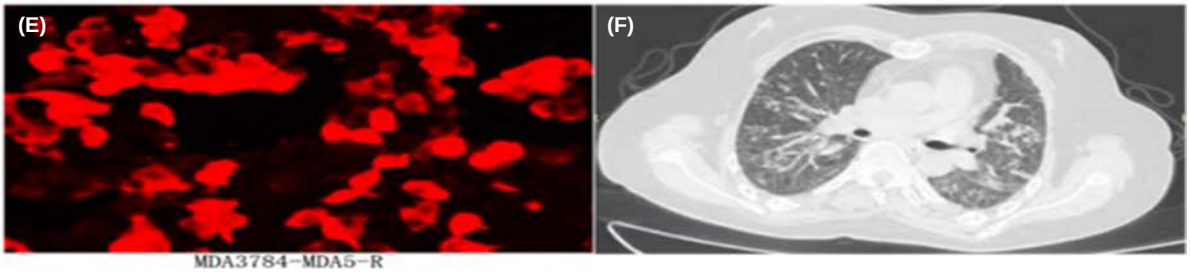
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2010 American College of Rheumatology / European Alliance of Associations for Rheumatology RA classification criteria.[18,19] Thus, the diagnosis was DM–RA overlap syndrome. The patient was previously healthy, and the only underlying disease before DM was RA. The patient complained of irregular medications for RA. As the disease progressed, DM overlapped. The specific cause of DM is still unclear, and more basic and clinical studies are needed to confirm it. The patient died 1 month after discharge from the hospital and failed to complete electromyography and muscle biopsy, which fully explained the rapid progression of the disease. To treat the disease, clinicians must develop a scientific treatment plan, and the patient must cooperate with the treatment to meet the standard procedure.

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How to cite this article: Xiong H, Tan Q, Luo F, Yuan X, Ma W, Yao X. Anti-MDA5 and anti-SSA/Ro52 antibodies double-positive dermatomyositis overlapping with rheumatoid arthritis-associated interstitial lung disease: A case report. *Int J Rheum Dis*. 2022;25:1437-1440. doi: 10.1111/1756-185X.14430

Received: 17 June 2022 | Revised: 5 September 2022 | Accepted: 22 September 2022

DOI: 10.1111/1756-185X.14452

International Journal of Rheumatic Diseases WILEY

CASE REPORT

Erythema nodosum with incidental calciphylaxis secondary to zoledronic acid and denosumab

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Abstract

Erythema nodosum (EN) is the most common clinical presentation of panniculitis, an inflammatory process that affects subcutaneous cellular tissue, characterized by the acute appearance of painful erythematous nodules predominantly in the lower extremities. An unusual case of EN is presented below, secondary to the administration of zoledronic acid (ZA) and denosumab, in which incidental histopathological findings of calciphylaxis were also found.

KEYWORDS
calciphylaxis, erythema nodosum, panniculitis, zoledronic acid

1 | CASE DESCRIPTION

A 55-year-old overweight woman with a history of endometrioid carcinoma, gastric bypass, hysterectomy and bilateral salpingo-oophorectomy, removed breasts fibroadenoma, secondary osteoporosis and T12 vertebrae pathological fracture, presented to the emergency department with a 9 days history of holocranial headache, fatigue and generalized myalgias after the administration of 5 mg zoledronic acid (ZA) for her osteoporosis treatment. Despite paracetamol management, pain in the inferior extremities exacerbated, limiting her gait. Physical examination revealed edema and erythematous nodules on both inferior extremities, painful to touch, of an average 10 mm diameter (Figure 1A,B). The rest of the physical exam was normal.

Chronic medications taken by the patient were 1500 mg calcium citrate +2200 IU vitamin D daily, vitamin B_{12} applied monthly and 60 mg denosumab application every 6 months. Initial imaging and laboratory studies revealed a lower extremities Doppler ultrasound negative for deep vein thrombosis, a normal chest X-ray, a normal blood count, a mild elevation of erythrocyte sedimentation rate (38 mm/h) and C-reactive protein (0.694 mg/dL). Renal function was preserved (blood urea nitrogen and creatinine 11 mg/dL and 0.52 mg/dL respectively), ionic calcium level was on the lower range limit. No other abnormalities on initial tests were found.

An erythema nodosum (EN) diagnosis was made, possibly due to a drug-related reaction given the temporal administration of ZA and the denosumab chronic use (Table 1). The patient was started on salicylic acid 500 mg three times a day, paracetamol and tramadol 1000 mg and 50 mg three times a day, respectively, and physical rest was recommended for management of EN. A punch biopsy showed fibroconnective tissue thickening on the septae separating fat lobules and intramural calcium deposit on subcutaneous tissue blood vessels (Figure 2A), which was confirmed by a Von Kossa stain (Figure 2B).

Due to the incidental histopathologic finding of calciphylaxis, more tests were performed, including kidney and liver functions, infectious diseases, immunological and endocrinological tests as well. A mild vitamin D deficiency (calciferol 20.6 mg/dL, reference levels 30–40 ng/mL), hypocalcemia (total serum calcium 7.8 mg/dL, reference range 8.8–10.6 mg/dL), ionic calcium (1.1 mmol/L, reference 1.16–1.32 mmol/L) and 24-hour severe hypocalciuria (12.5 mg/24 h, reference 100–300 mg/24 h) were found. No other laboratory abnormalities were documented.

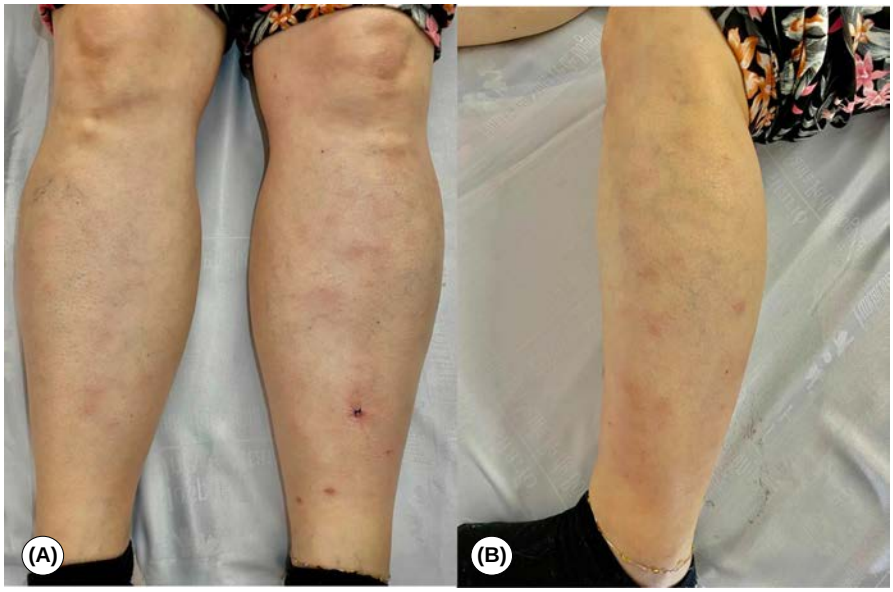

FIGURE 1 (A) and (B) Bilateral erythematous nodules on the pretibial region, lateral and posterior side of the legs. Biopsied zone is seen on the lower portion of the pretibial region on the left leg.

TABLE 1 Causes of erythema nodosum

	In favor	Against
Infections	—	IgM cytomegalovirus, IgM epstein barr virus, anti-hepatitis C virus and anti-human immunodeficiency vius negative
Drugs	Temporal relationship with ZA administration and symptomatic improvement with discontinuation of the drug. Denosumab having as target RANKL, causing RANK receptor inhibition, decreased bone resorption and down regulation of osteoprotegerin.	—
Sarcoidosis	—	Normal chest X-ray
Pregnancy	—	Hysterectomy and bilateral salpingo-oophorectomy
Inflammatory bowel disease	—	Absence of gastrointestinal symptoms
Vaccination	—	No recent vaccination
Autoimmune disease	—	Negative antinuclear antibodies and rheumatoid factor
Paraneoplastic syndrome	—	Acute presentation, absence of constitutional symptoms, resolution of symptoms with anti-inflammatory treatment

Abbreviations: IgM, immunoglobulin G; RANKL, receptor activator of nuclear factor kappa-B ligand; ZA, zoledronic acid.

Pharmacological treatment was continued with aspirin and analgesia, and calcium replacement was started with calcium citrate 1500 mg + vitamin D 200 IU daily. Two weeks after hospital admission the patient did not report more symptoms, and lesions showed improvement, so she was discharged home. A month post-discharge, a perilesional control skin biopsy showed normal fat cutaneous tissue and normal subcutaneous vasculature, no skin lesions were observed, thus it was considered a complete resolution of the condition.

2 | DISCUSSION

Erythema nodosum is the most frequently found clinical presentation of panniculitis. Typically, it presents as an acute eruption of erythematous, painful plaques or nodules, predominantly located on the pretibial region.[1] This entity may be idiopathic or triggered by multiple factors, such as infections, drugs, systemic diseases, or malignant neoplasms.[2] The overall prognosis is very good, and in most patients the lesions resolve almost completely without leaving any ulcer or residual scar.[1,2]

As mentioned earlier, drug-induced EN is an important etiology of this disease. In this particular case, it appears the ZA and denosumab together act as the trigger agents, the first due to its action on osteoclast activity and temporal relationship between the beginning of symptoms, and the second because of its receptor activator of nuclear factor kappa-B (RANK) inhibition, causing down-regulation of osteprotegerin. ZA is a bisphosphonate with high efficacy in binding bone hydroxyapatite, especially at sites where there is an elevated osteoclastic bone resorption.[3] It is widely known for its use in

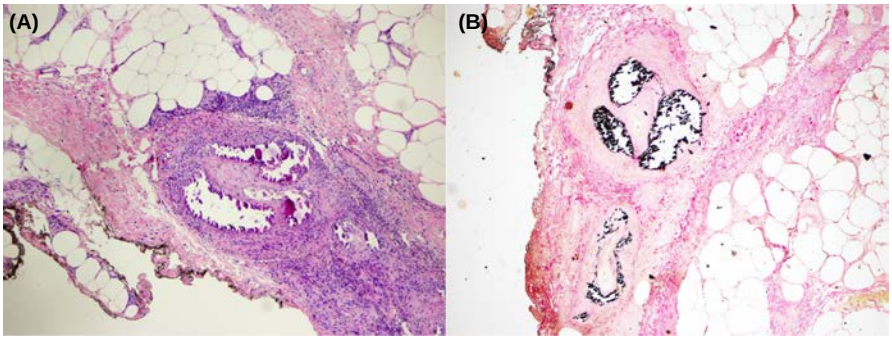

FIGURE 2 (A) Hematoxylin and eosin stain. Thickening of the fibrous connective tissue separating fat lobules and calcified material on medium- and small-caliber blood vessels. (B) Von Kossa stain which confirms calcification of the intimal region of the subcutaneous tissue arteries.

postmenopausal osteoporosis and among the frequently described side effects is osteomyalgia, arthralgia, gastrointestinal tract symptoms, and renal toxicity.[3,4] Cutaneous-reported side effects of ZA are scarce, and they range between pruritus and maculopapular rash, with a strong temporality that begins within the first 5–10 days of treatment initiation, and usually has a definite and limited course.[3] In mice there has been seen parathyroid hormone infusion associated with induced calciphylaxis,[5] therefore we believe ZA in conjunction with denosumab increasing RANK ligand expression caused calciphylaxis seen in this patient.

The diagnosis of EN is mainly clinical and it can be confirmed via histopathology.[2] Characteristically, as in this case, the subcutaneous fat septae are thickening and infiltrated, without signs of vasculitis.[1] However, it is striking that, additionally, we observed the presence of intramural calcium in the vessels of the subcutaneous cellular tissue with intimal proliferation and fibrosis, findings that are suggestive of calciphylaxis.[6] Calciphylaxis is a disorder that mainly affects patients with end-stage chronic kidney disease and is rarely seen in people with normal kidney function.[7] In the later, the main risk factors are: female gender, obesity, diabetes mellitus, hypercalcemia, hyperphosphatemia, hyperparathyroidism, supplementation with vitamin D and phosphate binders,[8,9] many of these encountered in this patient.

Although early calciphylaxis lesions may resemble EN, they tend to evolve into ulcers with eschar formation,[7] but this did not happen in our patient. Furthermore, in the control biopsy no persistent intramural calcifications were detected, which leads us to consider that it was a temporary finding concomitant to EN associated with the administration of ZA. Considering the pathophysiology proposed for EN, although still unknown, in this case, the ZA added to the denosumab usage, could act as a potential antigen that triggered a hypersensitivity reaction that generates the deposition of immune complexes in the venules of the septae of the subcutaneous fat.[2] The level of complexity that this case entails given the chronology of symptoms, the clinical presentation and the histopathological findings, invites us to identify this pathology earlier by recognizing possible triggers and risk factors, as well as the correct exclusion of other differential diagnoses, achieving adequate clinical and pathological correlation.

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How to cite this article: Pérez Haded I, Bayona D'vera JS, Blanco Espinoza AS, Llamas Castellanos BC, Rolón Cadena MC. Erythema nodosum with incidental calciphylaxis secondary to zoledronic acid and denosumab. *Int J Rheum Dis*. 2022;25:1441-1443. doi: 10.1111/1756-185X.14452

Received: 18 June 2022 | Accepted: 15 August 2022

DOI: 10.1111/1756-185X.14432

International Journal of Rheumatic Diseases WILEY

CLINICAL IMAGE

Tender cutaneous nodules of the legs—What is the diagnosis?

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A 51-year-old female, fish canning industry worker, with a personal history of arterial hypertension, dyslipidemia, and peripheral venous insufficiency, presented with a 3 month history of painful erythematous nodules with a scaly surface located bilaterally on the posterior side of her legs (Figure 1). No other concomitant symptoms and relevant epidemiological or family medical history were present. She reported no previous infections, trauma, or new medications. On physical examination, she had multiple tender nodules with perilesional erythema on the posterior-medial aspect of both legs. Laboratory studies revealed:

- Normal complete blood count;
- C-reactive protein 0.7 mg/dL (normal <0.5);
- Erythrocyte sedimentation rate 8 mm in first hour (normal <20);
- Normal results on renal, liver, and thyroid function tests;
- Normal levels of calcium (serum and urinary) and angiotensin-converting enzyme;
- Negative serology of human immunodeficiency virus and hepatitis B and C viruses and negative autoantibody test results;
- Tuberculin skin test was positive with 18 mm of induration;
- Chest X-ray: no acute pleuroparenchymal changes.

What differential diagnoses should be considered?

Tender cutaneous nodules of the legs are commonly seen in clinical practice. In view of this clinical presentation with several weeks of evolution, the following diagnoses should be taken into account: erythema induratum (EI), erythema nodosum, cutaneous polyarteritis nodosa, subcutaneous bacterial, fungal or mycobacterial infections, pancreatic panniculitis and malignant subcutaneous infiltrates.[1,2] A biopsy of a recent-stage lesion of the subcutaneous tissue is essential to specific diagnosis. Histopathological examination demonstrated predominant lobular panniculitis, granulomatosis with multinucleated giant cells of the Langhans type, and foci of caseification necrosis, aspects compatible with the diagnosis of EI (Figure 2A) and the search for acid-fast bacilli was negative. The combination of clinical signs, skin biopsy findings in conjunction with negative test results for lesional infection, and a positive tuberculin skin test led to the diagnosis of tuberculosis-related EI.

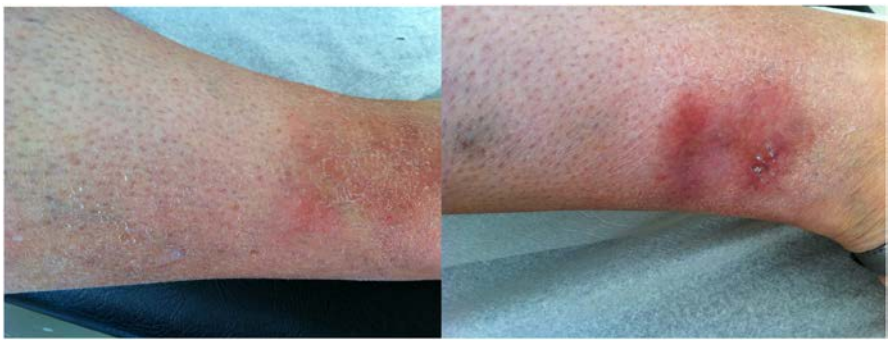

FIGURE 1 Macroscopic skin changes with tender, round, erythematous lesions localized to the lower limbs

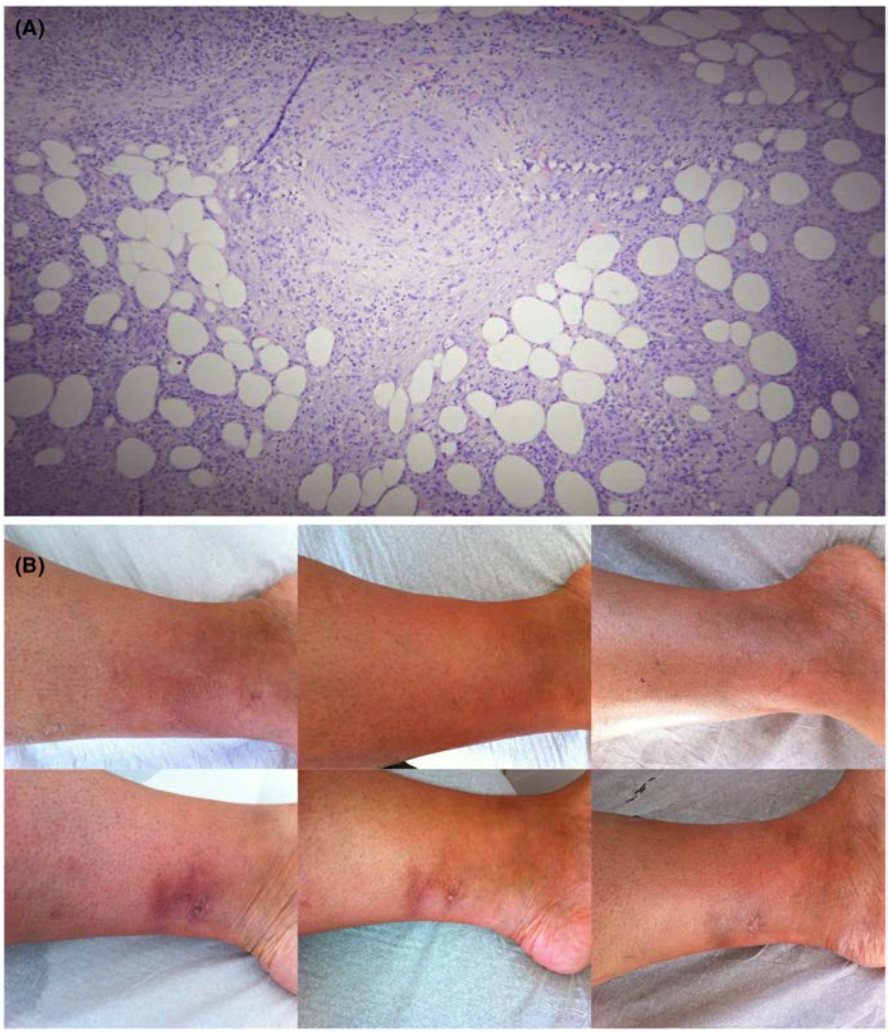

FIGURE 2 A, Histopathological findings of the skin biopsy tissue. B, Temporal evolution of skin lesions after initiation of treatment

Erythema induratum can be divided into three clinical variants: tuberculosis-associated EI, EI associated with other diseases and drugs, and idiopathic EI. Tuberculosis-associated EI is classified as a tuberculid, a type of hematogenous cutaneous tuberculosis. This condition is extremely rare, comprising 1%-1.5% of all extrapulmonary tuberculosis.[3,4] The treatment used in a tuberculid does not differ from the treatment of other types of tuberculosis, with a fixed-dose combination of anti-tuberculosis drugs for a period of 6 months. Over the months, the lesions improved, as shown in Figure 2B, and at the end of the treatment only a small scar and post-inflammatory hyperpigmentation were present.

CONFLICT OF INTEREST

None declared.

CONSENT

Patient consent obtained.

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Received: 12 July 2022 | Revised: 26 August 2022 | Accepted: 31 August 2022

DOI: 10.1111/1756-185X.14439

International Journal of Rheumatic Diseases

WILEY

CLINICAL IMAGE

COVID limb on FDG-PET/CT imaging

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1 | MYSTERY SUMMARY

A 79-year-old male patient is undergoing treatment for allergic respiratory disease in the pulmonology outpatient clinic. He was vaccinated with the first dose of Spikevax (Moderna) in the left deltoid muscle in early July 2021. Severe myalgia developed in the left upper limb the day after the vaccination. Neither neurological abnormalities nor skin lesions were identified. However, elevated serum creatine kinase level (458 U/L) was identified. Fluoro-2-deoxy-D-glucose (FDG) -positron emission tomography and computed tomography scan (PETC/CT) 2 weeks after the vaccination demonstrated hypermetabolism in the left shoulder with the forearm prominent (maximum standardized uptake value 7.5) but did not show lymphadenopathy in the axillary region (Figure 1, arrow). The symptoms of myalgia subsided gradually without medical intervention.

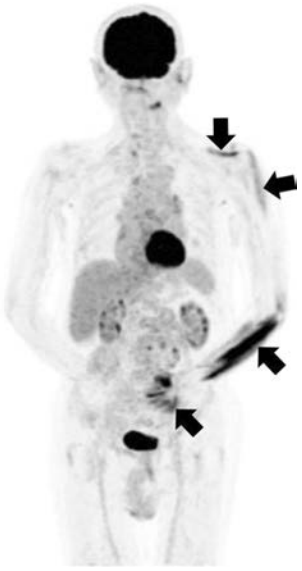

FIGURE 1 Fluoro-2-deoxy-D-glucose-positron emission tomography and computed tomography scan demonstrated hypermetabolism in the left shoulder with the forearm prominent but did not show lymphadenopathy in the axillary region

2 | ANSWER SECTION

COVID-19 arm, characterized by rash, myalgia, and tenderness surrounding the injection site, developed quite commonly after Spikevax injection.[1] Increased FDG uptake at the axillary lymph nodes on PET/CT scan has been noticed. Most cases of COVID-19 arm resolve spontaneously. Topical steroid and oral antihistamines are used in some individuals for symptomatic relief. Here, we report the Spikevax-relevant diffuse and persistent inflammation of muscles, illustrated by the FDG PET/CT scan. For this reason, we have named the image finding "COVID-19 limb".

AUTHOR CONTRIBUTIONS

All authors had access to the data and a role in writing this manuscript.

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How to cite this article: Kuo S-Y, Chu C-C, Lu C-C. COVID limb on FDG-PET/CT imaging *Int J Rheum Dis*. 2022;25: 1446-1447. doi: 10.1111/1756-185X.14439

Received: 29 July 2022 | Accepted: 30 July 2022

DOI: 10.1111/1756-185X.14420

International Journal of Rheumatic Diseases WILEY

COMMENT

Long-term safety and efficacy of methotrexate in patients with palindromic rheumatism

Dear Editor,

We read with great interest the article by Ghassembaglou et al. reporting the long-term outcomes of methotrexate (MTX)-based treatments for palindromic rheumatism (PR) using MTX.[1] We agree with the authors that MTX may be effective to relieve symptoms of PR, and would like to highlight some points.

In most real-world studies on MTX for rheumatic diseases including rheumatoid arthritis,[2] ankylosing spondylitis, juvenile idiopathic arthritis,[3,4] Sjogren's syndrome, psoriasis,[5] osteoarthritis,[6] and fibromyalgia[7], including the present study,[1] participants in most arms did not only receive MTX as a monotherapy. Instead, both groups took hydroxychloroquine (HCQ) at baseline. Additionally, neither HCQ nor MTX was the first-line drug for PR; as such, whether the participants used other first-line medications or treatments may be further included in the analysis. We look forward to seeing future studies conducting a subgroup analysis in which medications other than MTX are controlled during the entire course of treatment in order to eliminate the confounders, or at least analyses accounting for co-medications.[8]

In addition, the authors assessed the effect of MTX by measuring whether the participants stopped attacks and flare-ups for 12 weeks[1]. To augment such a novel finding, future studies adopting blood samples or imaging modalities including ultrasound and magnetic resonance imaging (MRI) may allow for the detection of serum anti-citrullinated protein antibodies (ACPAs)[9] and the presence of pannus or bone erosion, thereby providing more details on the response to MTX-based treatments in patients with PR.

To sum up, we are profoundly impressed by the significant study of treating PR patients with MTX.[1] Given the complicated medical history of PR, future studies taking co-medications into consideration and using measurements based on serology, inflammatory biomarkers,[10] and image findings may broaden our knowledge on the safety and efficacy of conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), including MTX, in patients with PR.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Received: 23 March 2022 | Revised: 25 August 2022 | Accepted: 22 September 2022

DOI: 10.1111/1756-185X.14453

International Journal of Rheumatic Diseases WILEY

APLAR GRAND ROUND CASE

Sarcoidosis with axial spondyloarthritis: A case-based review of simultaneous occurrence

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Abstract

This article presents a 47-year-old female patient who was concurrently diagnosed with sarcoidosis and axial spondyloarthritis. The coexistence of spondyloarthritis and sarcoidosis, the involvement of bone and sacroiliac synovium in sarcoidosis, and treatment options were discussed.

KEYWORDS
axial spondyloarthritis, common etiopathogenesis, co-occurrence, sarcoidosis

1 | INTRODUCTION

Although coexistence of sarcoidosis and axial spondyloarthritis (SpA) has been reported in the literature, simultaneous occurrence is very rare. At the same time, the majority of cases reported as simultaneous occurrence could not be classified as SpA according to The Assessment of Spondyloarthritis International Society (ASAS) criteria,[1] because of the lack of back pain features and imaging data.[2] In the case of asynchronous concomitance, the long interval between the onset of the two conditions supports the presence of a simple coincidence.[2] However, when the common pathological features of both diseases are taken into account, the possibility of an association between these diseases, especially when they occur simultaneously, comes to the fore. This study presents a 47-year-old woman who was diagnosed simultaneously with sarcoidosis and axial SpA. The clinical implications of the co-occurrence are discussed in light of the current literature by examining the pathophysiological mechanisms of both conditions.

2 | CASE REPORT

The patient, who had been suffering from low back pain for 5 years, presented to our outpatient clinic as her symptoms had been worsening for 6 months. The patient's history revealed that until the last 6 months the pain occurred occasionally and disappeared, especially when the patient was physically active. There was no nocturnal pain or pain that caused waking from sleep, and the patient's discomfort subsided when she rested. However, in the last 6 months, the pain had become severe and frequently woke the patient from sleep. The patient's complaints did not change with rest or exercise. The patient had lost 15 kg in the past 6 months and suffered from night sweats. The patient was not on a diet, and there was no other clinical history that could explain the weight loss (eg, diarrhea, depression, or medications). On physical examination, pain provocation tests for the sacroiliac joint were positive. No peripheral arthritis was noted. The straight leg raise test was negative bilaterally. Deep tendon reflexes were normoactive bilaterally and muscle strength was full. The tone of the paravertebral muscles was normal. Lumbar spine movements were severely limited by pain, so optimal assessment of range of motion was not possible. Laboratory tests revealed a C-reactive protein of 48.25 mg/L, an erythrocyte sedimentation rate of 40 mm/h, mild leukopenia (white blood cell count 3.74×10^9/L), and microcytic anemia (Hb: 11.86 g/L). HLA-B27 antigen and Brucella agglutination tests were negative. Magnetic resonance imaging (MRI) examination revealed multiple hyperintense lesions (osteitis) and chronic inflammatory findings in the short tau inversion recovery and T2-weighted sequences in the bilateral sacroiliac joints (Figure 1). Spinal MRI revealed hypointense signal changes in T1-weighted sequences and hyperintense changes in T2-weighted sequences extending from the endplates to the corpora throughout the spine (Figure 2). The patient was screened for malignancy due to the late age of admission, extensive involvement of the entire spine, marked acute-phase response, and existing

systemic symptoms. Breast and neck ultrasonography, mammography, computerized tomography of the neck, tumor markers, and gynecological examination were normal. Posteroanterior chest radiograph showed bilateral hilar lymphadenopathy and reticulonodular parenchymal lesions (Figure 3). Computerized tomography of the thorax showed mediastinal lymphadenopathies, patchy fibroatelectatic soft-tissue lesions with irregular margins in both lungs, and multiple centrilobular nodules. Abdominal computerized tomography showed lymphadenopathies in the celiac region. Lactate dehydrogenase, angiotensin-converting enzyme, and calcium levels were normal. Histological examination of mediastinal lymph nodes showed granulomas without necrosis and the patient was diagnosed with sarcoidosis. Cardiological, neurological, dermatological, cervical, and ophthalmological examination did not reveal any other sarcoidosis involvement. The patient was referred to a pulmonologist who did not initiate treatment for the disease, which appeared to be in remission. The patient was started on indomethacin treatment (150 mg/day) for low back pain and a dramatic response was obtained. The patient, who had bilateral active sacroiliitis on sacroiliac MRI, elevated C-reactive protein, and positive response to nonsteroidal anti-inflammatory drug (NSAID) treatment, was diagnosed with axial SpA according to ASAS criteria. Disease activity was classified as very high (both C-reactive protein and erythrocyte sedimentation rate) according to the ASAS-endorsed disease activity score (ASDAS).[3] The patient, who continued to take indomethacin, experienced headache and hypertension, so treatment was discontinued and diclofenac (150 mg/day) was started. After treatment, the night sweats and weight loss stopped, and the pain decreased significantly. The patient is currently followed up with low disease activity related to axial SpA under diclofenac treatment.

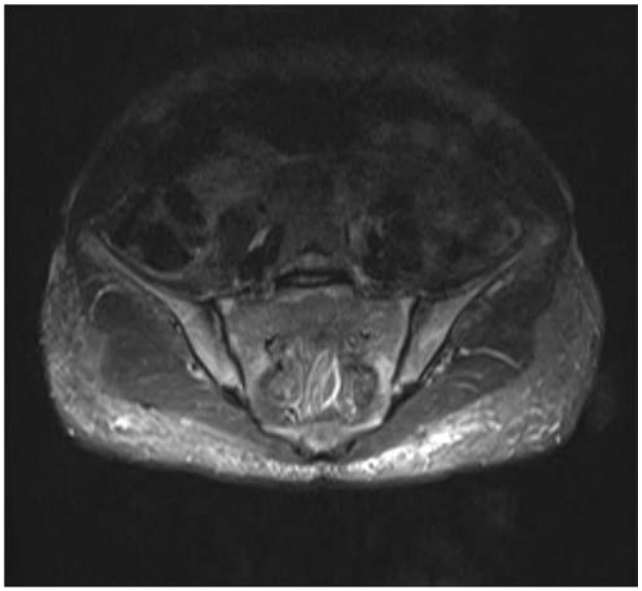

FIGURE 1 Sacroiliac magnetic resonance image: multiple hyperintense lesions (osteitis) in T2-weighted sequences in the bilateral sacroiliac joints.

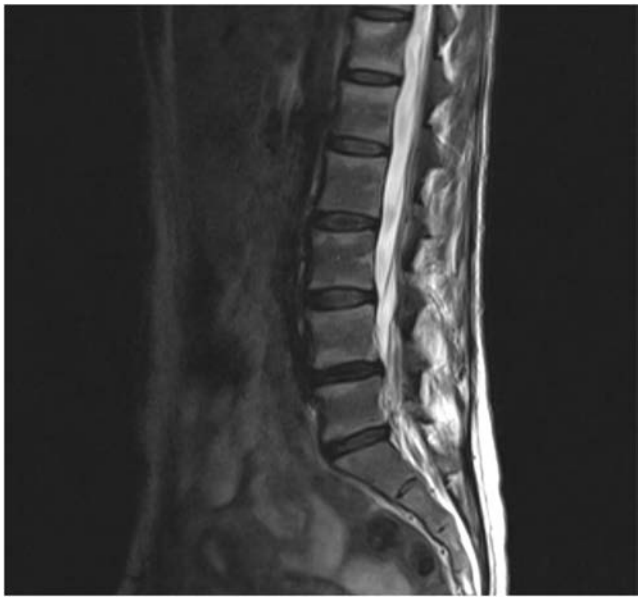

FIGURE 2 Spinal magnetic resonance image: hyperintense signal changes in T2-weighted sequences extending from the endplates to the corpora throughout the spine.

3 | DISCUSSION

Sarcoidosis is a granulomatous disease of unknown etiology and can affect all organs, especially the lungs, with mediastinal lymph nodes.[4] In the presence of lesions of the spine and sacroiliac joints associated with inflammatory low back pain in a patient with sarcoidosis, the following three possibilities may be considered:

1. A coincidence with SpA,
2. Involvement of the pelvis and vertebrae by sarcoidosis.
3. Involvement of the sacroiliac synovium by sarcoidosis.

Studies have shown that the incidence of axial SpA is increased 3.8-fold in patients with sarcoidosis compared with the normal population.[5] In SpA-sarcoidosis coincidence, patients are predominantly female and the presence of HLA-B27 antigen is about 46%.[2,6] Cross-sectional studies in the literature have shown inflammatory low back pain, and sacroiliitis in the pre-existing sarcoidosis population,[6–8] and the majority of case reports have also shown that sarcoidosis is added in patients who already have sacroiliitis.[9–12] The average 10-year interval observed in these studies can be interpreted as a finding suggesting the presence of two separate diseases.[2] However, the simultaneous occurrence raises the possibility that the two diseases are linked. The presence of high $CD4^+$ T-cell counts, interstitial lung disease, uveitis, and infliximab response in both diseases, as well as possible bacterial and viral infections, suggest the presence of common etiological and pathological mechanisms.[5,13–15] On the other hand, granulomatous lesions that may occur during anti-tumor necrosis factor therapy are drug-induced sarcoidosis-like reactions and should not be considered as a coincidence.[16]

Bone involvement in sarcoidosis occurs in 3%-13% of patients. About half are silent and are usually detected 2.8 years after the diagnosis of sarcoidosis. Patients with bone involvement are more likely to have mediastinal and extra mediastinal lymph node involvement, skin involvement, lung parenchymal involvement, and hypercalcemia. Pelvic bone involvement comprises 44% of all skeletal involvement.[17] Involvement of the pelvic bones in sarcoidosis tends to be unilateral and is associated with more aggressive and extra-mediastinal disease.[2] Vertebral bone involvement is characterized by lytic or osteoblastic patchy lesions, similar to metastatic diseases.[18,19] SpA, on the other hand, is characterized by lesions at the anterior-posterior vertebral body corners or by lesions spreading from the disk and endplates into the corpus.[20] Our case fits SpA involvement rather than sarcoidosis because of the osteitis predominantly located in the vertebral end plates and the absence of the patchy lesions.

Although it is theoretically possible for sarcoidosis to cause synovitis of the sacroiliac joint, this is controversial. This condition can only be distinguished from SpA coincidence by biopsy. To our knowledge, no biopsy-proven synovitis has been reported.

While infliximab has been successful in treating the coincidence of sarcoidosis and SpA, sarcoidosis recurrence has been observed with etanercept.[13,21,22] The increased number of T helper type 17 cells in granulomas suggests that interleukin-17 inhibitors may be beneficial in the treatment of sarcoidosis, and the beneficial effects of increased interleukin-17 production in Löfgren syndrome point to further studies on this topic.[23,24] Bone involvement of sarcoidosis responds well to steroid therapy, whereas a response to NSAIDs is typical for the presence of SpA.[17,20]

In our case, the absence of extrapulmonary involvement of sarcoidosis, the presence of bilateral sacroiliitis, the radiological morphometry of the spinal lesions, and the low back pain with a dramatic response to NSAID suggest a coincidence of sarcoidosis with SpA rather than its presence alone. Moreover, our case is unique in terms of the simultaneous occurrence of this coincidence. However, the presence of silent pulmonary sarcoidosis despite a very active axial SpA in our case suggests that these two conditions occur as two distinct diseases, although they are seen simultaneously. It is very likely that this situation was caused by a common trigger. It is known that the genetic disorders observed in both diseases are different (HLA class 1 in SpA and HLA class 2 in sarcoidosis). In this case, the common trigger could have activated a pathway other than the major histocompatibility antigens. The simultaneous coincidence in our HLA-B27-negative case also supports this contention. In this regard, further studies are needed to investigate non- major histocompatibility complex pathways in sarcoidosis-SpA coincidence.

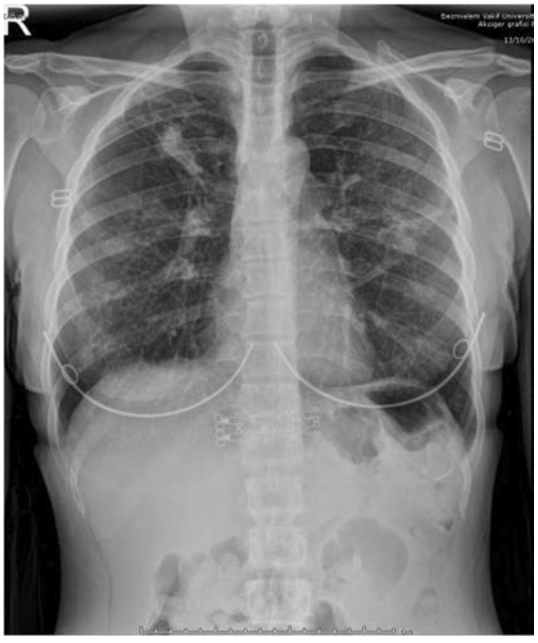

FIGURE 3 Chest X-ray: bilateral hilar lymphadenopathy and reticulonodular parenchymal lesions.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation and data collection were performed by SS and EK. The first draft of the manuscript was written by BI and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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How to cite this article: Ince B, Kultur E, Sayilir S, Kucukakkas O. Sarcoidosis with axial spondyloarthritis: A case-based review of simultaneous occurrence. *Int J Rheum Dis*. 2022;25:1450-1453. doi: 10.1111/1756-185X.14453