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LETTER TO THE EDITOR

Pelvic Floor Dysfunction in Systemic Lupus Erythematosus: Clinical and Laboratory Features, Risk Factors, and Prognostic Analysis

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Dear Editor,

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by pathogenic autoantibodies, systemic inflammation, immune complex deposition, and direct tissue damage [1]. Its clinical manifestations range from mucocutaneous lesions and arthralgia to life-threatening major organ involvement, imposing substantial burdens on patients' physical health, psychological well-being, and socioeconomic status [2]. SLE exhibits considerable population heterogeneity and a marked predilection for women, particularly those of childbearing age [3, 4]. Pelvic floor dysfunction (PFD) encompasses disorders such as urinary incontinence, defecatory dysfunction, and pelvic organ prolapse resulting from structural or functional impairment of the pelvic floor [5]. These conditions are highly prevalent among women in China and adversely affect both physical and mental health [6]. Limited public awareness of PFD and low healthcare-seeking behavior contribute to underdiagnosis and undertreatment in clinical practice [7]. Beyond established risk factors such as parity and obesity, connective tissue disorders have been increasingly recognized as contributors to PFD pathogenesis [8–10]. A higher prevalence of PFD has been observed in SLE patients compared with the general population [8]. However, data on the clinical characteristics and risk factors of PFD in this specific population remain scarce. In this study, we assessed PFD using three validated instruments: the Pelvic Floor Impact Questionnaire-7 (PFIQ-7), the Pelvic Floor Distress Inventory-20 (PFDI-20), and the International Consultation on Incontinence Questionnaire Short Form (ICI-Q-SF). This study aimed to characterize the clinical and laboratory features of SLE

patients with PFD, identify independent risk factors through multivariate logistic regression, and prospectively evaluate treatment outcomes and pelvic floor recovery.

We consecutively enrolled SLE patients from the Department of Rheumatology and Immunology at the First Affiliated Hospital of the University of Science and Technology of China between 2023 and 2025. All patients met the 2019 EULAR/ACR classification criteria and provided written informed consent [11]. The PFD group consisted of patients who satisfied two concurrent criteria: (a) at least one clearly reported PFD-related symptom (stress, urgency, or mixed urinary incontinence; a sensation of vaginal bulge, prolapse, or heaviness; defecatory difficulty or fecal incontinence; or chronic pelvic pain), and (b) a positive result at established cut-offs on the validated PFDI-20, PFIQ-7, and ICI-Q-SF instruments [12–14]. (c) In a subset of patients with prominent symptoms or diagnostic uncertainty, standardized diagnostic procedures (urodynamic studies, pelvic floor ultrasound) had been performed as part of routine clinical care. These were not mandatory diagnostic criteria but provided objective corroboration in a subset of patients. Controls were required to both deny all PFD-related symptoms and score negative on all three questionnaires. Exclusion criteria included: PFD attributable to alternative etiologies (such as iatrogenic injury, primary neurological disorder, pregnancy, or lactation), PFD predating SLE onset, incomplete data, or inability to complete assessments. After applying exclusion criteria, 109 eligible SLE patients with confirmed PFD constituted the case group. An

initial pool of 205 SLE patients without PFD was identified as potential controls. Following 1:2 propensity score matching based on age, BMI, obstetric history, and menopausal status, 107 cases and 191 controls were included in the subsequent conditional logistic regression analysis. Demographic and clinical data included age, BMI, obstetric history, menopausal status, and comorbidities. Clinical features encompassed disease duration, SLEDAI-2K score [15], and major organ involvement (interstitial lung disease, lupus nephritis, hemolytic anemia, antiphospholipid syndrome). Laboratory parameters included complete blood count, CRP, ESR, hepatic and renal function, 24-h urine protein, complement C3/C4, autoantibody profile (ANA, anti-dsDNA, anti-ENA), and lymphocyte subsets (CD4+, CD8+). Imaging reviewed included chest CT and echocardiography for interstitial lung disease, serositis, and pulmonary arterial hypertension. All patients were diagnosed with SLE according to the 2019 EULAR/ACR classification criteria. The presence and impact of PFD were assessed using three standardized instruments: the PFIQ-7, which evaluates the disease-specific quality of life; the PFDI-20, which quantifies symptom severity in the domains of pelvic organ prolapse, colorectal-anal dysfunction, and urinary distress; and the ICI-Q-SF, which measures the frequency and severity of UI. Statistical analyses were performed using SPSS version 27.0 and R version 4.5.2. Standardized mean differences were used to assess baseline balance between groups. Continuous variables are expressed as mean $\pm$ SD or median (IQR), with comparisons using *t*-test or Mann–Whitney U test. Categorical variables are presented as frequencies (percentages), compared by χ^2 or Fisher's exact test. Correlations were analyzed using Pearson's or Spearman's coefficient. Variables with *p* < 0.05 in univariate analysis were included

in multivariate logistic regression to identify independent predictors. For propensity score matching, a caliper width of 0.02 was used.

Among 109 SLE patients diagnosed as having PFD, 102 (93.6%) presented with urinary symptoms. Stress urinary incontinence (SUI) was the most prevalent condition, affecting 69.7% of cases, while urgency urinary incontinence (UUI) and mixed urinary incontinence (MUI) each accounted for 11.9%. Defecatory dysfunction was reported by 25 patients (22.9%), with diarrhea (10.1%) and constipation (9.2%) being the most frequent symptoms. Additionally, 21 patients described other pelvic symptoms, including abdominal distension or pain (11.0%) and uterine prolapse (8.3%). Among this cohort, the diagnosis of PFD was independently confirmed in 23 patients: 15 through standardized diagnostic procedures and 8 via previously documented medical records. After propensity score matching, 298 patients were included (107 PFD cases, 191 controls), with all covariates well-balanced (*p* > 0.05). As shown in Table 1, univariate analysis identified that SLEDAI-2K score (OR = 1.093, 95% CI: 1.032–1.159, *p* = 0.003), serum uric acid level (OR = 1.003, 95% CI: 1.000–1.005, *p* = 0.007), cumulative corticosteroid dose (OR = 1.015, 95% CI: 1.003–1.027, *p* = 0.013), ESR (OR = 1.012, 95% CI: 1.002–1.021, *p* = 0.016), and diuretic use (OR = 2.000, 95% CI: 1.112–3.599, *p* = 0.021) were significantly associated with the risk of PFD (*p* < 0.05). Arthritis showed a borderline significant association with increased risk (OR = 1.655, 95% CI: 0.999–2.742, *p* = 0.050). No significant associations were observed for CRP, interstitial lung disease, or positive Coombs test (*p* > 0.05). Multivariate analysis confirmed that, after further adjustment for other variables, only SLEDAI-2K score (OR = 1.078, 95% CI: 1.009–1.151, *p* = 0.026) and cumulative corticosteroid

TABLE 1 | Conditional univariate and multivariate logistic analysis for the development of PFD in patients with SLE.

Parameter	Univariate logistic analysis		Multivariate logistic analysis	
	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value
SLEDAI-2K	1.093 (1.032–1.159)	0.003*	1.078 (1.009–1.151)	0.026*
Uric acid, μ mol/L	1.003 (1.000–1.005)	0.007*	1.002 (0.999–1.004)	0.205
Cumulative glucocorticoid dose, g	1.015 (1.003–1.027)	0.013*	1.021 (1.008–1.035)	0.002*
ESR, mm/h	1.012 (1.002–1.021)	0.016*	1.009 (0.999–1.019)	0.066
Diuretics	2.000 (1.112–3.599)	0.021*	1.184 (0.595–2.357)	0.631
Arthralgia	1.655 (0.999–2.742)	0.050	/	/
Interstitial pneumonia	1.013 (0.998–1.028)	0.084	/	/
Coombs test positive	2.014 (0.823–4.929)	0.125	/	/
CRP, mg/L	1.545 (0.873–2.734)	0.135	/	/
Age, years	/	/	1.006 (0.965–1.048)	0.788
BMI, kg/m ²	/	/	1.010 (0.937–1.088)	0.791
Number of pregnancies, <i>n</i> (%)	/	/	1.210 (0.708–2.070)	0.486
Postmenopause, <i>n</i> (%)	/	/	1.121 (0.496–2.536)	0.783

Note: **p* < 0.05. Odds ratios (OR) with 95% confidence intervals (CI) from conditional univariate and multivariate logistic regression analyses identifying factors associated with PFD in patients with SLE. Odds ratios (OR) and 95% confidence intervals (CI) are presented with three decimal places to preserve precision, particularly for values whose interpretation (e.g., proximity to the null value of 1.00) could be affected by rounding. Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PFD, pelvic floor dysfunction; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index-2000.

dose (OR=1.021, 95% CI: 1.008–1.035, $p=0.002$) remained independent risk factors for PFD. Of the 109 eligible patients, 89 completed the 6-month follow-up, as 12 were lost to follow-up and 8 died during this period. Sixteen patients with clinically significant PFD symptoms received targeted pelvic floor intervention plus standard SLE treatment, while the remaining 73 received SLE-directed therapy alone and formed the longitudinal cohort. In this cohort, all four primary outcome measures demonstrated significant improvement from baseline. The proportion of patients with moderate to severe disease activity declined from 76.7% to 21.9%. Parallel improvements were observed in PFD-specific scores: the proportion reporting no symptomatic impact increased substantially on the PFIQ-7 (from 1.4% to 38.4%), PFDI-20 (from 1.4% to 28.8%), and ICI-Q-SF (from 4.1% to 37.0%). Median SLEDAI-2K score decreased significantly from 10.0 to 3.0 ($p<0.001$), with concomitant significant reductions in PFIQ-7 (9.5 to 4.8), PFDI-20 (14.6 to 8.3), and ICI-Q-SF (4.0 to 4.0) (all $p<0.001$). Correlation analysis revealed that Δ PFIQ-7 showed a significant positive correlation with Δ SLEDAI-2K ($r=0.362$, $p<0.01$), and Δ ICI-Q-SF demonstrated a weaker but significant correlation ($r=0.261$, $p<0.05$), indicating that improvement in pelvic floor symptoms tracked with SLE disease control, as shown in Figure 1.

Urinary symptoms, especially SUI, were the most common manifestations among 109 SLE patients with PFD, consistent with evidence linking a higher systemic immune-inflammation index to SUI risk [16]. Elevated ESR in our cohort supports inflammation as a key mechanism connecting SLE and PFD, further corroborated by altered pelvic floor cytokine expression (e.g.,

TNF- α , IL-6) [17]. Multivariable analysis identified SLEDAI-2K and cumulative glucocorticoid dose as independent predictors, indicating that active disease directly compromises pelvic floor integrity, while prolonged steroid use may exacerbate damage through muscle atrophy and osteoporosis [18]. These findings underscore the importance of early PFD screening in patients with high disease activity or long-term high-dose glucocorticoid exposure. Six-month follow-up showed that reductions in SLEDAI-2K were associated with significant improvements in PFIQ-7, PFDI-20, and ICI-Q-SF scores, with positive correlations between Δ SLEDAI-2K and Δ PFIQ-7/ Δ ICI-Q-SF. Although the change in PFDI-20 was not statistically significant, its downward trend suggests symptomatic benefit. These findings suggest that PFD in SLE reflects at least two partly distinct contributions. Longitudinally, improvement in pelvic floor symptoms tracked with reductions in disease activity, indicating that a component of symptom burden is linked to current, potentially reversible disease activity. In parallel, however, cumulative glucocorticoid exposure, rather than concurrent disease activity, was the factor that distinguished objectively confirmed cases from controls, consistent with a contribution from steroid-related structural and muscular damage that is less likely to be reversible. This dual pattern may explain why some patients experienced persistent pelvic floor symptoms despite SLE remission, and is further compatible with contributions from non-inflammatory factors such as age, parity, obesity, and psychosocial variables. Together, these observations support an integrated management strategy that combines control of SLE disease activity, judicious minimisation of cumulative glucocorticoid exposure, and tailored pelvic floor rehabilitation [19, 20].

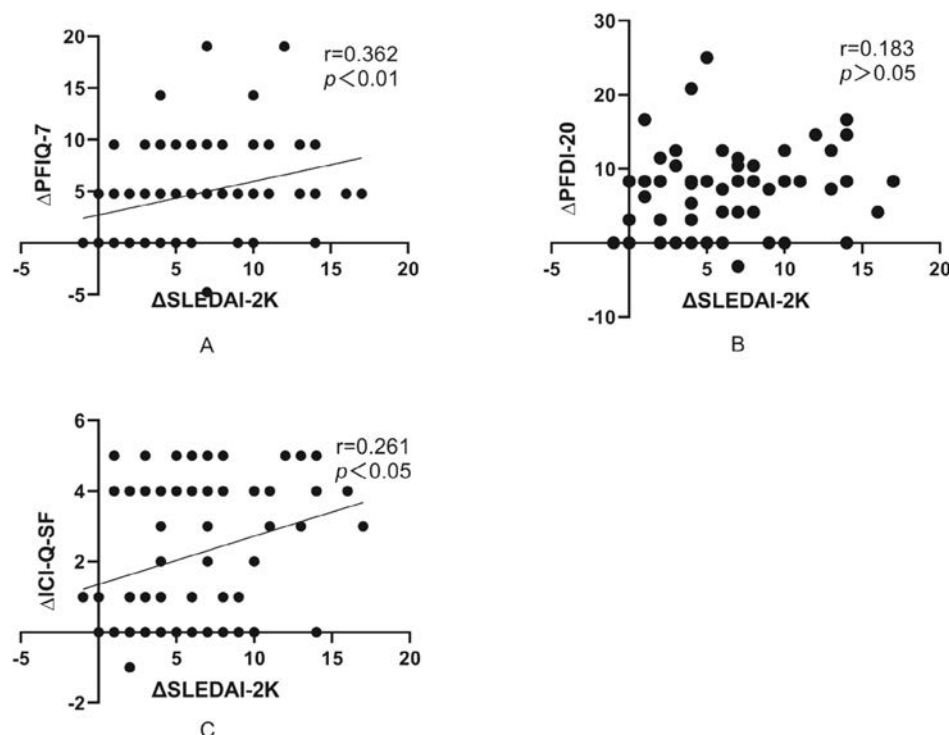


FIGURE 1 | Correlations between improvement in SLE disease activity and pelvic floor symptoms. Scatter plots show the relationship between the change in SLEDAI-2K (Δ SLEDAI-2K) and changes in (A) PFIQ-7, (B) PFDI-20, and (C) ICI-Q-SF scores. Solid lines represent linear regression. Significant positive correlations were observed with Δ PFIQ-7 ($r=0.362$, $p<0.01$) and Δ ICI-Q-SF ($r=0.261$, $p<0.05$). The correlation with Δ PFDI-20 was not significant ($r=0.183$, $p>0.05$). SLEDAI-2K, systemic lupus erythematosus disease activity index-2000; PFIQ-7, Pelvic Floor Impact Questionnaire-7; PFDI-20, Pelvic Floor Distress Inventory-20; ICI-Q-SF, International Consultation on Incontinence Questionnaire-Short Form.

Several limitations should be acknowledged. First, as a single-center and partly retrospective study, its generalizability requires validation in broader populations. Second, PFD assessment relied primarily on questionnaires. Objective structural or functional testing (e.g., three-dimensional pelvic floor ultrasound, urodynamic study) was available in only a subset and was not standardized across the cohort, potentially leading to misclassification of mild cases. We mitigated this by requiring concordant symptomatic and questionnaire-based evidence for cases and a symptom-free, questionnaire-negative definition for controls, and a sensitivity analysis restricted to the 23 objectively or records-confirmed cases confirmed that the association with cumulative glucocorticoid dose remained essentially unchanged (adjusted OR 1.019, $p=0.04$). Nonetheless, prospective studies incorporating standardized objective PFD measures such as urodynamic testing and pelvic floor ultrasound in larger, ideally multicenter cohorts are needed to confirm these findings. Third, because controls were defined by absent symptoms and negative questionnaire screening, some granular activity descriptors documented in cases (e.g., myositis, vasculitis) were not uniformly recorded in controls, and myositis occurred in only one case. These could not be compared across groups. As they are infrequent and not established PFD risk factors, their omission is unlikely to have affected our findings. Fourth, the moderate sample size, particularly the limited number of patients receiving targeted interventions, constrains the statistical power for subgroup and interventional analyses. Loss to follow-up and deaths during follow-up may introduce survival bias. However, non-completers differed from completers only in being older and having higher baseline inflammatory burden, so any such bias would most plausibly lead to modest overestimation rather than underestimation of longitudinal improvement. Fifth, our laboratory features were restricted to routine clinical parameters (autoantibodies, complement, ESR/CRP, and lymphocyte subsets). We did not perform advanced immunophenotyping including cytokine profiling, immune cell phenotyping of pelvic floor tissues, the Type I interferon signature, or BAFF quantification, nor did we investigate the underlying molecular mechanisms linking SLE and PFD. Future multicenter studies incorporating multidimensional functional assessment and these advanced immunological and molecular analyses are warranted to elucidate the pathophysiology of this comorbidity. Finally, given the observational design, our findings indicate association rather than causation. Residual confounding by disease severity cannot be fully excluded despite propensity score matching and multivariable adjustment, and prospective studies are needed to establish causality.

In summary, PFD in SLE patients predominantly presents as SUI. Our findings suggest two partly distinct contributions: a component linked to current, potentially reversible disease activity, and a component associated with cumulative glucocorticoid exposure, reflecting less readily reversible structural and muscular damage. Consistent with the former, PFD symptoms may improve as disease activity is controlled. Therefore, rigorous control of disease activity, judicious minimization of cumulative glucocorticoid exposure, and early screening and tailored intervention are crucial to prevent PFD and improve quality of life in this patient population.

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described in this article. All authors declare that they have no conflicts of interest relevant to this work. This study was approved by the Research Ethics Committee of the First Affiliated Hospital (Anhui Provincial Hospital) of the University of Science and Technology of China (approval number 2025KY-321). All participants provided written informed consent prior to enrollment.

Yours sincerely,

Zhu Chen, Cheng Peng.

Author Contributions

Yuanyuan Zhang: Investigation, formal analysis, writing – original draft. Zheng Fang: Investigation, data curation. Yuru Hu: Investigation, data curation. Cheng Peng: Conceptualization, methodology, resources, supervision, writing – review and editing. Zhu Chen: Conceptualization, methodology, resources, supervision, project administration, writing – review and editing. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets generated and/or analyzed during the current study are not publicly available owing to patient privacy and ethical restrictions. De-identified datasets may be obtained from the corresponding author upon reasonable request, subject to institutional ethical review approval.

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EDITORIAL

The Non-Coding Genome in Dermatomyositis: Alu Element Overexpression as a Potential Driver of the Interferon Signature

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1 | Introduction

Dermatomyositis (DM) has long been recognized as a distinct clinicopathological entity within the spectrum of idiopathic inflammatory myopathies (IIMs), primarily characterized by its profound type I interferon (IFN) signature [1]. As recent discussions on IIM classification have emphasized, conventional clinicopathological categories are increasingly being complemented by serological, genetic, and molecular frameworks that better capture disease heterogeneity [2]. However, the precise “upstream” trigger for this IFN surge has remained elusive. While viral mimicry has been hypothesized, recent breakthroughs in genomic sequencing—specifically the Telomere-to-Telomere (T2T) reference genome—have shifted our focus toward the “dark matter” of our own DNA: the noncoding genome [3].

2 | The “Viral Mimicry” From Within

A landmark study reveals that the pathogenic driver in DM may not be an external pathogen, but rather the uncontrolled expression of Alu elements [3]. These short interspersed elements (SINEs), which occupy approximately 10% of the human genome, have a high propensity to form endogenous double-stranded RNA (dsRNA). Under normal physiological conditions, the enzyme

ADAR (adenosine deaminase acting on RNA) “edits” these dsRNA structures—converting adenosine to inosine (A-to-I)—to prevent them from triggering innate immune sensors [4].

In DM muscle, however, a global elevation of Alu expression—including 577 unique Alu elements—appears to overwhelm the cellular editing capacity. This leads to an accumulation of unedited or insufficiently edited dsRNA that triggers MDA5-mediated inflammation [5]. MDA5 itself is also a known autoantigen in DM, thereby linking endogenous dsRNA sensing with the characteristic IFN cascade [6]. This internal “viral mimicry” model provides a compelling explanation for the prominent type I IFN signature of DM, supported by the observation that Alu overexpression appears relatively specific to DM and is not similarly enriched in other myositis subtypes such as inclusion body myositis (IBM) or antisynthetase syndrome (ASyS).

3 | Clinical Relevance and Methodological Rigor

The clinical implications of these genomic findings are substantial but still emerging. The correlation between Alu expression, interferon-stimulated genes, and inflammatory cell signatures suggests that the “Alu editome” may capture an important layer of immune activation in DM and could eventually contribute to

Tang-Kai Cheng and Teng-Chieh Hsu contributed equally to this work.

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molecular subtyping or disease monitoring. Furthermore, the enrichment of edited Alu elements on chromosome 19 highlights a nonrandom genomic architecture that may intensify dsRNA sensing and IFN activation in DM [3].

However, translating the Alu signature into clinical practice will require validation in larger, longitudinal, and disease-subtype-stratified cohorts. Future studies should carefully account for treatment exposure, disease duration, biopsy site, autoantibody status, and baseline disease activity to avoid confounding and bias when linking genomic signatures to clinical outcomes [7].

4 | Current Application and Future Directions

Currently, the clinical application of these findings lies in refining our diagnostic categories, improving molecular phenotyping, and generating testable hypotheses regarding therapeutic responses. The relative specificity of the Alu signature in DM supports the concept that molecular signatures may complement conventional clinicopathological and autoantibody-based classification frameworks in IIMs [2].

Moving forward, research must address the “why”: What triggers the sudden overexpression of these retroelements? Future research directions should include:

- Epigenetic regulation: Investigating alterations in DNA methyltransferase activity or other epigenetic factors that normally keep Alu elements silenced [8].
- Therapeutic editing: Exploring whether enhancing ADAR activity or utilizing RNA-targeted therapies can neutralize immunogenic dsRNA.
- Autoantibody pathogenesis: Determining whether interactions between immunogenic Alu-dsRNA and RNA-sensing proteins such as MDA5 contribute to the breakdown of immune tolerance and the development of myositis-specific autoantibodies.

5 | Conclusion

The discovery of the Alu-driven IFN signature marks a pivotal moment in myositis research. By integrating advanced genomic insights with rigorous clinical validation, we are moving closer to a future where dermatomyositis classification, monitoring, and treatment are guided by the molecular mechanisms that drive disease.

Author Contributions

Tang-Kai Cheng and Teng-Chieh Hsu drafted the manuscript. James Cheng-Chung Wei conceptualized the study, provided supervision, and critically revised the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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EDITORIAL

Fibromyalgia and Autoantibodies

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1 | Introduction

Fibromyalgia syndrome (FMS) is a clinical syndrome defined by chronic widespread pain accompanied by non-restorative sleep, cognitive difficulties colloquially termed “fibrofog,” profound fatigue, and a range of somatic and autonomic symptoms [1]. For more than three decades, the dominant explanatory model has centered on central sensitization; heightened excitability and reduced inhibition within central nociceptive pathways [2]. While this framework successfully accounts for many clinical observations, including temporal summation, secondary hyperalgesia and the frequent presence of comorbidities such as migraine and irritable bowel syndrome, it leaves important peripheral findings unexplained and has limited the development of mechanism-based therapies.

Diagnostic criteria themselves reveal biological heterogeneity. The 1990 American College of Rheumatology (ACR) classification criteria required the presence of at least 11 of 18 tender points together with widespread pain [3]. The subsequent 2010/2011 criteria, revised in 2016, abandoned the tender-point examination in favor of a widespread pain index (WPI) and a symptom-severity score (SSS) [4]. Patients classified by the two systems only partially overlap [5]. While the majority fulfill both sets of criteria, a non-trivial proportion satisfy only the 1990 or only the 2016 definition. Individuals meeting solely the 2016 criteria more often carry comorbid neuropsychiatric diagnoses including post-traumatic stress disorder, restless-legs syndrome, and chronic fatigue syndrome, suggesting that the 2016 phenotype may capture a more centrally driven or multisystem distress state, whereas the classic 1990 phenotype may be enriched for peripheral nociceptive drivers [5]. Differences in comorbidity do not by themselves establish distinct pain mechanisms; hence, should be regarded as hypothesis-generating. Recognition of this

heterogeneity is a prerequisite for interpreting serological and histopathological studies.

1.1 | Small-Fiber Pathology in Fibromyalgia

A substantial body of skin-biopsy evidence demonstrates that approximately 40%–50% of patients who fulfill contemporary fibromyalgia criteria exhibit reduced intra-epidermal nerve-fiber density (IENFD). The pattern is frequently non-length-dependent, distinguishing it from the distal predominant small fiber neuropathy (SFN) of diabetes or idiopathic length-dependent neuropathy [6]. These structural findings are best described as small fiber pathology rather than a classical, clinically defined small fiber neuropathy, and the two should not be equated. Some patients additionally display electrodiagnostic or clinical features suggestive of a chronic inflammatory demyelinating polyneuropathy (CIDP) spectrum disorder and have been reported to respond symptomatically to intravenous immunoglobulin (IVIg) [7]. Bilateral complex regional pain syndrome (CRPS), although a clinically distinct disorder, can share features with fibromyalgia, and such patients may fulfill the 1990 criteria. The two conditions may therefore share pathogenic mechanisms in selected individuals.

Autoantibodies directed against autonomic receptors have been identified in a subset of CRPS patients, particularly antibodies with functional activity at β_2 -adrenergic and muscarinic acetylcholine receptors [8]. Passive transfer of patient immunoglobulin into rodents can reproduce key sensory and autonomic features of CRPS, yet the phenotype is critically dependent on prior trauma to the recipient limb and on interleukin-1 signaling [9]. Thus antibodies alone are insufficient and they appear to amplify an already prepared peripheral substrate. Despite initial

enthusiasm for IVIg in both CRPS and fibromyalgia-associated SFN [7], larger randomized trials have generally failed to demonstrate consistent benefit, underscoring that antibody-mediated mechanisms operate only in a minority of patients and that additional disease-modifying factors are required.

1.2 | Potential for Antibodies to Cause Fibromyalgia and Pain

Analogous antibody mediated pain states provide important context. Anti-NMDA-receptor [10], anti-CASPR2 [11], and anti-GFAP [12] antibodies, best known for their association with autoimmune encephalitis and neuropsychiatric lupus, can produce severe neuropathic pain even when central inflammatory signs are modest. These observations establish that neuronal or glial surface antibodies are capable of generating pain phenotypes that clinically resemble fibromyalgia [13].

Experimental evidence for an autoantibody contribution to fibromyalgia itself emerged from passive-transfer studies. When IgG purified from the serum of patients with FMS is administered to mice, the animals rapidly develop mechanical and cold hypersensitivity, reduced spontaneous locomotor activity and a measurable reduction in cutaneous IENFD [14]. Parallel injections of IgG from healthy controls produce none of these changes. The sensory abnormalities resolve over subsequent weeks as human IgG is cleared, confirming that the effect is antibody dependent rather than the result of residual inflammatory mediators. Immunohistochemical analysis shows that patient IgG accumulates selectively within dorsal root ganglia (DRG). Binding is concentrated on satellite glial cells (SGCs); the specialized glia that completely envelop sensory-neuron somata, while neuronal binding itself is minimal. Concurrent upregulation of GFAP within SGCs indicates functional activation of these cells.

Subsequent quantitative studies in independent Swedish and Canadian cohorts have shown that a subset of fibromyalgia patients (approximately one-third to one-half) harbor elevated titers of IgG that bind cultured murine SGCs or human DRG tissue sections [15]. Patients with the highest anti-SGC IgG levels report significantly greater pain intensity on visual-analogue scales and higher scores on the Fibromyalgia Impact Questionnaire; they also exhibit lower pressure-pain thresholds. Antibody levels do not correlate with disease duration or with total circulating IgG, arguing against a non-specific acute-phase phenomenon. Interestingly, polysymptomatic distress scores that incorporate the symptom severity items of the ACR 2016 criteria tend to be higher among antibody-negative individuals. This dissociation implies that anti-SGC antibodies preferentially drive the sensory-pain dimension of the syndrome, whereas the broader symptom complex—fatigue, cognitive complaints, and affective distress—may be governed by additional central or systemic mechanisms. Significant overlap in binding intensity between patients and controls further indicates antigenic and titer heterogeneity. Not every patient with fibromyalgia possesses pathogenic anti-SGC antibodies, and not every antibody that binds SGCs is necessarily pathogenic [15]. Because the serology is cross-sectional, it cannot establish whether SGC binding drives pain or is instead a secondary epiphenomenon

of general neuroinflammation, and causality cannot be inferred from association alone. The identity and specificity of the SGC antigen also remain uncertain. Although passive transfer shows that patient IgG can induce a pain-related phenotype, and separate studies show that some patients harbor SGC-binding IgG that tracks with symptom severity [16], these findings do not by themselves establish that the SGC-binding antibodies are the pathogenic IgG fraction responsible for the transferred phenotype. Small-fiber pathology and anti-SGC antibody positivity may not fully overlap, with many patients exhibiting one without the other. So, the two should be quantified separately rather than assumed to coincide.

1.3 | How Antibodies to Satellite Glial Cells Produce Pain: The Role of Fc Receptors

The cellular and molecular cascade that links SGC-bound antibody to nociceptor hyperexcitability is only partially elucidated. SGCs and the sensory neurons they envelop are electrically and chemically coupled through gap junctions and paracrine signaling. Activated SGCs release ATP, pro-inflammatory cytokines, nerve growth factor, and other mediators that sensitize adjacent neuronal membranes. In addition, primary sensory neurons themselves can up-regulate Fcγ receptors, particularly FcγRI, after injury or under inflammatory conditions [17, 18]. Antibody bound to the surface of neighboring SGCs could therefore engage neuronal FcγRI, potentially producing membrane depolarization and a lowered threshold for action-potential generation. This pathway is, however, biologically plausible rather than demonstrated in fibromyalgia. A second Fc receptor of critical importance is the neonatal Fc receptor (FcRn). FcRn is constitutively expressed within the DRG and is responsible for the local accumulation and prolonged half-life of IgG [18]. Pharmacological blockade of FcRn with the engineered Fc fragment efgartigimod reduces IgG deposition in the DRG after experimental nerve injury and attenuates mechanical hypersensitivity in preclinical models [19]. Because these FcRn data derive from other neuropathic pain models rather than from fibromyalgia, they should be regarded as a working hypothesis. They nonetheless raise the possibility that FcRn antagonists could be therapeutically useful in antibody-positive fibromyalgia [20].

Taken together, the data support a multi-hit model of antibody-mediated fibromyalgia pain. A permissive peripheral “soil” (prior trauma, metabolic syndrome, small-fiber injury or genetic susceptibility) is required; an appropriate “seed” (autoreactive IgG of sufficient titer and specificity for SGC surface antigens) must be present; and intact Fc-receptor signaling pathways must amplify the resulting nociceptive traffic [19]. These elements may need to coincide for a sustained pain phenotype to emerge, although this is a proposed rather than an established requirement. Whether all three are obligatory is uncertain. Passive-transfer experiments suggest that, at least in rodents, sufficient titers of pathogenic IgG may induce a pain phenotype even when the other factors are not deliberately introduced [14]. The framework may nonetheless help explain why clinical trials that enrolled unselected fibromyalgia populations have yielded mixed or negative results [20], if the therapeutic window is restricted to the antibody-positive subset.

1.4 | Implications and Future Directions

Several practical consequences follow. First, measurement of IgG reactivity against both SGC and sensory neurons, currently performed by immunofluorescence on SGC-enriched cultures or human DRG sections, offers a potential biomarker for patient stratification [13–15]. Because it is not yet clear whether these antibodies are pathogenic, bystanders, or even protective, future fibromyalgia trials should incorporate antibody status as a stratifying biomarker to define and compare subgroups rather than assume pathogenicity. Antibody-positive individuals might be evaluated in trials of FcRn antagonists, B-cell-depleting agents, short courses of IVIg, or plasma exchange. Second, evidence that peripheral, antibody-associated mechanisms contribute to pain in at least a subset of patients challenges the exclusive reliance on centrally acting agents (antidepressants, anticonvulsants, cognitive-behavioral therapy) and invites a more balanced therapeutic algorithm. Third, the incomplete concordance between antibody status and overall symptom burden reminds clinicians that central sensitization, autonomic dysregulation, sleep fragmentation, and psychosocial factors continue to shape the clinical picture even when autoantibodies are present.

Critical knowledge gaps remain. The precise molecular identity of the SGC surface antigen(s) and of the neuronal antigens recognized by patient IgG is still unknown. Its identification would enable the development of specific immunoassays and antigen-targeted therapies. Longitudinal studies are needed to determine whether anti-SGC antibody titers fluctuate with disease activity or predict treatment response. Finally, rigorous, biomarker-stratified randomized trials of immunomodulatory interventions are required before clinical recommendations can be made.

In summary, converging lines of evidence: passive-transfer experiments, quantitative serology linking antibody titer to pain severity, and mechanistic studies of Fc receptors, indicate that circulating IgG, including antibodies that bind satellite glial cells and sensory neurons, can contribute to pain-related mechanisms in at least a subset of patients with fibromyalgia. It would, however, be premature to conclude that a substantial fraction of fibromyalgia is specifically driven by these antibodies or by a defined antibody–Fc-receptor pathway. The process is neither universal nor sufficient in isolation. It operates within a framework that also encompasses small fiber pathology, genetic predisposition and central amplification. The traditional view of fibromyalgia as an exclusively central disorder therefore requires substantial revision. The enrichment of the 2016-only phenotype for neuropsychiatric comorbidity, together with its apparent underrepresentation of anti-SGC antibodies, is consistent with the 2016 criteria capturing a more centrally driven phenotype distinct from the peripherally enriched 1990 phenotype, although this mapping requires direct testing. Taken together, the evidence reinforces the view that fibromyalgia is likely not a single disease but a syndrome of multiple, distinct etiologies. Future research that identifies the relevant antigens, refines diagnostic assays and tests selective interruption of the antibody–Fc-receptor axis holds the promise of genuine mechanism-based therapy for this historically enigmatic condition.

Author Contributions

Lekshmi Minikumari Rahulan: methodology, visualization, writing – original draft, writing – review and editing. **Able Lawrence:** conceptualization, methodology, visualization, writing – review and editing, supervision.

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Conflicts of Interest

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Data Availability Statement

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LETTER TO THE EDITOR

Case Report: Cryoglobulinemic Vasculitis Relapse With Peripheral Neuropathy During HDV-RNA Positivity Despite HBV-DNA Suppression

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Dear Editor,

Cryoglobulinemic vasculitis is most commonly associated with hepatitis C virus infection, whereas hepatitis B virus (HBV)-related cases are uncommon and HBV/hepatitis delta virus (HDV) coinfection-associated cases remain exceedingly rare [1, 2]. HDV requires hepatitis B surface antigen (HBsAg) for assembly and propagation and is associated with more aggressive chronic viral hepatitis [3]. Whether active HDV replication can contribute to extrahepatic immune complex-mediated disease despite HBV-DNA suppression remains unclear. We report a case of cryoglobulinemic vasculitis relapse with new electrophysiologically confirmed peripheral neuropathy, occurring in temporal association with high-level HDV-RNA positivity despite persistently suppressed HBV-DNA.

A 62-year-old HBsAg-positive male patient was evaluated in 2019 for purpuric lesions on both lower extremities. Skin biopsy revealed leukocytoclastic vasculitis. Direct immunofluorescence demonstrated IgM, C3, and fibrinogen deposition along vessel walls (Figure 1A–C). Rheumatoid factor (RF) was 2090 IU/mL. Antineutrophil cytoplasmic antibody and anti-cyclic citrullinated peptide antibody were negative. No monoclonal band was detected in serum or urine. HBV-DNA was undetectable by quantitative PCR, anti-HCV was negative, total anti-HDV and anti-HDV IgM were negative, and HDV-RNA was undetectable. The markedly elevated RF level, in the absence of clinical or radiological evidence of arthritis, supported mixed cryoglobulinemic vasculitis rather than rheumatoid arthritis. The initially negative cryoglobulin result was not considered sufficient to exclude cryoglobulinemia, given the marked sensitivity of the assay to preanalytical handling conditions [4, 5]. These findings

were considered compatible with probable HBV-associated mixed cryoglobulinemic vasculitis. Because of HBsAg positivity and ongoing immunosuppression, tenofovir was administered as HBV-active antiviral therapy during immunosuppression from 2019 onward [6]. Methylprednisolone and azathioprine were also administered.

In July 2025, the patient presented with extensive lower-extremity purpura and paresthesia in the feet while continuing tenofovir therapy, after discontinuing methylprednisolone and azathioprine approximately one month earlier against medical advice. RF was 2220 IU/mL, C4 was 0 mg/dL, and C3 ranged between 43 and 55 mg/dL. HBsAg remained positive and HBV-DNA was again undetectable; however, HDV-RNA was positive at 1327970 copies/mL. During relapse, HCV and HIV testing were negative, serum and urine immunofixation showed no monoclonal component, liver tests were normal, and no hepatic decompensation was evident. HDV genotype was unavailable. Because all HDV markers were negative in 2019 and none were available until 2025, newly acquired infection could not be distinguished from infection previously below assay detection. FibroScan, liver biopsy, and formal fibrosis stage were unavailable.

A repeat cryoglobulin test performed under appropriate conditions was positive: the sample was transported at 37°C, a macroscopic precipitate formed after 3 days at 4°C, and the precipitate dissolved upon reheating to 37°C (Figure 1D,E). Cryoglobulin typing and cryocrit measurement could not be performed for technical reasons. Electrophysiological testing demonstrated a sensory-predominant, mixed axonal and demyelinating

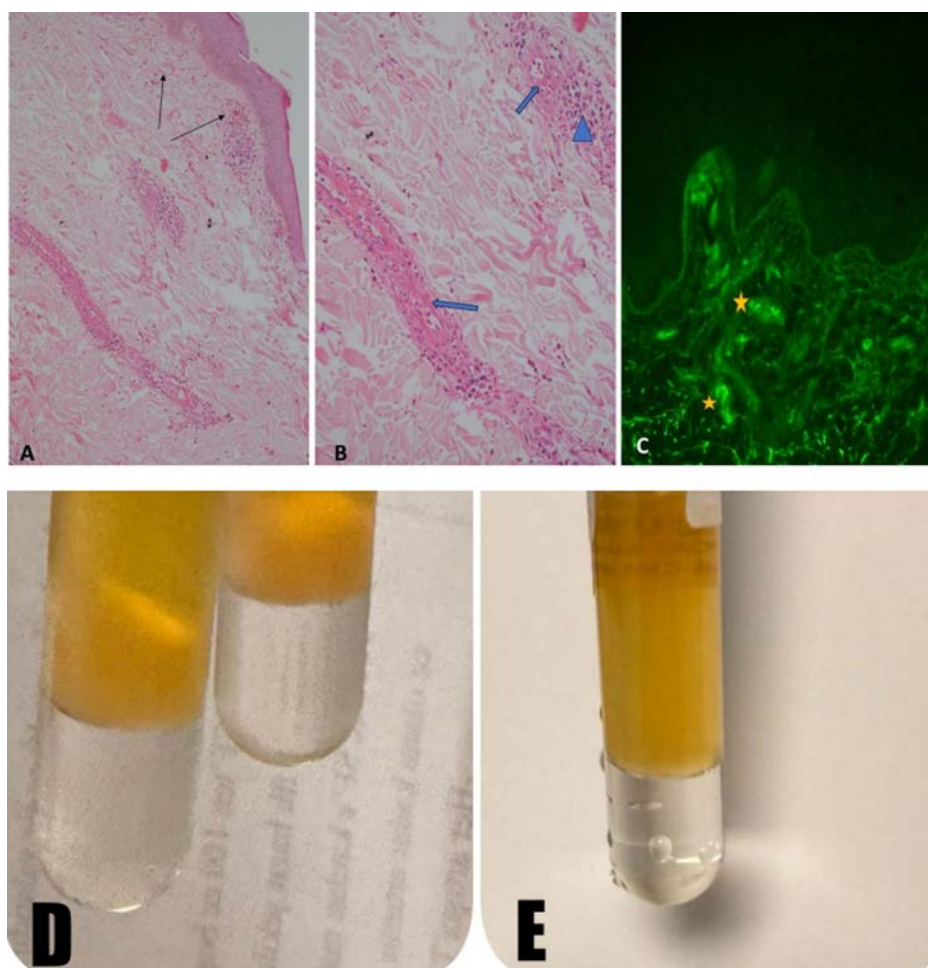


FIGURE 1 | Histopathological, immunofluorescence, and cryoglobulin test findings. (A) Skin biopsy showing normal epidermis and erythrocyte extravasation in the upper dermis. (B) Fibrinoid necrosis of the vessel wall, perivascular polymorphonuclear leukocyte infiltration, and leukocytoclasia. (C) Representative direct immunofluorescence image showing fibrinogen deposition in the vessel wall; IgM and C3 deposition were also detected in the same examination. (D) Macroscopic precipitate formed after the sample was transported at 37°C and incubated at 4°C for 3 days. (E) Dissolution of the precipitate upon reheating to 37°C supports the presence of cryoglobulins.

peripheral polyneuropathy. The normal 2022 study supported new neuropathy during relapse. Alternative explanations were considered: HCV and HIV testing were negative, immunofixation showed no monoclonal component, and renal function and urinalysis were unremarkable. The close temporal concurrence of new neuropathy with purpura, cryoglobulin positivity, profound C4 consumption, and markedly elevated RF made cryoglobulinemic vasculitis the most likely explanation.

Tenofovir was continued, and systemic glucocorticoid therapy equivalent to prednisolone 1 mg/kg/day was initiated. The purpuric lesions markedly regressed; however, neurological symptoms persisted. Consensus recommendations support rituximab for cryoglobulinemic neuropathy [7]. Because the disease was severe but not immediately life-threatening, targeted B-cell therapy was favored over broader cytotoxic immunosuppression with cyclophosphamide; limited experience also supports rituximab in HBV-associated Type II cryoglobulinemia [8]. Because B-cell depletion carries a substantial risk of HBV reactivation in HBsAg-positive patients, treatment was coordinated with hepatology; tenofovir was continued, HBV-DNA remained suppressed, and close biochemical and virological monitoring was

undertaken [6]. Rituximab was given as two 1-g intravenous doses 2 weeks apart [7]. No new purpuric lesions developed after treatment.

At follow-up in February 2026, the cryoglobulin test became negative, and HBV-DNA remained undetectable. Although HDV-RNA positivity persisted, the viral load decreased to 387 470 copies/mL. The patient remains clinically stable while receiving low-dose methylprednisolone, tenofovir, and symptomatic treatment for neuropathic pain. The longitudinal disease course is summarized in Figure 2.

Taken together, the concurrence of high-level HDV-RNA positivity with cryoglobulin positivity, marked C4 consumption, purpuric relapse, and new electrophysiologically confirmed peripheral neuropathy raises the possibility that active HDV replication may contribute to cryoglobulinemic vasculitis activity in selected HBsAg-positive patients. This temporal association is clinically relevant but does not establish causality.

The inability to type the cryoglobulin prevented distinction between Type II and Type III mixed cryoglobulinemia and

Clinical course and virological timeline

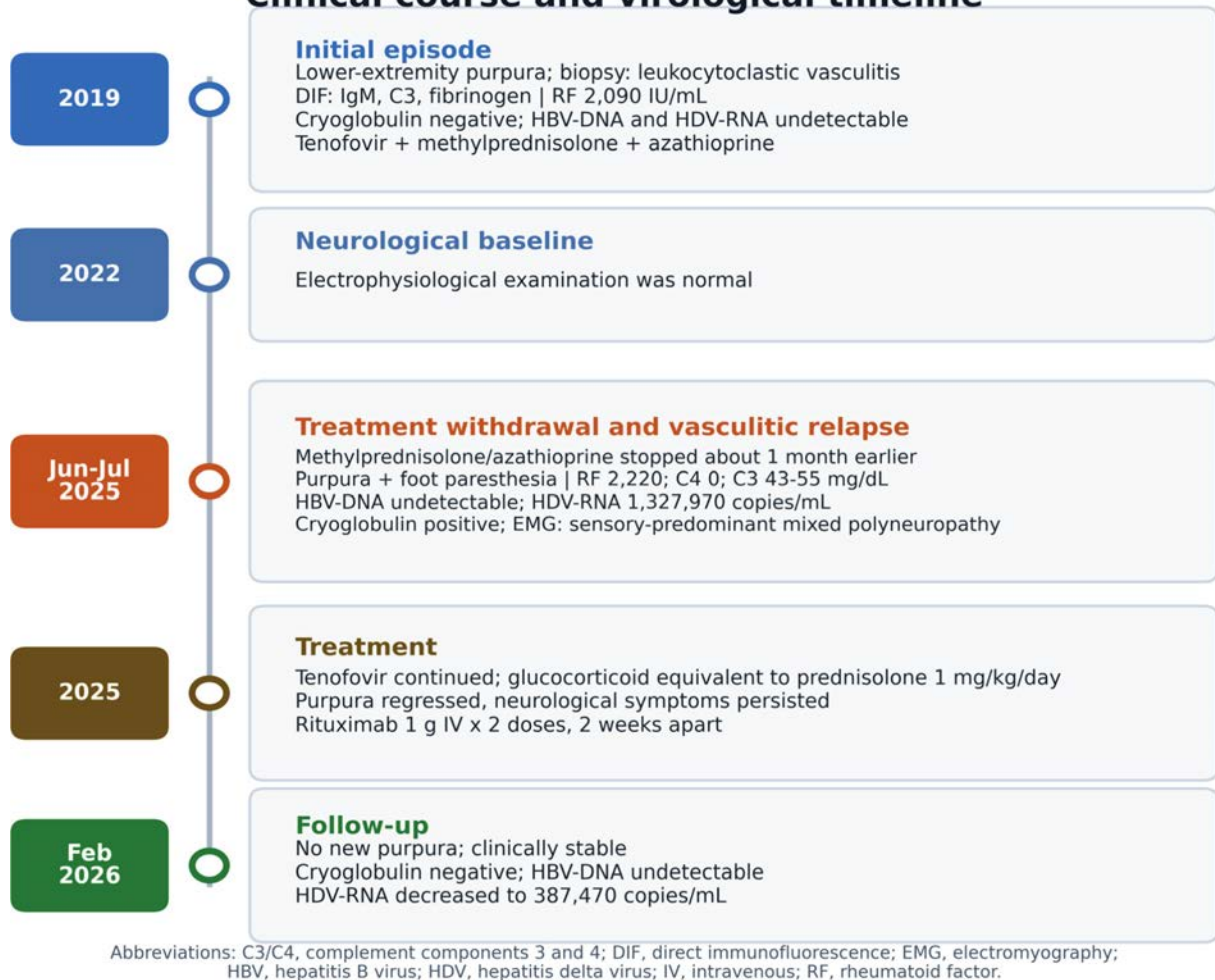


FIGURE 2 | Chronological course of cryoglobulinemic vasculitis, virological findings, treatment, and follow-up. The timeline summarizes the initial presentation, baseline neurological assessment, 2025 relapse, immunosuppressive treatment, and February 2026 follow-up.

determination of whether the cryoprecipitate contained a monoclonal IgM rheumatoid factor component. Accordingly, whether the process reflected a predominantly clonal B-cell response could not be determined, and a specific HBV/HDV-related immune-complex mechanism could not be characterized [4, 5]. The absence of cryocrit measurement also prevented quantitative correlation of cryoglobulin burden with disease activity and treatment response. Additional limitations include the absence of nerve biopsy, incomplete retrospective documentation of a comprehensive metabolic and toxic neuropathy evaluation, unavailable HDV genotype, inability to distinguish newly acquired from previously undetected HDV infection, and absence of formal fibrosis staging by elastography or liver biopsy. Because the relapse occurred after discontinuation of methylprednisolone and azathioprine, withdrawal of immunosuppression may also have contributed. HDV replication should therefore be interpreted as a temporal association rather than evidence of causality.

Therefore, in HBsAg-positive patients at risk for HBV/HDV infection, cryoglobulinemic vasculitis should be actively investigated in the presence of purpura, high RF levels, C4 consumption, and peripheral neuropathy; HDV-RNA should be

assessed even when HBV-DNA is suppressed. In severe neurological involvement, rituximab may be considered with concurrent high-barrier HBV-active antiviral therapy and close hepatological-virological monitoring. The potential role of HDV-directed antiviral therapy, including bulevirtide, should also be considered in collaboration with hepatology, although evidence in HDV-RNA-positive cryoglobulinemic vasculitis remains limited [2, 3].

Author Contributions

Fuat Albayram: conceptualization; data curation; writing – original draft; writing – review and editing. **Servet Yolbaş:** investigation; resources. **Sezgin Zontul:** writing – review and editing. **Rabia Sağır:** writing – review and editing. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Funding

The authors have nothing to report.

Consent

Written informed consent was obtained from the patient for publication of this case report and the associated clinical, laboratory, histopathological, and cryoglobulin test images for scientific purposes.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting this case report are not publicly available due to patient confidentiality and ethical restrictions. They may be made available from the corresponding author upon reasonable request and subject to appropriate ethical approvals.

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LETTER TO THE EDITOR

Case Report: Childhood Scleredema Following Streptococcal Infection Mimicking Systemic Sclerosis

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Correspondence: Bablu Kumar Gaur (drbkgaur@gmail.com)**Received:** 5 June 2026 | **Revised:** 2 September 2026 | **Accepted:** 14 September 2026

Dear Editor,

Childhood scleredema is a rare entity characterized by diffuse, non-pitting induration of the skin, most commonly occurring in children following streptococcal infections [1]. Its clinical resemblance to systemic sclerosis often leads to diagnostic confusion [2]. Unlike scleroderma (systemic sclerosis), the hands and feet are usually spared, and there is no associated Raynaud's phenomenon or internal organ involvement. However, due to overlapping features such as skin thickening and tightening, scleredema may closely mimic scleroderma, leading to diagnostic confusion [3, 4]. We report a case of a 6-year-old female who presented with progressive skin thickening over the face, neck, and chest and upper back following a recent episode of fever with sore throat. The initial clinical suspicion was systemic sclerosis or scleroderma; however, further evaluation revealed negative autoimmune markers, including anti-Scl-70, and elevated antistreptolysin O titers. Skin biopsy demonstrated thickened collagen bundles with mucin deposition, confirming the diagnosis of post-streptococcal scleredema. She was managed conservatively with appropriate antibiotics and showed gradual clinical improvement over time, consistent with post-infectious scleredema. Here, we describe a case of post-streptococcal scleredema in a child presenting with features mimicking systemic sclerosis, highlighting the diagnostic challenges and reviewing the available literature.

A 6-year-old female child presented with complaints of progressive tightening and thickening of the skin over the face, neck, and upper back for 4 weeks' duration. The onset was insidious and gradually progressive. The condition initially involved the face and, over the course of 1 month, progressed symmetrically

to involve the neck, chest and upper back as well as the upper limbs. There was no associated restriction in the range of motion of the trunk, fingers, limbs, shoulders, or neck. The onset of symptoms was preceded by a 10-days history of fever, cough, and sore throat, which had been managed conservatively at home with over-the-counter medications. There was no history of associated skin rash, dysphagia, or other systemic manifestations. His past medical history was unremarkable. There was no history suggestive of connective tissue disease, including Raynaud phenomenon, digital ulcers, joint stiffness, dysphagia, or respiratory difficulty. The child's developmental milestones were normal, and her immunization status was appropriate for age.

On examination, the child was febrile and hemodynamically stable. Cutaneous examination revealed diffuse, symmetrical, non-pitting induration involving the face, neck, shoulders, upper limbs, chest, and upper back (Figure 1). The skin was thickened with a woody consistency, and there was restriction of neck movements due to skin tightening. Notably, there was no involvement of the fingers, and features such as sclerodactyly, telangiectasia, or calcinosis were absent. Oral cavity examination showed a congested posterior pharyngeal wall with enlarged and erythematous tonsils, without exudates, suggestive of recent pharyngotonsillitis. The remainder of the systemic examination was unremarkable. Anthropometric assessment revealed a body mass index (BMI) of 10.6 kg/m² (< 3rd percentile), weight of 14 kg (below -2 SD), and height of 115 cm (between median and -1 SD). As per Indian Academy of Pediatrics (IAP) growth charts, this indicates underweight status with normal stature for age, and a markedly low BMI suggestive of thinness (wasting).

Abbreviations: ASO, antistreptolysin O; BMI, body mass index; IAP, Indian Academy of Pediatrics.

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Vital parameters included a temperature of 39.2°C, heart rate of 147 beats per minute, blood pressure of 106/72 mmHg, and respiratory rate of 24 breaths per minute.

Laboratory investigations showed hemoglobin of 12.7 g/dL, total leukocyte count of $19.10 \times 10^9/L$ with 74% neutrophils and 24% lymphocytes, and platelet count of $181 \times 10^9/L$. The erythrocyte sedimentation rate was mildly elevated at 20 mm/h. Liver function tests were within normal limits, including serum glutamic pyruvic transaminase (SGPT) 34 IU/L, serum glutamic oxaloacetic transaminase (SGOT) 32 IU/L, alkaline phosphatase 203 U/L, total bilirubin 0.5 mg/dL, and serum albumin 3.8 g/dL. Renal function tests and urine microscopy were within normal limits. Chest X-ray, electrocardiography, echocardiography, and abdominal ultrasonography revealed no abnormalities. Immunological evaluation revealed serum IgM level of 1.86 g/dL and IgG level of 1060 mg/dL, both within normal limits for age. Antistreptolysin O (ASO) titre was elevated at 560 IU/mL,

supporting recent streptococcal infection. Throat swab culture grew *streptococcus pyogenes*. Autoimmune workup showed anti-Scl-70 IgG level of <2 RU/mL, which was within the negative range; antinuclear antibody and anti-centromere antibodies were also negative. A provisional diagnosis of post-infectious scleredema was made. The child was managed with a 10-day course of oral amoxicillin-clavulanate along with adequate hydration and supportive care. A skin biopsy was performed, which demonstrated thickening of the dermis with broad, swollen collagen bundles separated by mucin deposition. There was no evidence of significant fibrosis or adnexal atrophy. These histopathological findings were consistent with scleredema (Figure 2). Based on the clinical presentation, antecedent history of upper respiratory infection, elevated ASO titres, and characteristic histopathological features, a diagnosis of post-streptococcal scleredema was established. She was discharged after 10 days and has since been on regular follow-up. Over the course of 1 year, her skin gradually regained normal elasticity,



FIGURE 1 | (A, B) Clinical photograph showing diffuse, symmetrical, non-pitting induration involving the face, neck, shoulders, upper limbs, and chest, consistent with scleredema.

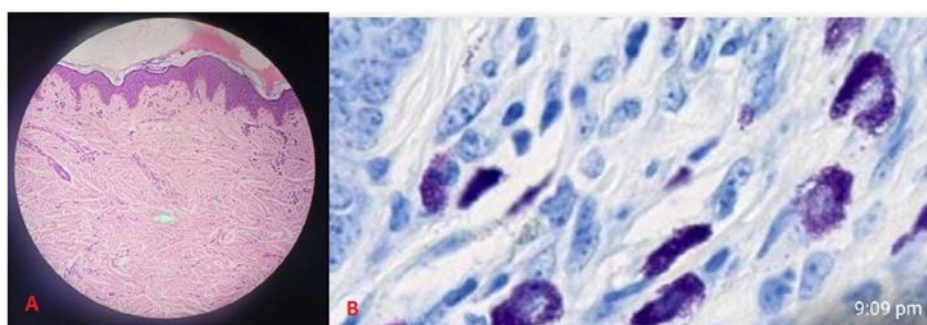


FIGURE 2 | Histopathological sections of skin showing (A) relatively normal epidermis with diffuse dermal thickening, and (B) higher magnification revealing widened spaces between collagen bundles due to prominent mucin deposition, without significant inflammation—features suggestive of scleredema (H&E; A: $\times 40$, B: $\times 400$).

with significant symptomatic improvement. The ASO titre was reassessed during follow-up and was 312 IU/mL.

Scleredema is a rare fibromucinous connective tissue disorder of unclear etiology. It is characterized by increased synthesis of Type I collagen and glycosaminoglycans by fibroblasts within the reticular dermis [1]. It is classified into three types as described by Rongioletti and Rebra: Type I (post-infectious), commonly seen in children following streptococcal infection and usually self-limiting; Type II, associated with paraproteinemia and a chronic progressive course; and Type III (scleredema diabeticorum), occurring in patients with long-standing diabetes and characterized by persistent skin induration, typically over the neck and upper back [2]. In this case, the fever was considered attributable to the concomitant *Streptococcus pyogenes* pharyngotonsillitis, supported by the clinical findings and positive throat culture, rather than being a primary manifestation of scleredema. There is no well-established direct association between malnutrition and the development of scleredema. It is more commonly associated with diabetes mellitus, obesity, and preceding streptococcal infections [3]. In this patient, the wasting may have reflected the underlying malnutrition rather than being a recognized predisposing factor for scleredema.

Childhood scleredema following streptococcal pharyngotonsillitis is a rare and infrequently reported entity in the literature. Goreal et al. described a 6-year-old child who developed diffuse skin induration after streptococcal pharyngitis, with elevated ASO titers and complete spontaneous resolution [5]. Similarly, Majumder et al. reported an adolescent with extensive skin involvement mimicking early systemic sclerosis, highlighting the diagnostic challenge; however, the absence of Raynaud's phenomenon and negative autoantibodies helped in differentiation [6]. Additional pediatric reports, including Shrestha et al. and Rani et al., further emphasize that scleredema can present as diffuse swelling in children and may pose a diagnostic challenge, but generally carries a favorable prognosis with conservative management [7, 8]. Furthermore, Murthy et al. reported a case of scleredema in a young child showing a favorable response to systemic corticosteroids, suggesting that while the condition is often self-limiting, selected cases may benefit from steroids [9]. Collectively, most of these reports support that post-streptococcal scleredema in children typically presents with acute-onset, non-pitting skin induration and follows a favorable, self-resolving course.

The differentiation between scleredema and scleroderma is clinically significant. Scleroderma (systemic sclerosis) is a chronic autoimmune disorder with potential multisystem involvement, requiring long-term immunosuppressive therapy. In contrast, scleredema, particularly the post-infectious type, is usually self-limiting and benign. Key distinguishing features include sparing of acral areas, absence of vascular phenomena such as Raynaud's, lack of internal organ involvement, and the presence of mucin deposition on histopathology. The role of laboratory investigations is supportive rather than diagnostic. Elevated ASO titers indicate recent streptococcal infection, as seen in the present case. Autoimmune markers such as ANA and anti-Scl-70 are typically negative in scleredema, helping exclude scleroderma [10].

Management of Type I scleredema is largely supportive, as the condition is typically self-limiting. Most cases resolve spontaneously within months to 2 years. Antibiotic therapy targeting streptococcal infection may be administered when indicated, along with symptomatic treatment and physiotherapy to maintain mobility. Supportive measures such as emollients and physiotherapy help maintain skin flexibility and prevent functional limitation. In refractory or severe cases, modalities such as phototherapy (UVA-1), methotrexate, or corticosteroids have been tried with variable success, although their use is rarely required in pediatric cases [9, 11].

Post-streptococcal scleredema should be considered in children presenting with diffuse, non-pitting skin induration. Early identification is essential to avoid unnecessary aggressive immunosuppressive therapy and to provide appropriate reassurance regarding its generally benign and self-limiting course.

Author Contributions

S.J., B.C. and G.V.J. was involved in patient management and the review of literature. B.K.G. also contributed to the preparation of the draft manuscript. B.K.G. performed the critical review and will act as the corresponding author and guarantor.

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Funding

The authors have nothing to report.

Ethics Statement

We declare that this case report did not require approval from an ethics committee or institutional review board.

Consent

Written informed consent was obtained from the parents.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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LETTER TO THE EDITOR

Short-Interval Discordance Between Fatigue and Joint Counts in Rheumatoid Arthritis

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Dear Editor,

Fatigue is a core patient-reported outcome in rheumatoid arthritis (RA), but it is only partly explained by inflammatory markers and joint examination [1–3]. Most longitudinal studies have assessed fatigue over several weeks or months [4, 5]. We examined change in fatigue visual analogue scale (VAS) at the second routine assessment, which often occurred within days, and evaluated whether a decrease in fatigue occurred in parallel with changes in swollen joint count (SJC) and tender joint count (TJC).

We conducted a retrospective single-centre study of hospitalized patients with RA using a de-identified routine-care database from the Department of Rheumatology, Dongzhimen Hospital, Beijing University of Chinese Medicine. Records included in the analysis spanned 12 May 2022 to 18 December 2024. Eligible inpatient records were identified consecutively, and all records meeting the study-specific criteria were included without sampling. Patients were eligible if RA was diagnosed according to the 2010 ACR/EULAR criteria or confirmed by a rheumatologist and fatigue VAS was recorded at baseline and at the second assessment [6]. Baseline was the assessment used for cohort entry, and the second assessment was the next recorded routine-care assessment after baseline. Each patient contributed one assessment pair. Reporting followed STROBE guidance [7]. Fatigue was recorded on a 0–10 VAS, with 0 indicating no fatigue and 10 extreme fatigue. SJC and TJC were obtained from routine 28-joint examinations covering the bilateral shoulders, elbows, wrists, metacarpophalangeal joints, proximal interphalangeal joints and knees. A fatigue decrease was defined as a reduction of at least 1 point on the 0–10 fatigue VAS, consistent with published estimates of minimally important improvement in RA [8]. Because a ≥ 1 -point decrease was not possible from a

baseline fatigue VAS of 0, the primary fatigue-responder analysis was restricted to patients with baseline fatigue VAS ≥ 1 ($n = 189$). The 30 patients with baseline fatigue VAS = 0 were retained in the full-cohort analyses and classified as having no ≥ 1 -point decrease. The four-pattern fatigue–joint count classification used the full cohort ($N = 219$). The primary joint-count comparator was any decrease in SJC or TJC. Sensitivity analyses required simultaneous SJC and TJC decrease, at least 20% reduction in either count, or at least 20% reduction in both counts. Continuous paired outcomes were summarized at both assessments and as within-patient change (second assessment minus baseline) and were compared using the Wilcoxon signed-rank test. Paired CRP and ESR analyses used complete paired observations only; missing laboratory values were not imputed. Binary proportions were reported with Wilson 95% confidence intervals. Exploratory logistic regression models were fitted separately for each candidate predictor. Models for disease-activity measures were adjusted for age, sex, body mass index (BMI) and baseline fatigue VAS. In the BMI model, BMI was the predictor of interest and age and sex were the only covariates. These regression analyses were exploratory and were not adjusted for multiple comparisons. DAS28, CDAI and SDAI were treated as established RA disease-activity indices [9–11].

The analysis included 219 patients; 156 (71.2%) were women, and the mean age was 61.87 years (SD 11.90). Second-assessment dates were available for 187 patients. Two records with chronologically inconsistent or negative intervals were excluded from the interval summary, leaving 185 patients with a median interval of 7 days (IQR 6–9). All 219 patients had paired fatigue VAS, SJC and TJC measurements and contributed to the paired fatigue–joint count analyses; the interval summary was therefore restricted to 185 patients.

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TABLE 1 | Short-interval changes and principal fatigue outcomes.

Panel A: paired continuous measures				
<i>n</i>	Baseline, median (IQR)	Second assessment, median (IQR)	Within-patient change, median (IQR)	<i>p</i>
219	3.00 (1.50–5.00)	2.00 (1.00–4.00)	–1.00 (–1.00 to –1.00)	<0.001
163	13.03 (4.03–37.98)	6.13 (1.40–19.15)	–5.99 (–23.32 to 5.55)	<0.001
144	36.00 (21.00–62.25)	30.00 (17.00–58.00)	–6.00 (–30.25 to 16.00)	0.084
219	10.00 (4.00–16.00)	10.00 (4.00–16.00)	0.00 (–6.50 to 7.00)	0.954
219	13.00 (8.00–18.00)	12.00 (8.00–18.00)	0.00 (–7.00 to 7.00)	0.868
Panel B: principal binary fatigue outcomes				
Outcome	Denominator		<i>n</i> (%)	95% CI
≥ 1-point fatigue decrease, baseline fatigue VAS ≥ 1 (primary)	189		166 (87.8%)	82.4–91.8
≥ 1-point fatigue decrease, full cohort (descriptive)	219		166 (75.8%)	69.7–81.0
Fatigue decrease without any SJC or TJC decrease	219		77 (35.2%)	29.1–41.7

Note: The reassessment interval was available for 185 patients and had a median of 7 days (IQR 6–9). Within-patient change was calculated as second assessment minus baseline. CRP and ESR analyses included complete paired observations only; missing values were not imputed. *p* Values for paired continuous outcomes were obtained using the Wilcoxon signed-rank test. Proportion 95% CIs were calculated using the Wilson method. Abbreviations: CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range; SJC, swollen joint count; TJC, tender joint count; VAS, visual analogue scale.

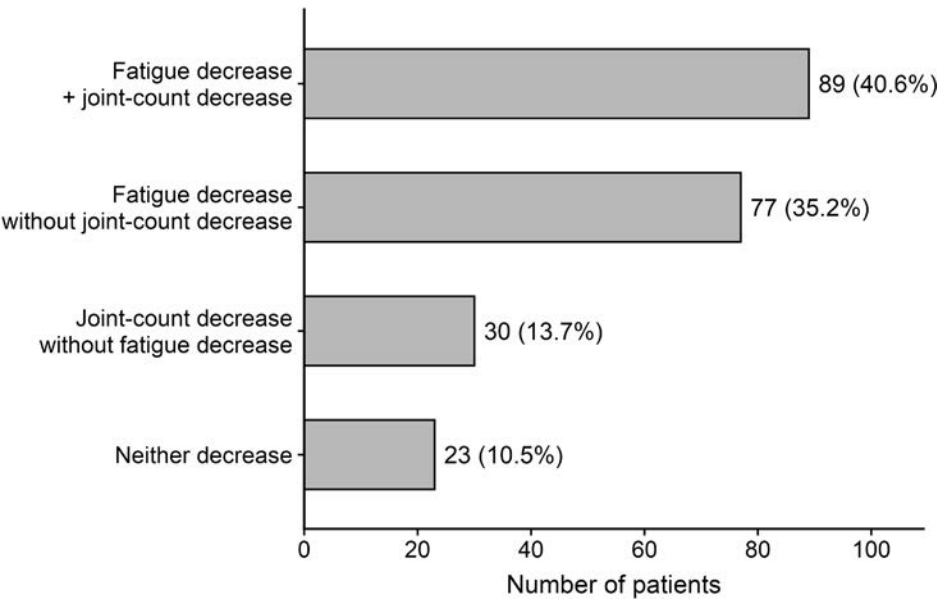


FIGURE 1 | Classification of ≥ 1-point fatigue decrease and any joint-count decrease in the full cohort (*N* = 219). A fatigue decrease was defined as a decrease of at least 1 point on the fatigue VAS. Any joint-count decrease was defined as a decrease in SJC or TJC. The four categories are mutually exclusive and exhaustive. This comparator was descriptive and was not intended as a validated clinical joint-response criterion.

Fatigue VAS decreased from a median of 3.00 (IQR 1.50–5.00) to 2.00 (IQR 1.00–4.00; *p* < 0.001). CRP also decreased among the 163 patients with paired CRP measurements (Table 1), whereas ESR in 144 paired observations did not show a statistically significant group-level change. SJC and TJC likewise showed no statistically significant group-level change, although 119 of 219 patients (54.3%, 95% CI 47.7–60.8) had a decrease in at least one joint count at the individual level. Among the 189 patients with baseline fatigue VAS ≥ 1, 166 achieved a ≥ 1-point fatigue decrease (87.8%, 95% CI 82.4–91.8), which was the primary

fatigue-responder estimate. In the full cohort, the corresponding descriptive estimate was 166/219 (75.8%, 95% CI 69.7–81.0).

The four fatigue-joint count patterns are shown in Figure 1. Fatigue decreased together with any joint-count decrease in 89 patients (40.6%, 95% CI 34.3–47.3), whereas 77 (35.2%, 95% CI 29.1–41.7) had a fatigue decrease without a decrease in either SJC or TJC. Thirty patients (13.7%, 95% CI 9.8–18.9) had a joint-count decrease without a fatigue decrease, and 23 (10.5%, 95% CI 7.1–15.3) had neither. Under the alternative joint-count

definitions, the fatigue-only pattern occurred in 105/219 patients (47.9%, 95% CI 41.4–54.5) when both SJC and TJC were required to decrease, 85/219 (38.8%, 95% CI 32.6–45.4) when a $\geq 20\%$ reduction in either count was required, and 115/219 (52.5%, 95% CI 45.9–59.0) when a $\geq 20\%$ reduction in both counts was required.

Higher baseline DAS28-CRP (adjusted odds ratio [OR] 1.55, 95% CI 1.02–2.35), DAS28-ESR (OR 1.81, 95% CI 1.14–2.88), and SJC (OR 1.09 per joint, 95% CI 1.02–1.17) were associated with failure to achieve a 1-point fatigue decrease. Associations for CDAI, SDAI, and TJC were weaker, and ESR and C-reactive protein alone were not associated with this outcome.

The results show partial, rather than complete, concordance between early changes in fatigue and joint counts. The absence of a statistically significant group-level change in SJC or TJC does not imply that individual joint counts were unchanged, because decreases and increases can coexist within the cohort. This is consistent with evidence that RA fatigue reflects interacting inflammatory, physical, and psychosocial factors [2, 3, 12–14]. The finding does not indicate that fatigue is independent of RA activity: baseline composite disease activity and joint burden were associated with fatigue at the second assessment. It does indicate that a short-term change in fatigue cannot be inferred from joint-count change alone.

Several limitations are relevant. Valid reassessment intervals were available for 185 of 219 patients; therefore, the median 7-day interval does not describe the entire cohort. Treatment changes during the short interval were not captured consistently, so the observed fatigue decrease cannot be attributed to a specific therapy. Rest, sleep, clinical contact, measurement variation, and regression to the mean may also have contributed. The study was conducted at one centre; fatigue was measured with a single-item VAS, and information on sleep, mood, and physical activity was unavailable. The broad primary joint-count definition was addressed with stricter sensitivity analyses, but single-joint differences and routine examination variability may affect individual classifications, and none of these definitions constitutes a validated short-term joint-response criterion.

In routine reassessment, fatigue VAS may complement joint examination and composite disease-activity measures. A decrease in fatigue over a few days should be interpreted as a patient-reported change, not as evidence of inflammatory control or treatment efficacy.

Author Contributions

Yidi Huang contributed to study conception, data curation, statistical analysis, interpretation, and drafting. Lü Liu, Hangwei Wan, and Cunxiang Xie contributed to data collection, data checking, and manuscript revision. Hailong Wang supervised the study design, quality control, interpretation, and final revision. All authors read and approved the final manuscript.

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Use of artificial intelligence tools: Language-editing assistance was used during manuscript preparation. The authors reviewed the final text and verified all data, analyses, interpretations, and references.

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Ethics Statement

The Ethics Committee of Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine approved the study (approval no. 2025DZMEC-036-02). The database was de-identified, and the requirement for informed consent was waived by the ethics committee.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets are not publicly available because they contain potentially sensitive clinical information and are subject to institutional data-governance restrictions. De-identified data may be available from the corresponding author upon reasonable request and with permission from the relevant institutional authority.

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ORIGINAL ARTICLE

Artificial Intelligence–Based Scoring System and Exploratory Multiomics in Ankylosing Spondylitis: Hip Severity and Age-at-Severe-Hip-Involvement Stratification

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ABSTRACT

Introduction: Accurate diagnosis and assessment of hip involvement are important for the clinical management of ankylosing spondylitis (AS). This study aimed to develop and internally validate an artificial intelligence–based Ensemble Scoring System (ESS) for AS diagnosis and hip-disease severity assessment and to explore its association with heterogeneous immune-cell infiltration and age at first documented severe hip involvement.

Methods: A total of 5721 patients with pelvic radiographs and linked clinical records were retrospectively included. Participants were divided into training and held-out validation cohorts at a 7:3 ratio strictly at the patient level, with no patient or image overlap. Deep-learning features extracted by a convolutional neural network were integrated with clinical variables using XGBoost to construct task-specific ensemble deep-learning (EDL) models. Exploratory proteomic, transcriptomic, single-cell RNA-sequencing, immunohistochemical, and peripheral-blood analyses were performed to investigate AS-related immune infiltration. An exploratory immune ensemble (IE) score was constructed using the monocyte proportion and EDL score. Kaplan–Meier analysis was used to examine the association between the IE score and age at severe hip involvement.

Results: The EDL model achieved AUCs of 0.978 for distinguishing AS from non-AS, 0.990 for identifying hip arthropathy among patients with AS, and 0.996 for identifying BASRI-Hip grade ≥ 3 among patients with hip arthropathy. Monocyte percentage was an influential feature across the three EDL classification tasks. Exploratory multiomics and immunohistochemical analyses showed higher LYN expression in AS than in the corresponding non-AS fracture-control groups and suggested that LYN expression was concentrated predominantly in monocytes. A high IE score was associated with earlier severe hip involvement, with estimated median

Abbreviations: AI, artificial intelligence; AS, ankylosing spondylitis; AUC, area under the curve; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASO, basophil; BASOP, basophil percentage; BASRI-hip grade, bath ankylosing spondylitis radiology hip grade; CNN, convolutional neural network; DICOM, digital imaging and communications in medicine; DL, deep learning; DLF, deep learning features; EDL, ensemble deep learning; EOP, eosinophils percentage; ESR, erythrocyte sedimentation rate; ESS, Ensemble Scoring System; Grad-CAM, gradient-weighted class activation mapping; HAAS, Hip Arthropathy in Ankylosing Spondylitis; IE, immune ensemble; JPEG, Joint Photographic Experts Group; KNN, K-nearest neighbors; LASSO, least absolute shrinkage and selection operator; LFQ, label-free quantification; LightGBM, Light Gradient Boosting Machine; LMPHP, lymphocyte percentage; LR, logistic regression; LYN, LYN Proto-Oncogene, Src Family Tyrosine Kinase; MCHC, mean corpuscular hemoglobin concentration; ML, machine learning; MONOP, monocyte percentage; MPV, mean platelet volume; NEUT, neutrophil count; NPV, negative predictive value; PDW, platelet distribution width; PLT, platelet; PPV, positive predictive value; RBC, red blood cell; RDWCV, red cell distribution width variance coefficient; RF, random forest; ROC, receiver operating curve; scRNA-seq, single-cell RNA sequencing; SVM, support vector machine; THA, total hip arthroplasty; t-SNE, t-Distributed Stochastic Neighbor Embedding; WBC, white blood cell count.

ages at first documented severe hip involvement of 36 and 50 years in the high- and low-IE-score groups, respectively. This finding represents exploratory risk stratification and does not establish independent prediction of biological disease progression.

Conclusions: The ESS showed strong internal validation performance for AS diagnosis and hip-disease severity classification. Exploratory analyses linked LYN dysregulation in monocytes with heterogeneous immune infiltration, while the IE score was associated with earlier severe hip involvement. Prospective multicenter external validation is required before clinical application.

1 | Introduction

Ankylosing spondylitis (AS) is an inflammatory condition characterized by hallmark manifestations of axial spine joint pathological ossification, boasting a global prevalence between 0.1% and 1.4% [1]. Typical manifestations of AS encompass inflammatory back pain, restricted spinal mobility, along with peripheral and extra-articular symptoms. As AS progresses, the pathological formation of new bone culminates in bony ankylosis of the axial spine, resulting in sacroiliac arthritis, spinal ankylosis, and hip arthropathy [2]. Approximately 24%–36% of AS individuals suffer with hip arthropathy. Such patients, referred to as those with Hip Arthropathy in Ankylosing Spondylitis (HAAS), often exhibit poorer functional status and more severe clinical symptoms, predisposing them to increased axial disease severity [3]. The 1984 modified New York criteria for diagnosing AS have gained wide acceptance in clinical settings and their efficacy has been corroborated. Nevertheless, these criteria cannot evaluate AS severity and are unable to predict the trajectory of HAAS. Prolonged diagnosis and treatment commencement can increase the burden on both individuals and society, culminating in significant socioeconomic implications [4]. Hence, identifying AS, discerning HAAS, and predicting disease outcomes is of paramount importance.

In the contemporary research landscape, significant attention has been paid to understanding the pathological mechanism and clinical diagnosis of AS. Prior efforts have sought to identify molecular biological markers to understand AS pathogenesis [5]. However, the clinical adoption of these markers remains elusive due to high costs and insufficient validation across substantial sample sizes. A plethora of clinical investigations have been focused on AS diagnosis, developing clinical predictive frameworks that have demonstrated reasonable predictive prowess [6]. These models, however, need further refinement, with the integration of multi-omics data potentially enhancing their depth and breadth. A prevailing hypothesis postulates that the inflammatory response, catalyzed by heterogeneity in immune cell infiltration, underpins AS pathogenesis. Research insights suggest that this heterogeneous immune cell infiltration might instigate aberrant osteogenic differentiation, leading to pathological new bone creation. Our preliminary investigations corroborated the linkage between AS pathogenesis and immune cell dysregulation, identifying *LYN* (*LYN* Proto-Oncogene, Src Family Tyrosine Kinase) as a pivotal gene orchestrating this heterogeneous immune cell infiltration. Consequently, heterogeneous immune cell infiltration in AS patients might emerge as a valuable biomarker for elucidating AS pathogenesis. Over the past decade, the medicinal landscape has witnessed seismic advancements in the realm of Artificial Intelligence (AI), particularly in facets like computer-assisted screening, decision augmentation, and precision diagnostics. In 2023, our findings

posited that Convolutional Neural Networks (CNN) were adept at recognizing sacroiliac joint anomalies in AS patient pelvic X-rays, birthing an AI-augmented system rooted in CNN to bolster clinical AS diagnostics [7]. Moreover, the research highlights AI's importance in forecasting lymph node metastasis preoperatively and refining surgical determinations and customizing treatment plants [8]. Nonetheless, these AS-centric investigations grapple with limitations, notably the looming specter of overfitting owing to paucity in sample sizes or the challenges presented by an absence of multi-omics data analysis [9].

Recent studies have suggested that integrating artificial intelligence with multiomics data may facilitate disease classification, risk stratification, and biological interpretation. Accordingly, this study aimed to develop and internally validate an artificial intelligence-based ESS for AS diagnosis and hip-disease severity assessment. We further performed exploratory multiomics analyses to characterize AS-related heterogeneous immune infiltration and examined whether the exploratory IE score was associated with different trajectories of age at severe hip involvement.

2 | Materials and Methods

2.1 | Patients

The study protocol received approval from the Ethics Committee. All participants furnished signed informed consent forms, ensuring the study's adherence to the Declaration of Helsinki principles. The work has been reported in line with the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS) criteria [10].

Between September 2012 and August 2022, we retrospectively included 5721 patients, comprising 2087 patients with AS and 3634 non-AS patients. Available data included pelvic radiographs, baseline clinical characteristics, routine blood measurements, and erythrocyte sedimentation rate (ESR) values. All AS-diagnosed patients met the modified New York criteria. We excluded AS patients presenting with other conditions such as tumors, severe systemic inflammatory diseases, or having a history of other autoimmune disorders. Conversely, non-AS patients with no history of systemic inflammation or any autoimmune condition, including SpA, were included.

For the exploratory time-to-event analysis, severe hip involvement was defined as the first documented radiographic assessment showing BASRI-Hip grade ≥ 3 or bony ankylosis of the hip joint. In addition, routine blood-test data on monocyte percentage (MONOP) were collected from 8826 patients, including 3557 patients with AS and 5269 non-AS patients, to validate the findings related to monocyte infiltration. Figure S1 presents the overall study framework.

2.2 | Deep Learning Analysis and Interpretability

A total of 5721 pelvic radiographs from 5721 unique patients, including 2087 patients with AS and 3634 non-AS patients, were divided into training and held-out validation cohorts at a 7:3 ratio. The split was performed strictly at the patient level before any model-dependent preprocessing. Each patient contributed one radiograph and one linked clinical record; therefore, no patient, radiograph, or linked clinical record appeared in both cohorts. The same initial patient-level allocation was retained when constructing the AS-diagnosis, hip-arthropathy, and BASRI-Hip severity datasets, and patients were not reassigned between cohorts for different tasks.

Ten candidate deep-learning architectures were evaluated exclusively within the training cohort. ResNet50 showed the best training-cohort cross-validation performance and was selected without reference to the held-out validation cohort. Image preprocessing parameters and model-selection decisions were determined using the training cohort and then applied unchanged to the held-out validation cohort. The validation cohort was used only for final performance evaluation.

The 10 candidate architectures were DenseNet121, DenseNet169, DenseNet201, ResNet18, ResNet34, ResNet50, ResNet152, VGG11, VGG13, and VGG16. Detailed ROC curves and confusion matrices for ResNet50 are provided in Figure S6, whereas the corresponding results for the DenseNet, alternative ResNet, and VGG architectures are presented in Figures S7–S9. DICOM images were converted to JPEG format, pixel values were standardized to a range of 0–1000, and images were resampled to 448×448 pixels. The Adam optimizer was used with an initial learning rate of 0.001 and a batch size of 64. Early stopping and L2 regularization were applied to reduce overfitting. All deep-learning models were implemented in PyTorch. Eight deep-learning features were extracted from the fully connected layer of the final ResNet50 model.

Gradient-weighted class activation mapping was used to visualize the regions contributing to the CNN predictions. Grad-CAM generated class-activation maps using gradients from the final convolutional layer, thereby providing a visual interpretation of the model output.

2.3 | Machine Learning and the Construction of Ensemble Deep Learning Model

Clinical variables were linked to the corresponding deep-learning features using unique patient identifiers. Z-score parameters, correlation filtering, feature selection, algorithm selection, and hyperparameter optimization were performed using the training cohort only. Variables with pairwise Spearman correlation coefficients greater than 0.90 were removed. Primary feature selection was subsequently performed using LASSO regression in the training cohort, and the training-derived feature set was fixed for all subsequent model fitting and held-out validation.

Training-selected variables were entered into 10 machine-learning algorithms: logistic regression, naive Bayes, support

vector machine, K-nearest neighbors, random forest, Extra Trees, XGBoost, LightGBM, gradient boosting, and AdaBoost.

Ten machine-learning algorithms were compared using 10 repetitions of stratified 10-fold cross-validation within the training cohort. Each repetition used a different random seed, resulting in 100 internal cross-validation estimates for each algorithm. The final algorithm and hyperparameters were selected exclusively from the training process. The fitted model was then applied unchanged to the held-out validation cohort. Performance was evaluated using ROC-AUC, sensitivity, specificity, accuracy, positive predictive value, negative predictive value, confusion matrices, and decision-curve analysis.

2.4 | Sample Collection and Analysis of Multi-Omics Sequencing Data

Our previous research indicated a connection between heterogeneous immune cell infiltration in AS and monocytes, aligning with the findings in this study where MONOP emerged as a high-weight predictor [11]. Therefore, to further investigate the relationship between heterogeneous immune cell infiltration in AS and monocytes, we conducted multi-omics studies.

We procured tissue samples from 24 patients during surgery for exploratory multi-omics analyses. The AS group included patients with ankylosing spondylitis and heterotopic ossification of the hip undergoing THA. The comparison group comprised patients undergoing surgery for acetabular fractures and is therefore termed the non-AS fracture-control group rather than a healthy-control group. Proteomics included 12 samples, transcriptomics included 6 samples, and single-cell transcriptomics included 6 samples. Because these sample sizes were small, these analyses were considered hypothesis-generating.

2.4.1 | Proteomics

We selected 6 AS patients undergoing THA and 6 non-AS fracture controls with acetabular fractures. The baseline clinical characteristics of patients included in the proteomic analysis are presented in Table S5. Hip ligament tissue was collected during surgery and analyzed using label-free quantification (LFQ); detailed procedures are provided in the Supporting Information S1. Protein-expression profiles were normalized, background-adjusted, and log2-transformed. Between-group differences were assessed using Student's *t*-test with a nominal $p < 0.05$ threshold. No multiple-testing correction was applied; therefore, these proteomic findings are exploratory and require confirmation in larger cohorts.

2.4.2 | Transcriptomics

Hip ligament samples were obtained from 3 AS patients undergoing THA and 3 non-AS fracture controls undergoing surgery for acetabular fractures. The baseline clinical characteristics of patients included in the transcriptomic analysis are presented in Table S6. RNA was extracted immediately after collection; detailed procedures are provided in the Supporting

Information S1. Expression data were normalized, background-corrected, and log2-transformed using the Bioconductor package *affy*. Between-group differences were assessed using Student's *t*-test with a nominal $p < 0.05$ threshold. No multiple-testing correction was applied; therefore, these transcriptomic findings are exploratory.

2.4.3 | Single-Cell RNA Sequencing

Three AS patients undergoing THA and 3 non-AS fracture controls with acetabular fractures were included. The baseline clinical characteristics of patients included in the single-cell RNA-sequencing analysis are presented in Table S7. Bone-marrow samples were rapidly processed for RNA extraction. After principal-component analysis, clustering and gene labeling were visualized using t-SNE, followed by focused analysis of the monocyte/macrophage-associated cluster. Cell types were annotated using SingleR [12].

Processed expression matrices for GSE73754 (GPL10558, Illumina HumanHT-12 V4.0 expression beadchip) and GSE25101 (GPL6947, Illumina HumanHT-12 V3.0 expression beadchip) were downloaded from the Gene Expression Omnibus database [13, 14]. The deposited normalized expression matrices were used. Expression values were log2-transformed where necessary, and probe identifiers were mapped to gene symbols according to the corresponding platform annotations. Differential expression was assessed using Student's *t*-test, with a nominal $p < 0.05$ threshold. No multiple-testing correction was applied; therefore, these analyses were considered exploratory.

2.5 | Immune Cell Infiltration Analysis

CIBERSORT [15] is a computational tool designed to assess the composition of immune cells, representing the immune cell components in a matrix format. This algorithm employs Monte Carlo sampling techniques to determine the *p*-value of the deconvolution for each specimen. The cumulative proportion of immune cell compositions for each sample in the GEO dataset amounted to 100%. We employed CIBERSORT to analyze immune cell infiltration in samples from the GSE25101 dataset. Our objective was to investigate the disparities in immune cell infiltration between AS and non-AS patients and to understand the association between genes and immune cells.

2.6 | Immunohistochemistry Analysis

Hip ligament samples from 6 AS patients with bony hip ankylosis undergoing THA constituted the AS group, and samples from 6 patients undergoing surgery for acetabular fractures constituted the non-AS fracture-control group. Immunohistochemistry was used to compare LYN expression between groups. Baseline characteristics are shown in Table S2. LYN antibody information and staining procedures are provided below. ImageJ was used to quantify LYN positivity, and an independent-samples *t*-test was performed in GraphPad Prism 9.

2.7 | Construction of the Ensemble Scoring System

The Ensemble Scoring System (ESS) comprises the ensemble deep learning (EDL) score and the immune ensemble (IE) score. For each classification task, XGBoost combined the selected standardized deep-learning and clinical variables. The EDL score can be expressed in simplified form as EDL score = $f_{\text{XGBoost}}(z(\text{DLf}), z(\text{clinical variables}))$, where f_{XGBoost} is the fitted probability output of the task-specific model. The complete feature list and importance weights are provided in the Supporting Information S1. The IE score was calculated as IE score = MONOP/EDL score, where MONOP was expressed as a proportion ranging from 0 to 1 and the EDL score was the predicted probability generated by the BASRI-Hip severity-classification model. The IE score was considered a post hoc descriptive ratio of MONOP to the model-estimated probability of severe hip involvement. Because MONOP was also included among the predictors of the EDL model, the IE score was not considered independent of the EDL score and was not used to claim incremental predictive value beyond the EDL score alone. This exploratory index requires prospective validation.

2.8 | Statistics

Statistical analyses were performed using SPSS version 22.0, GraphPad Prism version 9.0, and R software. Continuous variables are presented as mean (standard deviation) or median (interquartile range), as appropriate. Student's *t*-test was used for normally distributed continuous variables, whereas the Mann-Whitney *U* test was used for non-normally distributed variables. Categorical variables are presented as numbers and percentages and were compared using the chi-square test or Fisher's exact test, as appropriate. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant.

No multiple-testing correction was applied to the exploratory proteomic and transcriptomic analyses. Accordingly, these findings were interpreted as nominal and hypothesis-generating.

Information on treatment status, including the use of nonsteroidal anti-inflammatory drugs, biologics, disease-modifying antirheumatic drugs, and corticosteroids, was available. However, detailed information regarding treatment duration, dose, adherence, and longitudinal treatment changes was incomplete. Treatment variables were therefore not incorporated into the primary Kaplan-Meier analysis, which should be considered exploratory and unadjusted. Residual confounding associated with treatment exposure cannot be excluded.

For the time-to-event analysis, age was used as the time scale. The event was the first documented radiographic assessment showing BASRI-Hip grade ≥ 3 or bony ankylosis of the hip joint. Patients who did not meet this endpoint were censored at the age of their last available pelvic radiograph. High- and low-IE-score groups were defined using the median IE score of the analysis cohort. Kaplan-Meier curves were generated using the R packages *survival* and *survminer*. This exploratory and unadjusted analysis evaluated the association between the IE score and age at first documented severe hip involvement and did not establish independent prediction of biological disease progression.

3 | Results

3.1 | Interpretability and Performance of the Deep Learning Model

Given that the decision-making framework of a DL model can often seem opaque or be viewed as a “black box,” visualization of the model becomes pivotal. To this end, we employed the gradient-weighted class activation mapping (Grad-CAM) approach to enhance the model’s interpretability. Figure 1 presents the primary input image for the DL model, the resulting heatmap, and the confusion matrix that assesses the model’s

performance. Initially, we input the pelvic X-ray images from 5721 patients (comprising 2087 AS patients and 3634 non-AS patients) into the DL model to differentiate AS from non-AS cases. Figure 1A displays a pelvic X-ray from an AS patient, revealing noticeable sacroiliac joint alterations, such as indistinct articular surfaces and bony ankylosis. These atypical changes were recognized by the DL model and visualized in the heatmap (Figure 1B). The model’s performance was evaluated using the confusion matrix and ROC curve, yielding an AUC of 0.948 (Figure 1C and Figure S2). Subsequently, we fed the model with pelvic X-ray images from 2087 AS patients to discern the presence of hip lesions. Figure 1D portrays a pelvic X-ray of an AS

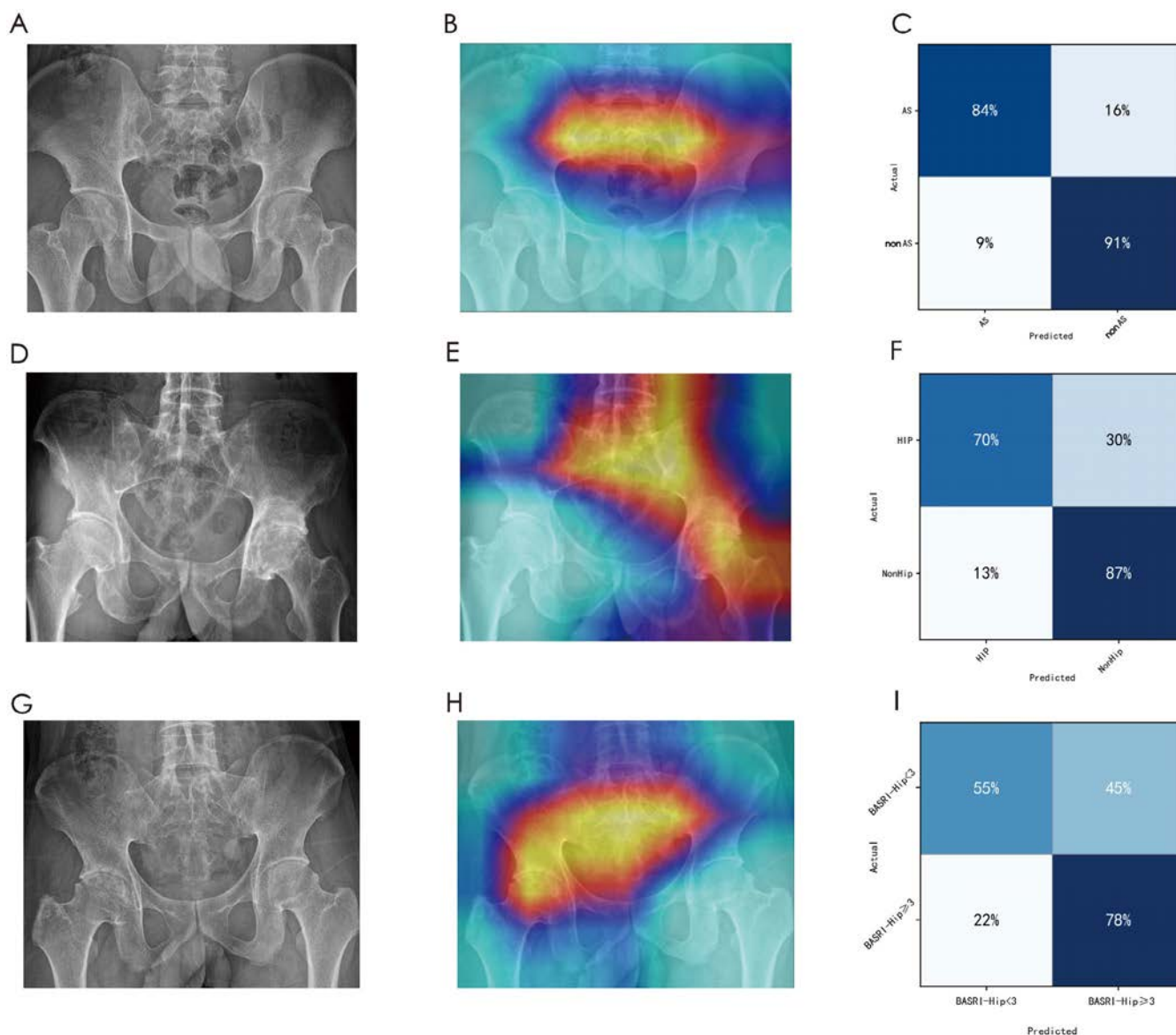


FIGURE 1 | Interpretability and Performance of the DL model. (A) Pelvic X-ray of an AS patient. (B) CNN identified abnormalities on Pelvic X-ray in AS patients, and the sacroiliac joint of the patient was marked as a hot area in red. (C) Confusion matrix presents the performance of the DL model in identifying AS and non-AS patients. (D) Pelvic X-ray of an AS patient with left hip arthropathy. (E) CNN identified abnormalities on Pelvic X-ray in AS patients with left hip arthropathy, and the sacroiliac joint and left hip joint were highlighted in red. (F) Confusion matrix presents the performance of the DL model in identifying AS patients with left hip arthropathy. (G) Pelvic X-ray of an AS patient with BASRI-Hip Grade 3. (H) CNN identified abnormalities on Pelvic X-ray in AS patients with BASRI-Hip grade ≥ 3 , and the sacroiliac joint and right hip joint were highlighted in red. (I) Confusion matrix presents the performance of the DL model in identifying AS patients with BASRI-Hip grade ≥ 3 . AS, Ankylosing Spondylitis; BASRI-Hip grade, Bath Ankylosing Spondylitis Radiology hip grade.

patient exhibiting a lesion in the left hip joint. Both the sacroiliac and left hip joint abnormalities were identified by the model and depicted in a heatmap (Figure 1E). The confusion matrix and ROC curve validated the model's performance, with an AUC of 0.856 (Figure 1F and Figure S3). Lastly, the pelvic X-ray images of 631 AS patients diagnosed with hip arthropathy were introduced into the DL model to determine the severity of hip lesions. Figure 1G showcases a pelvic X-ray of an AS patient whose right hip joint presented a lesion classified as BASRI-Hip Grade 3. This deviation was identified by the DL model, and its visualization is evident in the heatmap (Figure 1H). The model's effectiveness in discerning AS patients with BASRI-Hip ≥ 3 was emphasized by the confusion matrix and ROC curve, recording an AUC of 0.741 (Figure 1I and Figure S4).

3.2 | Construction and Performance of Ensemble Deep Learning Models

An overview of the three task-specific EDL classification models, including their input populations, sample sizes, endpoints, and held-out validation AUCs, is provided in Table 1. After combining deep learning features with clinical variables for 5721 patients, we applied the preprocessing and feature-selection procedure described above. Figure 2A shows the correlation structure, and Figure 2B,C shows LASSO selection. Final predictors were determined by LASSO in the training cohort; Ten machine-learning algorithms were compared for distinguishing AS from non-AS. XGBoost performed best (Figure 2D and Table S1). In the held-out validation cohort, the XGBoost EDL

TABLE 1 | Summary of EDL classification tasks and held-out validation performance.

Task	Input population	N	Endpoint	Model	Held-out validation AUC
AS diagnosis	AS and non-AS patients	5721	AS versus non-AS	EDL (XGBoost)	0.978
Hip arthropathy	Patients with AS	2087	Hip arthropathy present versus absent	EDL (XGBoost)	0.990
Severe hip involvement	AS with hip arthropathy	631	BASRI-Hip ≥ 3 versus < 3	EDL (XGBoost)	0.996

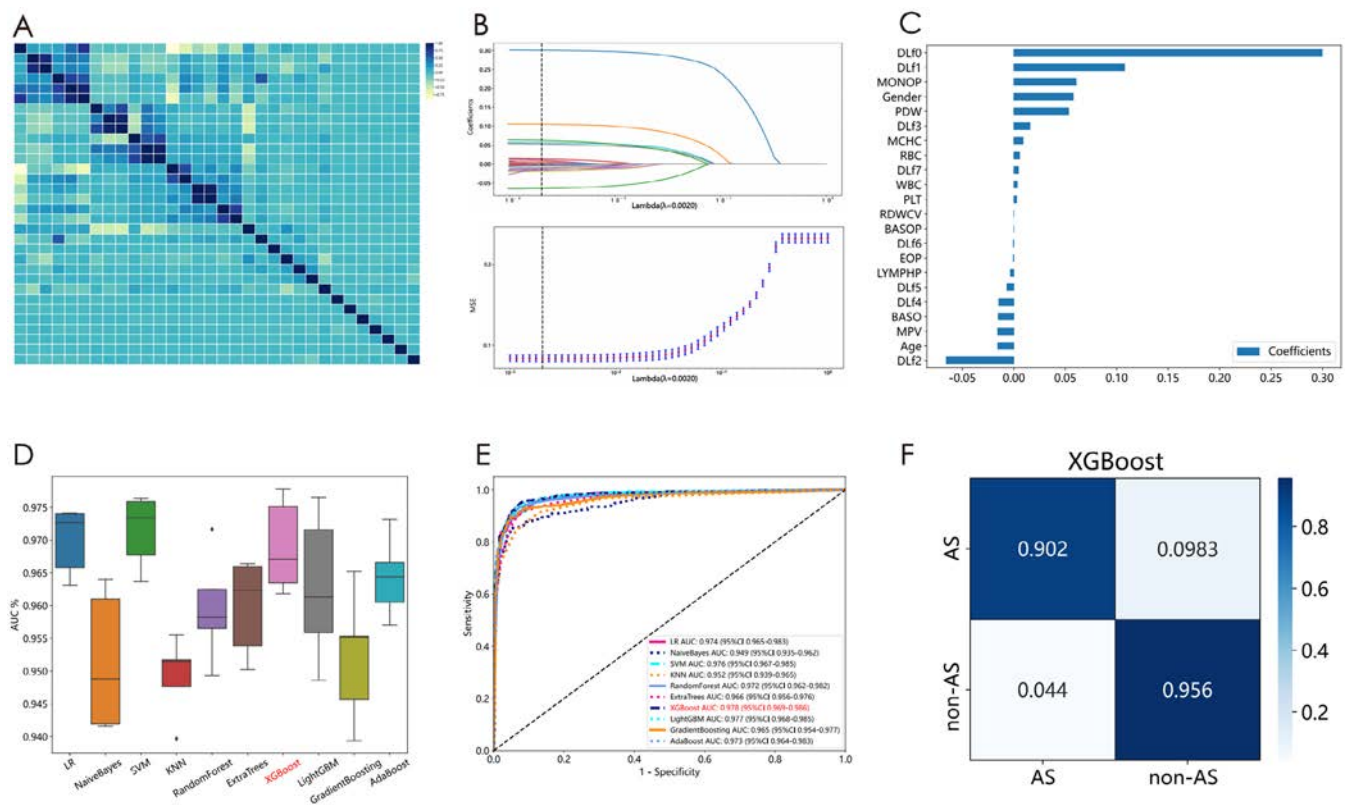


FIGURE 2 | Construction and performance of EDL models. (A) Correlation heatmap of the variables in the EDL models. (B) LASSO coefficient profiles of the predictive factors and the results of LASSO regression, using 10 repetitions of stratified 10-fold cross-validation within the training cohort to determine the optimal penalty parameter lambda. (C) The variable importance ranking for constructing the EDL models. (D) Performance of 10 different machine learning algorithms used to construct EDL models. (E) ROC of 10 different machine learning algorithms. (F) Confusion matrix presents the performance of the EDL model. AS, Ankylosing Spondylitis; AUC, area under the curve; KNN, K-nearest neighbors; LightGBM, Light Gradient Boosting Machine; LR, logistic regression; MSE, mean squared error; SVM, Support Vector Machine.

model achieved an AUC of 0.978 for distinguishing AS from non-AS patients (Figure 2E), and the corresponding confusion matrix is shown in Figure 2F.

3.3 | Ensemble Deep Learning Model to Identify AS Patients With Hip Arthropathy

Figure 3A presents a visual heatmap from the DL model highlighting an AS patient with bilateral hip lesions, possessing a BASRI-Hip grade of 4. Figure 3B depicts the variables identified by the LASSO regression for the construction of an EDL model aiming to recognize AS patients with hip joint lesions. The ROC curves for both the DL and EDL models in determining AS patients with hip joint lesions are shown in Figure 3C. In the held-out validation cohort, the DL and EDL models achieved AUCs of 0.856 and 0.990, respectively, for identifying hip arthropathy among patients with AS. The confusion matrix for the EDL model, used to ascertain if AS patients exhibited hip arthropathy, is displayed in Figure 3D. As presented in Figure 3E and Table S3, the ESR levels in AS patients with hip arthropathy (AS Hip group) significantly exceeded those without hip arthropathy (AS non-Hip group) ($p < 0.05$). The EDL Score for the AS Hip group was also markedly elevated compared to the AS non-Hip group ($p < 0.001$) (Figure 3F and Table S3). A notable positive correlation between ESR and EDL Score is depicted in Figure 3G.

Figure 4A showcases a heatmap from the DL model, identifying an AS patient with left hip arthropathy holding a BASRI-Hip grade of 3. The LASSO regression-identified variables for developing an EDL model to gauge the severity of hip arthropathy in AS patients are illustrated in Figure 4B. Figure 4C presents the ROC curves of both the DL and EDL models when identifying AS patients with hip arthropathy of BASRI-Hip ≥ 3 . In the held-out validation cohort, the DL and EDL models achieved AUCs of 0.741 and 0.996, respectively, for identifying BASRI-Hip grade ≥ 3 . Figure 4D illustrates the confusion matrix for the EDL model in determining AS patients with hip arthropathy of BASRI-Hip ≥ 3 . As evidenced in Figure 4E and Table S4, the ESR of AS patients with hip arthropathy graded as BASRI-Hip ≥ 3 significantly surpassed those graded as BASRI-Hip < 3 ($p < 0.05$). The EDL Score for the BASRI-Hip ≥ 3 group was also notably higher than that of the BASRI-Hip < 3 group ($p < 0.001$) (Figure 4F and Table S4). A marked positive association between ESR and EDL Score is illustrated in Figure 4G.

3.4 | EDL Model Revealed That Monocytes Were Associated With Hip Arthropathy in AS Patients

In this research, we employed the XGBoost algorithm to develop the EDL model. Figure S10 illustrates the weightage and ranking of individual features within the EDL model. Figure S10A highlights the feature weights in the EDL model when distinguishing between the AS and non-AS patients. The most influential three features include Deep Learning feature 0 (DLf0), Deep Learning feature 1 (DLf1), and monocyte percentage (MONOP). Figure S10B details the feature weights in the EDL model when differentiating between the AS Hip and AS non-Hip groups. The top-ranking three features are neutrophil count (NEUT),

MONOP, and DLf1. Figure S10C provides the feature weights of the EDL model when identifying patients within the BASRI-Hip ≥ 3 and BASRI-Hip < 3 groups. The three dominant features are MONOP, white blood cell count (WBC), and eosinophils percentage (EOP). It's noteworthy that MONOP plays a significant role in the EDL model's categorization of AS patients.

3.5 | Identification of the Monocyte-Associated Gene LYN by Multiomics Analysis

From surgical procedures, we obtained 24 samples for exploratory multi-omics analysis: 12 from AS patients undergoing THA and 12 from non-AS fracture controls undergoing surgery for acetabular fractures. Figure 5A shows the radiograph and pathological specimen from an AS patient with BASRI-Hip Grade 4 undergoing THA. In each omics comparison, LYN expression was higher in the AS group than in the corresponding non-AS fracture-control group: proteomics of hip ligament (6 AS vs. 6 controls; Figure 5B), transcriptomics of hip ligament (3 AS vs. 3 controls; Figure 5C), and single-cell transcriptomics of bone marrow (3 AS vs. 3 controls; Figure 5D), all at nominal $p < 0.05$. These small-sample findings are exploratory. Single-cell RNA sequencing identified 13 clusters (Figure 5E), and LYN expression was concentrated predominantly in monocytes (Figure 5F,G).

3.6 | Validation of Immune Cell Infiltration Analysis and Clinical Significance of the Immune Ensemble Score

Immunohistochemical analysis included hip-ligament samples from six patients with AS-related hip arthropathy and six non-AS fracture controls. LYN expression and LYN-positive staining were higher in the AS group than in the non-AS fracture-control group ($p < 0.001$; Figure 6A–C). LYN was also differentially expressed between AS and non-AS samples in GSE73754 (Figure 6D). Proteomic LYN abundance was positively correlated with MONOP ($p < 0.05$; Figure 6E).

In the clinical cohort of 8826 patients, MONOP was slightly higher in patients with AS than in non-AS patients (0.077 ± 0.023 vs. 0.074 ± 0.022 ; $p < 0.001$; Figure 6F and Table S8). The exploratory IE score differed across BASRI-Hip grades (Figure 6G). In the unadjusted Kaplan–Meier analysis, the high-IE-score group showed an earlier age at Severe hip involvement than the low-IE-score group, with estimated median ages of 36 and 50 years, respectively (Figure 6H). These results indicate an exploratory association with Timing of severe hip involvement and do not establish independent prediction of biological disease progression.

4 | Discussion

4.1 | Orientation and Overview of Ensemble Scoring System

Correct diagnosis and evaluation of ankylosing spondylitis (AS) are important for symptom management and assessment of structural severity. Multi-omics analyses may help characterize

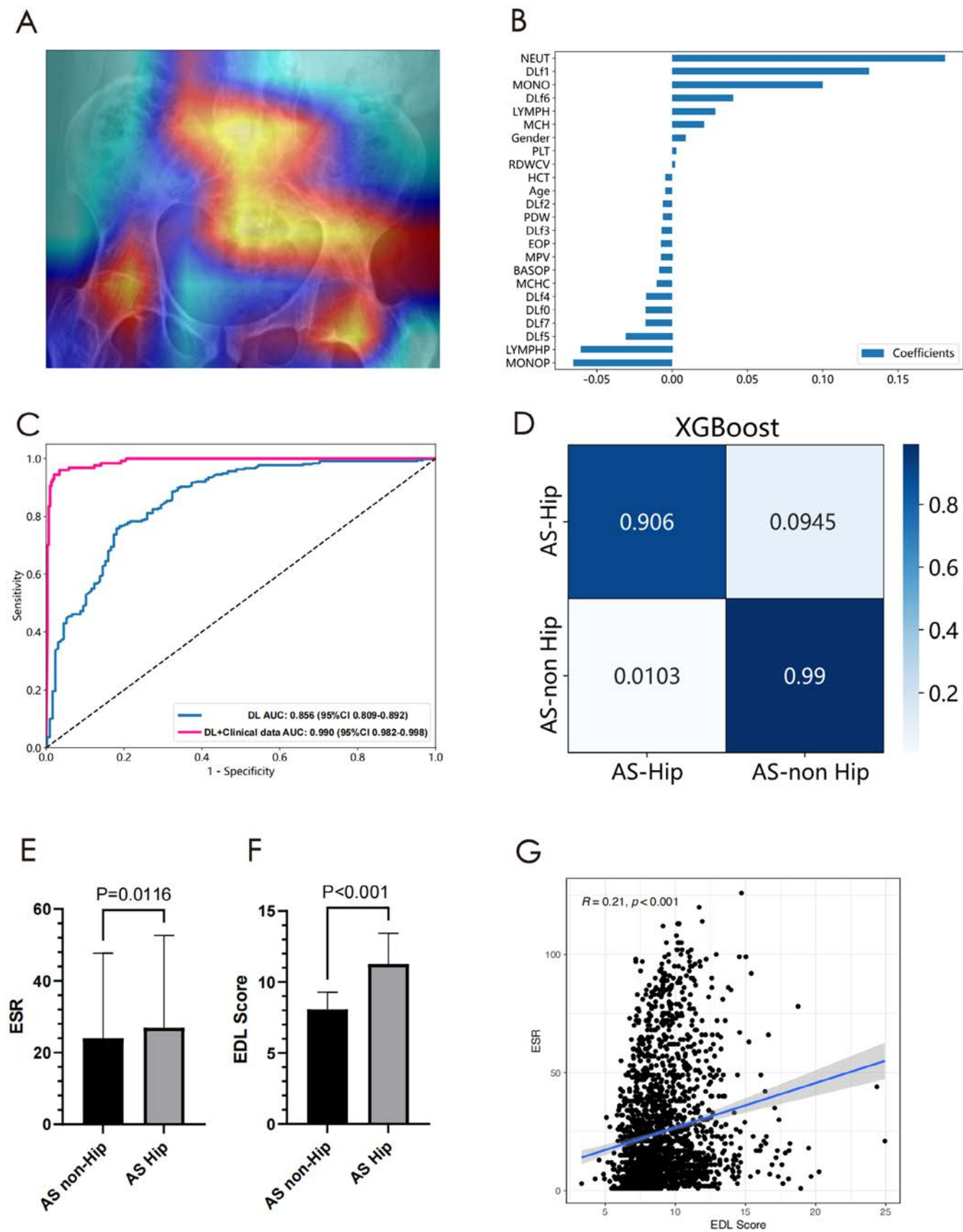


FIGURE 3 | Legend on next page.

FIGURE 3 | EDL model to identify AS patients with hip arthropathy. (A) CNN identified abnormalities on Pelvic X-ray in AS patients with bilateral hip arthropathy, and the sacroiliac joint and bilateral hip joint of the patient was marked as a hot area in red. (B) The variable importance ranking for constructing the EDL models. (C) ROC for DL and EDL models. (D) The confusion matrix presents the performance of the EDL model in identifying hip arthropathy among patients with AS. (E, F) Comparison of ESR and EDL scores between patients with AS with and without hip arthropathy. (G) Correlation analysis of ESR and EDL score in AS patients. AS, Ankylosing Spondylitis; BASO, basophil; BASOP, basophil percentage; DL, Deep Learning; DLf, Deep Learning feature; EDL, Ensemble Deep Learning; EOP, eosinophil percentage; ESR, erythrocyte sedimentation rate; LMPHP, lymphocyte percentage; MCHC, mean corpuscular hemoglobin concentration; MONOP, monocyte percentage; MPV, mean platelet volume; PDW, platelet distribution width; PLT, platelet; RBC, red blood cell; RDWCV, red cell distribution width variance coefficient.

the heterogeneous immune microenvironment and identify candidate biomarkers. In this study, we developed an AI-based Ensemble Scoring System (ESS) for AS and used XGBoost to identify MONOP as an influential variable. The ESS showed strong diagnostic and severity-classification performance, while the exploratory IE score was associated with different ages at severe hip involvement. These findings require external and prospective validation.

4.2 | Ensemble Deep Learning Model in the Diagnosis of AS

The merger of artificial intelligence (AI) and healthcare has emerged as a focal point of research within the medical domain [16, 17]. Existing literature reveals that AI, particularly in the realm of digital radiology imaging, can effectively support clinicians in their diagnostic endeavors [18, 19]. Both deep learning and machine learning are subsets of AI, and our preliminary research has ventured into these domains. By deploying pelvic radiographs and a convolutional neural network (CNN) oriented deep learning algorithm, we efficiently discerned between AS and non-AS patients. Utilizing an array of machine learning algorithms, we harnessed clinical data to forge a predictive model for AS. To refine the diagnostic model for AS, informed by these two investigations, we amalgamated deep learning and machine learning techniques to develop an ensemble deep learning (EDL) model, encompassing data from 5721 patients (2087 AS and 3634 non-AS patients). The EDL model emerged after integrating Deep Learning features (DLf) with clinical markers. The model's AUC stood at 0.978, signifying its potent predictive capacity for AS. Furthermore, the EDL model proficiently differentiated between Hip Arthropathy in Ankylosing spondylitis (HAAS) and non-HAAS patients, as illustrated in Figure 3C. Similarly, the EDL model adeptly identified AS patients with a BASRI-Hip ≥ 3 (Figure 4C). The EDL model synthesized the attributes of both deep learning and machine learning. However, deep learning, in essence, remains a black-box model; despite its exceptional outputs, its inference pathway remains elusive, yielding non-interpretable results. To mitigate this, we utilized CNN to render a visual representation, culminating in a heatmap. As Figure 1B,E,H depict, the CNN adeptly spotlighted sacroiliac joint lesions in AS patients' pelvic X-rays and likewise identified similar lesions in HAAS patients. This heatmap offers a more lucid insight into the decision-making trajectory of deep learning, manifestly echoing the precision of the EDL model. Algorithm selection remains paramount in machine learning. Machine learning, via algorithm-driven weighting, refines DLf and clinical parameters to identify the

most impactful variables. Following a comparative evaluation of diverse algorithms, XGBoost emerged as the optimal choice (Figure 2D,E). Notably, our study underscored the prowess of machine learning in unearthing diagnostic predictors tied to AS, thereby facilitating deeper inquiries into its pathogenesis. Figure S10 accentuates that MONOP, weighed via XGBoost, was pivotal in distinguishing AS patients, HAAS patients, and those with a BASRI-Hip ≥ 3 designation. This hints at anomalous MONOP levels potentially being intrinsically linked to the onset of AS. Consequently, the EDL model, by coalescing CNN's visual capabilities and machine learning's attribute screening prowess, showcased robust predictive accuracy for AS, suggesting promising implications for clinical applications.

Recently, Wei et al. developed an AI-based severity inspection model for AS using patients' X-ray images and the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS). Their study employed a two-stage deep learning framework (keypoint detection followed by mSASSS evaluation) and established a "Gold Standard" through three-expert consensus annotation, achieving a mean accuracy of 0.81 in mSASSS evaluation [20]. Their successful application of a two-stage deep learning framework further validates the feasibility of AI-driven radiographic assessment for AS, reinforcing the methodological foundation for studies like ours.

4.3 | Multi-Omics Analysis Identified Heterogeneous Immune Cell Infiltration in AS

AS is an inflammatory ailment, with immune cells serving as pivotal regulators of inflammation. Earlier research has posited that aberrant immune cell infiltration is intertwined with the pathogenesis of AS. The macrophage-mediated inflammatory response, when activating the Wnt/ β -catenin signaling pathway, can trigger the osteogenic differentiation of bone marrow mesenchymal stem cells in AS [21]. Moreover, our findings corroborated that MONOP held considerable significance in discerning AS patients, HAAS, and those with a BASRI-Hip ≥ 3 , as gauged through XGBoost. MONOP was an influential feature in the AS-diagnosis and hip-severity classification models. However, its role in longitudinal disease progression requires further investigation (Figure S10). In their developmental phase, monocytes partake in both homeostasis and the release of inflammatory agents [22, 23]. Research has indicated that inflammatory maladies like rheumatoid arthritis (RA), multiple sclerosis (MS), and systemic lupus erythematosus (SLE) exhibit a close association with monocytes [24]. The advancement of such inflammatory conditions

also has ties with monocytes, as elucidated by the work of Adrian Schreiber and colleagues, which underscored that the pro-inflammatory nature of monocytes instigates Th-17 cell polarization, culminating in the progression of ANCA-induced glomerulonephritis [25]. Hence, monocytes are integral to the pathogenesis and evolution of AS. We postulate

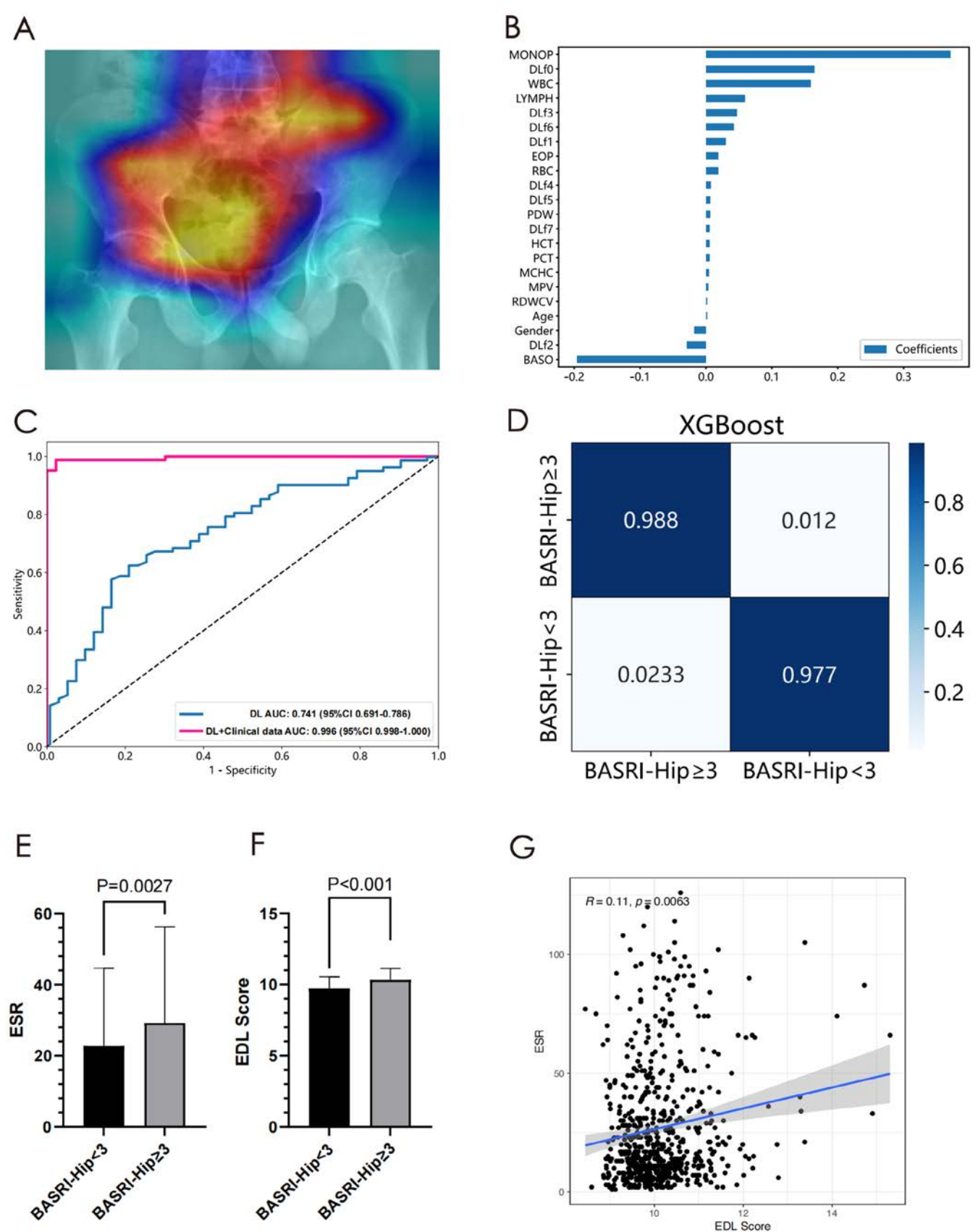


FIGURE 4 | Legend on next page.

FIGURE 4 | EDL model to identify AS patients with BASRI-Hip grade ≥ 3 . (A) CNN identified abnormalities on Pelvic X-ray in AS patients with right BASRI-Hip grade = 3, and the sacroiliac joint and right hip joint of the patient was marked as a hot area in red. (B) The variable importance ranking for constructing the EDL models. (C) ROC for DL and EDL models. (D) The confusion matrix presents the performance of the EDL model in identifying patients with BASRI-Hip grade ≥ 3 . (E, F) Comparison of ESR and EDL score between BASRI-Hip grade ≥ 3 and BASRI-Hip grade < 3 patients. (G) Correlation analysis of ESR and EDL score in AS patients with hip arthropathy. AS, Ankylosing Spondylitis; BASO, basophil; BASOP, basophil percentage; DL, Deep Learning; DLF, Deep Learning feature; EDL, Ensemble Deep Learning; EOP, eosinophil percentage; ESR, erythrocyte sedimentation rate; LMPHP, lymphocyte percentage; MCHC, mean corpuscular hemoglobin concentration; MONOP, monocyte percentage; MPV, mean platelet volume; PDW, platelet distribution width; PLT, platelet; RBC, red blood cell; RDWCV, red cell distribution width variance coefficient.

that monocyte dysregulation may be associated with heterogeneous immune infiltration and structural hip involvement in AS. *LYN* assumes a central role in modulating the inflammatory response; curtailing *LYN* kinase offers potential therapeutic avenues for conditions like small vessel vasculitis and nascent liver fibrosis [26]. Our preliminary investigations affirmed *LYN*'s position as a central gene intertwined with AS's immune cell infiltration. To further validate the connection between *LYN* dysregulation and diverse immune cell infiltration concerning AS progression, we delved into the multi-omics data of HAAS patients. Proteomics, transcriptomics, and single-cell transcriptomics insights collectively revealed heightened *LYN* expression in HAAS patients (Figure 5B–D). It has been documented that *LYN*'s dysregulation is linked with the progression of inflammatory diseases, given its crucial role in activating, proliferating, and steering immune cells during inflammation [27]. Our findings align with these prior conclusions. A deeper examination of single-cell transcriptomic data from HAAS patients spotlighted the predominant expression of *LYN* in monocytes (Figure 5F,G). Concurrently, Spearman's correlation analysis denoted a robust positive correlation between *LYN* expression and MONOP. These insights hint at the influence of *LYN* dysregulation in monocytes on the diverse immune cell infiltration observed in AS. Furthermore, immunohistochemical evaluations of hip ligaments in HAAS patients vindicated the findings derived from the single-cell transcriptomics analysis (Figure 6A–C).

4.4 | Clinical Interpretability of the Ensemble Scoring System in Ankylosing Spondylitis

At present, the primary diagnostic tools for AS encompass HLA-B27 testing, analysis of acute-phase reactants, assessment of clinical symptoms, and radiological examinations. Nonetheless, there are scenarios where patients, despite testing negative for HLA-B27, manifest unconventional early clinical signs and imaging results [28]; thus, complicating diagnosis for medical practitioners. While the modified New York criteria serve as the standard for diagnosing AS [29], they are tailored only to distinguish between AS and non-AS patients, lacking granularity in identifying variations in disease severity among AS patients. The BASDAI assesses disease activity, the BASFI evaluates functional ability, and the BASRI-Hip grades radiographic hip damage in patients with AS [30–32]. These diagnostic criteria and scoring benchmarks for AS offer limited clinical interpretability. In light of these limitations, we devised an Ensemble Scoring System (ESS) for AS, integrating AI and multi-omics insights. This ESS bifurcates into two segments: the ensemble deep learning (EDL) score and the immune ensemble (IE) score.

AS is characterized as a persistent inflammatory autoimmune disorder. Our objective was to elucidate the nexus between AS and the ESS. Our findings underscored that the EDL score was markedly elevated in the HAAS cohort compared to their non-HAAS counterparts. In a similar vein, the EDL score was distinctly higher in the BASRI-Hip ≥ 3 group relative to the BASRI-Hip < 3 faction. The EDL score showed potential for classifying radiographic hip-disease severity within the present retrospective cohort. Notably, only approximately 40%–50% of clinically active AS patients showcase increased erythrocyte sedimentation rate (ESR) levels [33]. Gauging disease activity and therapy responsiveness in AS remains intricate. Though ESR, a conventional marker for acute phase response evaluation, has been employed, its correlations with patient symptoms or radiological advancements aren't consistent. The advent of the EDL score, which amalgamates AI-driven radiological attributes with clinical features, warrants prospective evaluation in independent external cohorts.

Using exploratory multi-omics analyses, we identified an association between monocytes and AS-related immune infiltration and therefore incorporated MONOP into the IE score. IE scores differed between BASRI-Hip severity groups. In Kaplan–Meier analysis, a high IE score was associated with earlier severe hip involvement (estimated median age, 36 vs. 50 years). However, the age at first documented severe hip involvement may be influenced by the frequency and timing of clinical follow-up and radiographic assessment and may not represent the exact onset of structural hip progression. Thus, this result should be interpreted as hypothesis-generating risk stratification rather than confirmation that the IE score independently predicts biological disease progression. Prospective studies with standardized treatment data, longer follow-up, additional outcomes, and external validation are needed before clinical use.

4.5 | Integrating AI and Multi-Omics for Investigating Heterogeneous Immune Cell Infiltration in AS and Advancing Personalized Medicine

During the construction of the deep learning model using XGBoost, we noted that MONOP showed high feature importance across the three EDL classification tasks. Notably, MONOP ranked among the top three predictive factors for distinguishing between AS and non-AS patients, identifying AS patients with hip arthropathy, and recognizing AS patients with BASRI-Hip scores ≥ 3 (refer to Figure S10). Interestingly, previous research from our team also indicated a connection between heterogeneous immune cell infiltration in AS and monocytes, aligning

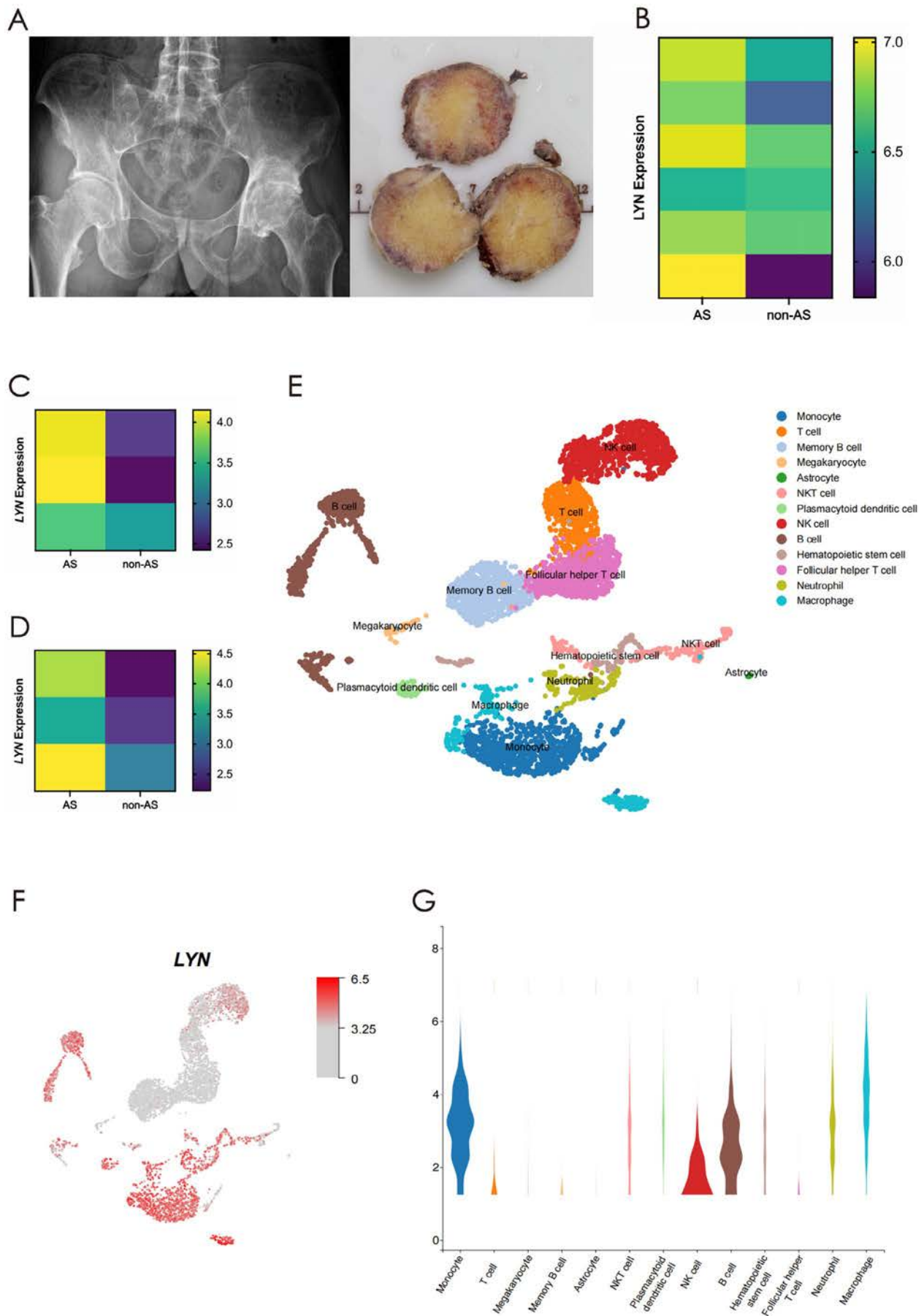


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FIGURE 5 | Multi-omics analyses identified LYN expression in monocytes. (A) Human hip ligament tissue collection. (B) Proteomic comparison of hip ligament from AS patients ($n=6$) and non-AS fracture controls ($n=6$). (C) Transcriptomic comparison of hip ligament from AS patients ($n=3$) and non-AS fracture controls ($n=3$). (D) Single-cell transcriptomic comparison of bone marrow from AS patients ($n=3$) and non-AS fracture controls ($n=3$). In panels B–D, LYN expression was higher in AS than in the corresponding control group at nominal $p < 0.05$; no multiple-testing correction was applied. (E) t-SNE plot showing 13 cell clusters. (F) Feature plot of LYN. (G) Violin plot of LYN expression across cell clusters. LYN, LYN proto-oncogene, Src family tyrosine kinase; AS, ankylosing spondylitis.

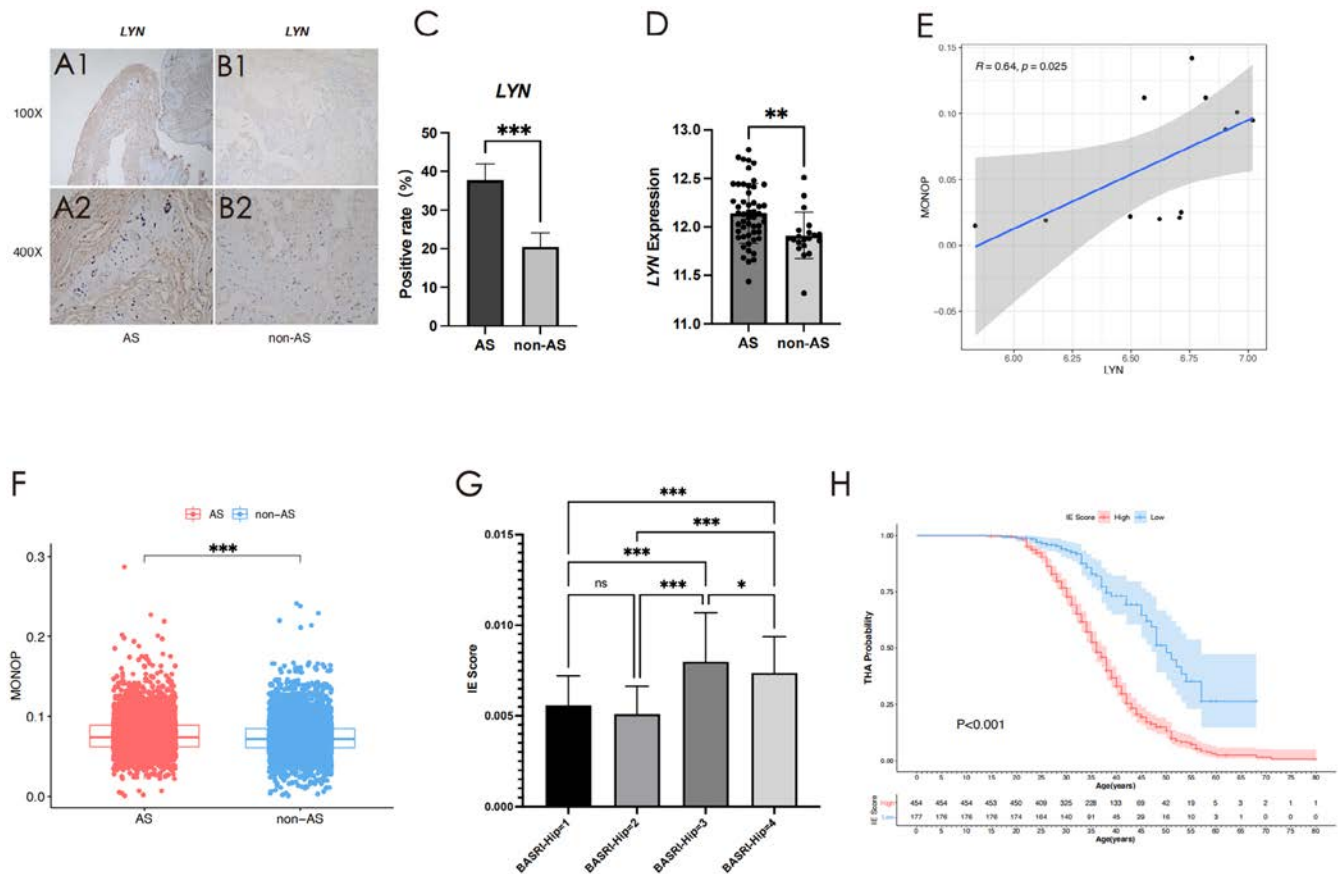


FIGURE 6 | Validation of AS-related immune infiltration and exploratory association between the IE score and age at Severe hip involvement. (A and B) Representative LYN immunohistochemical staining in hip-ligament samples from patients with AS-related hip arthropathy ($n=6$) and non-AS fracture controls ($n=6$). (C) Quantification of LYN-positive staining. (D) LYN expression in AS ($n=52$) and non-AS ($n=20$) samples from GSE73754. (E) Correlation between proteomic LYN abundance and MONOP. (F) MONOP in patients with AS ($n=3557$) and non-AS patients ($n=5269$). (G) Exploratory IE scores across BASRI-Hip grades. (H) Unadjusted Kaplan–Meier curves for age at Severe hip involvement, stratified by IE-score group. The analysis represents an exploratory association with Timing of severe hip involvement rather than independent prediction of biological disease progression.

with the deep learning model based on the XGBoost algorithm, where MONOP carried high predictive weight.

Existing research has demonstrated a link between the dysregulation of the immune microenvironment, stemming from the inflammatory response of monocytes in patients, and the pathological ossification in individuals with AS [34]. Furthermore, multidimensional perspectives on the immune microenvironment, effectively portrayed through multi-omics studies, have played a crucial role in exploring the diverse immune infiltration in AS [35]. To further investigate the relationship between heterogeneous immune cell infiltration in AS and monocytes, we conducted multi-omics studies. Using single-cell transcriptomics analysis, we identified 13 distinct cell clusters, with monocytes emerging as a pivotal constituent among these

clusters (Figure 5E). Furthermore, we observed predominant expression of the immunorelated hub gene LYN within monocytes (Figure 5F,G). Similarly, leveraging transcriptomic data, we conducted immune cell infiltration analysis in AS patients, revealing a significant elevation in monocyte proportions and differential expression of LYN (Figure S5). These findings were further validated through immunohistochemistry (Figure 6A–C) and a substantial dataset of clinical data comprising 3557 AS patients and 5269 non-AS patients (Figure 6F).

Our exploratory multi-omics analyses support monocytes as a component of heterogeneous immune infiltration in AS. IE scores differed across disease-severity groups (Figure 6G), and a high IE score was associated with earlier severe hip involvement in the retrospective cohort (Figure 6H).

In summary, this study developed an AI- and multi-omics-informed Ensemble Scoring System for AS diagnosis and severity assessment. The exploratory IE score may help stratify patients with different trajectories to severe hip involvement, but prospective external validation is needed.

4.6 | Limitation

This study has several limitations. First, the ESS was developed retrospectively and has not undergone independent external validation. Although patient-level separation, training-only feature selection, and repeated internal cross-validation were used to reduce information leakage and overfitting, the high validation AUCs require confirmation in independent multicenter cohorts. Second, the proteomic, transcriptomic, and single-cell RNA-sequencing analyses included small sample sizes, and no multiple-testing correction was applied; these findings should therefore be considered exploratory. Third, patients with acetabular fractures served as non-AS tissue controls. Trauma and perioperative inflammation may influence immune and gene-expression profiles, and these patients are not equivalent to healthy controls. Fourth, although treatment status was available, detailed information on treatment duration, dose, adherence, and longitudinal changes was incomplete and was not incorporated into the primary Kaplan–Meier analysis. Residual confounding by treatment therefore cannot be excluded. Fifth, the age at first documented severe hip involvement may be influenced by the frequency of clinical follow-up and radiographic assessment and may not represent the exact onset of structural hip progression. Finally, the IE score was constructed post hoc and requires prospective validation, assessment of calibration and clinical utility, and evaluation against conventional clinical predictors before clinical application.

5 | Conclusion

In summary, we developed and internally validated an artificial intelligence-based ESS for AS diagnosis and hip-disease severity classification. Exploratory multiomics and immunohistochemical analyses linked LYN dysregulation in monocytes with heterogeneous immune infiltration. The exploratory IE score was associated with earlier severe hip involvement but did not independently establish prediction of biological disease progression. Prospective multicenter external validation is required before clinical application.

Author Contributions

Conceptualization, Chenxing Zhou; Data curation, Jiang Xue, Tuo Liang and Xuhua Sun; Formal analysis, Shaofeng Wu, Sitan Feng, Chengqian Huang, Bin Zhang, Wendi Wei and Zhenwei Yang; Funding acquisition, Chenxing Zhou and Chong Liu; Investigation, Chengqian Huang, Jiarui Chen and Hao Li; Methodology, Chenxing Zhou, Xinli Zhan, Jichong Zhu and ShengSheng Huang; Project administration, Chenxing Zhou and Chong Liu; Resources, Jiarui Chen and Jiang Xue; Software, Chenxing Zhou, Jichong Zhu and Tianyou Chen; Supervision, Chong Liu; Validation, Chenxing Zhou, Tianyou Chen, Shaofeng Wu, Sitan Feng, Liyi Chen and Jie Jiang; Visualization, Bin Zhang and Chong Liu; Writing – original draft, Chenxing Zhou; Writing – review and editing, Xinli Zhan and Chong Liu.

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Ethics Statement

The study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (approval No. 2023-E352-01). All participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The original data in this study are available upon reasonable request. More inquiries can be directed to the corresponding authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Prediction performance of machine learning algorithms. **Table S2:** Clinical characteristics of patients in immunohistochemistry analysis. **Table S3:** ESR levels and EDL score in AS Hip and AS non-Hip groups. **Table S4:** ESR levels and EDL score in BASRI-Hip ≥ 3 and BASRI-Hip < 3 groups. **Table S5:** Clinical characteristics of patients in proteomics analysis. **Table S6:** Clinical characteristics of patients in transcriptomics analysis. **Table S7:** Clinical characteristics of patients in Single-cell RNA sequencing analysis. **Figure S1:** The graphical abstract of this study. **Figure S2:** The model's efficacy to identify AS and non-AS patients was depicted through the ROC curve, boasting an AUC of 0.948. **Figure S3:** The model's performance in identifying hip arthropathy among patients with AS was evaluated using the ROC curve, yielding an AUC of 0.856. **Figure S4:** The model's effectiveness in discern AS patients with BASRI-Hip ≥ 3 was depicted through the ROC curve, boasting an AUC of 0.741. **Figure S5:** Heterogeneous Immune Cell Infiltration diagram. Box plot diagram of the difference in immune cells between AS and non-AS patients in GSE 25101. **Figure S6:** Receiver Operating Characteristic (ROC) curves and confusion matrices were employed to evaluate the discriminative performance of ResNet 50 (A, B) in distinguishing AS patients from non-AS patients. **Figure S7:** Receiver Operating Characteristic (ROC) curves and confusion matrices were employed to evaluate the discriminative performance of DenseNet 121 (A, B), DenseNet 169 (C, D), and DenseNet 201 (E, F) in distinguishing AS patients from non-AS patients. **Figure S8:** Receiver Operating Characteristic (ROC) curves and confusion matrices were employed to evaluate the discriminative performance of ResNet 18 (A, B), ResNet 34 (C, D), and ResNet 152 (E, F) in distinguishing AS patients from non-AS patients. **Figure S9:** Receiver Operating Characteristic (ROC) curves and confusion matrices were employed to evaluate the discriminative performance of VGG 11 (A, B), VGG 13 (C, D), and VGG16 (E, F) in distinguishing AS patients from non-AS patients. **Figure S10:** Weightage and ranking of features within the EDL model based on XGBoost.



ORIGINAL ARTICLE

Development and Characterization of a Fully Human Recombinant MPO-ANCA Monoclonal Antibody as Candidate Quality Control Material

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ABSTRACT

Aim: MPO-ANCA detection suffers prominent inter-assay variability due to a shortage of standardized, sustainable quality control (QC) materials. Existing patient-pooled sera have limited supply, while chimeric monoclonal antibodies fail to fully mimic human autoantibodies. This study aimed to construct a fully human recombinant MPO-ANCA monoclonal antibody and systematically characterize its analytical performance as a candidate QC material for multi-platform MPO-ANCA immunoassay evaluation.

Methods: A human anti-MPO scFv phage display library was constructed using peripheral blood mononuclear cells obtained from MPO-ANCA-positive patients. Following antigen-specific biopanning and screening, the candidate clone H042 was selected and converted into a full-length human IgG1 monoclonal antibody. We verified its structural integrity, antigen specificity, pANCA immunofluorescence pattern, quantitative linearity, thermal/freezing–thaw stability, and cross-kit performance across enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA), multiplex flow immunoassay, and indirect immunofluorescence assay (IIFA) from multiple mainstream manufacturers.

Results: Purified H042 exhibited intact IgG structure, strong MPO binding affinity, and no cross-reactivity with PR3/GBM. It presented typical pANCA staining in IIFA and showed predictable quantitative response. H042 retained >89% residual activity under accelerated thermal and repeated freezing–thaw stress. Notably, H042 yielded consistent qualitative positivity on all tested kits of four mainstream methodologies without false-negative signals, despite inter-platform signal intensity differences.

Conclusion: The fully human monoclonal antibody H042 demonstrated favorable structural integrity, MPO specificity, and broad detectability across the evaluated commercial assay platforms. These characteristics support its potential as a scalable recombinant candidate QC material and an auxiliary tool for MPO-ANCA assay harmonization and laboratory quality assurance.

Meiyu Guo and Yeshuang Yuan contributed equally to this work.

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1 | Introduction

The detection of anti-neutrophil cytoplasmic antibodies (ANCA) targeting myeloperoxidase (MPO) is a cornerstone in the diagnosis and management of ANCA-associated vasculitis (AAV). Based on the 2022 American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) classification criteria, MPO-ANCA positivity carries substantial diagnostic weight, underscoring its central role in disease classification and clinical decision-making [1, 2]. Further, MPO-ANCA levels have been associated with renal involvement, relapse risk, and clinical outcomes, supporting their potential utility in longitudinal assessment [3, 4].

The 2017 revised international consensus on ANCA testing has endorsed high-quality antigen-specific immunoassays as the preferred method for MPO-ANCA detection [5]. However, substantial variability among commercial MPO-ANCA immunoassays remains a persistent and significant clinical challenge [6, 7]. This variability stems from multiple factors, including differences in antigen source and preparation, assay design, calibration strategies, and the use of arbitrary reporting units [8]. Such heterogeneity compromises inter-laboratory and cross-platform comparability, poses significant challenges for reliable longitudinal patient monitoring and multi-center clinical trials, and ultimately affects patient care.

A key contributor to this inter-assay variability is the absence of universally accepted, well-characterized, and reproducible reference and quality control materials. While significant progress has been made in this area, current options have inherent limitations. The Institute for Reference Materials and Measurements (IRMM) has developed certified reference materials (CRMs), ERM-DA476/IFCC for the calibration of MPO-ANCA IgG assays [9]. These CRMs, derived from pooled patient plasma and assigned mass unit values, have been instrumental in harmonizing results across different platforms [10]. However, as they are derived from finite biological sources, they are not inexhaustible and are primarily intended for calibrator value assignment rather than routine quality control.

Beyond CRMs, chimeric human-mouse monoclonal antibodies have been developed as potential reference materials for MPO-ANCA detection [11]. However, such chimeric antibodies cannot fully recapitulate the molecular, structural, and immunological characteristics of naturally occurring fully human MPO-ANCA autoantibodies isolated from vasculitis patients. In addition, the cross-platform and cross-manufacturer verification scope of these chimeric antibodies was limited. The majority of analytical evaluations were performed exclusively using immunoassay kits and instruments from a single manufacturer; the lack of systematic validation across multiple independent manufacturers and full coverage of routine clinical detection modalities limits conclusions regarding their applicability across diverse commercial assay platforms.

As such, the development of patient-derived fully human MPO-ANCA monoclonal antibodies remains an attractive strategy to provide materials with defined molecular

characteristics and potential biological relevance to naturally occurring autoantibodies. In this study, we report the successful development of a fully human recombinant MPO-ANCA monoclonal antibody, designated H042, from a patient-derived single-chain variable fragment (scFv) phage display library. Critically, H042 demonstrates consistent detectability across multiple commercial immunoassay platforms and maintains stable immunoreactivity under accelerated storage and repeated freeze-thaw conditions. These favorable analytical characteristics support the potential utility of H042 as a novel, non-serum-derived, fully human candidate quality control material for MPO-ANCA assay evaluation and laboratory quality assurance, contributing to the ongoing effort to standardize ANCA testing.

2 | Materials and Methods

2.1 | Study Participants and Sample Collection

Peripheral blood and serum specimens were collected from patients diagnosed with MPO-AAV and healthy controls (HCs) enrolled at Beijing Hospital. MPO-AAV was classified according to the 2022 ACR/EULAR classification criteria [1, 2]. Written informed consent was acquired from all participants before specimen collection. This study was reviewed and approved by the Ethics Committee of Beijing Hospital (Approval number: 2025BJYYEC-KY354-01), and all experimental procedures were performed following the principles outlined in the Declaration of Helsinki.

Peripheral blood samples isolated from MPO-ANCA-seropositive patients were utilized to construct a human single-chain variable fragment (scFv) phage display library. Serum samples obtained from healthy controls served as the blank serum matrix for preparing simulated quality control (QC) samples and supporting subsequent analytical characterizations, including cross-platform comparison and stability testing. All specimens were fully anonymized prior to laboratory analysis to protect participant privacy.

2.2 | Construction of Human scFv Phage Display Antibody Library

Peripheral blood samples were collected from a total of 10 MPO-AAV patients with strongly positive MPO-ANCA serostatus. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation (GE Healthcare Ficoll-Paque). Total RNA was extracted using TRIzol reagent (Invitrogen, USA) and reverse-transcribed into cDNA. Variable regions of immunoglobulin heavy (VH) and light (VL) chains were amplified by PCR and assembled into scFv constructs using overlap extension PCR. The scFv fragments were digested with SfiI and ligated into the phage display vector pComb3X (Sino Biological Inc., Beijing, China). Recombinant plasmids were electroporated into XL1-Blue competent cells (Sino Biological Inc., Beijing, China) to construct the human scFv phage display library [12, 13]. The library capacity was determined by colony counting. Colony PCR was performed to evaluate insertion efficiency, and representative clones were

analyzed by Sanger sequencing to assess sequence diversity and library quality.

2.3 | Antigen-Specific Biopanning and Screening of MPO-Reactive Clones

Recombinant human MPO protein (Cat. No. 11917-H08H, Sino Biological Inc., Beijing, China) was immobilized onto immunotubes and blocked with 4% skim milk in PBS. The constructed human scFv phage display library was incubated with immobilized MPO antigen for 1 h at room temperature (RT). After extensive washing with PBST, bound phages were eluted using pH 3.0 arginine-acetate buffer and neutralized with Tris-HCl (1 mol/L, pH 7.4). Eluted phages were amplified in TG1 cells with helper phage M13K07. Four consecutive rounds of biopanning were performed to enrich MPO-binding phage clones. Individual clones were screened by indirect ELISA, followed by sequencing and redundancy analysis. Candidate clones were prioritized based on binding intensity, sequence uniqueness, and reproducibility across repeated ELISA measurements. The H042 clone was selected as the final candidate based on its favorable analytical characteristics.

2.4 | Recombinant Expression and Purification of the H042 Monoclonal Antibody

The VH and VL sequences of the selected H042 clone, directly derived from the original patient-derived scFv clone, were cloned into human IgG1 heavy-chain and κ light-chain expression vectors. The recombinant antibody was generated by transient co-expression of paired heavy- and light-chain plasmids in HEK293F cells to obtain a full-length human IgG molecule. HEK293F cells were cultured in serum-free medium and transfected using a transient expression system. Cells were maintained at 37°C with 5% CO₂ under shaking conditions. M293TI-1 medium, SMS-293-SUPI feed solution, and TF1 transfection reagent (Sino Biological Inc., Beijing, China) were used according to the manufacturer's instructions. Feed supplement was added on days 1, 3, and 5 after transfection. Cell culture supernatants were collected and clarified by centrifugation (4000g, 30 min) followed by filtration using a 0.45 μ m filter. The recombinant H042 antibody was purified by Protein A affinity chromatography using Protein A resin. Eluted fractions were immediately neutralized and buffer-exchanged into PBS by dialysis or ultrafiltration. The purified H042 antibody was quantified and stored at 4°C until further analysis.

2.5 | SDS-PAGE Analysis

Purified H042 antibody (0.5 mg/mL in 10 mM PBS, pH 7.2) was analyzed under reducing and non-reducing conditions by SDS-PAGE. Samples were separated using 7.5% and 13% SDS-PAGE gels under reducing and non-reducing conditions, respectively. Protein molecular weight markers (Cat. No. #26610 and #26614, Thermo Fisher Scientific, USA) were used for molecular size estimation. After electrophoresis, gels were stained with Coomassie Brilliant Blue. Non-reducing SDS-PAGE was used to evaluate the integrity of the assembled IgG molecule, whereas

reducing SDS-PAGE was performed to confirm the expected heavy-chain and light-chain molecular sizes.

2.6 | Enzyme-Linked Immunosorbent Assay (ELISA)

Purified H042 was used for molecular characterization, whereas simulated QC samples were prepared by spiking H042 into IgG-depleted HC serum matrix for commercial assay evaluation. ELISA-based assays were performed to evaluate the MPO-binding activity, quantitative response characteristics, and cross-platform detection performance of H042. An IgG-free HC serum matrix was generated by immunoglobulin depletion using Protein A and Protein L affinity purification to minimize endogenous IgG interference and was used for the preparation of simulated quality control samples.

For recombinant MPO-binding analysis, recombinant human MPO antigen (Cat. No. 11917-H08H, Sino Biological Inc., Beijing, China) was used for indirect ELISA. Microplates were coated with recombinant MPO antigen at 1 μ g/mL overnight at 4°C and blocked with blocking buffer. Serially diluted H042 samples (0.05–1000 ng/mL) were added and incubated for 2 h at room temperature. Bound H042 was detected using HRP-conjugated goat anti-human IgG (H + L) secondary antibody (Cat. No. 109-036-088, Jackson ImmunoResearch, USA; dilution 1:5000). Signal detection was performed using TMB substrate, and absorbance was measured at 450 nm.

For quantitative evaluation of H042 in a clinical MPO-ANCA detection system, H042 samples were analyzed using a commercial MPO-ANCA IgG ELISA kit (Cat. No. EA 1211-9601 G, EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) according to the manufacturer's instructions. H042 samples were prepared using a three-fold serial dilution scheme. Detected signals were expressed as RU/mL, and concentration-response curves were generated. The relationship between H042 concentration and ELISA signal intensity was evaluated using second-order polynomial regression.

For ELISA platform comparison, simulated quality control samples were prepared by spiking H042 into IgG-free HC serum matrix and analyzed using commercial MPO-ANCA IgG ELISA systems, including Anti-MPO (pANCA) (Cat. No. ORG 519, ORGENTEC Diagnostika GmbH, Mainz, Germany), QUANTA Lite MPO IgG Reagents (Cat. No. 708700, Werfen, Bedford, MA, USA), and pANCA: myeloperoxidase (MPO) (Cat. No. EA 1211-9601 G, EUROIMMUN, Lübeck, Germany), and AESKULISA MPO (Cat. No. 3303, Aesku.Group, Wendelsheim, Germany). Results were interpreted according to manufacturer-defined cut-off (CO) values.

2.7 | Flow Cytometry-Based Multiplex Immunoassay

Simulated quality control samples prepared by spiking H042 into IgG-free HC serum matrix were analyzed using the Anti-MPO Antibody, Anti-PR3 Antibody, and Anti-GBM Antibody Detection Kit (Cat. No. LP030068, Shanghai Tellgen Life

Science Co. Ltd., Shanghai, China), MPO/PR3/GBM IgG Antibody Detection Kit on AtheNA Multi-Lyte System (Zeus Life Technology (Changzhou) Co. Ltd., Changzhou, China). Results were interpreted according to manufacturer-defined CO values.

2.8 | Indirect Immunofluorescence Assay (IIFA)

IIFA was performed using ethanol-fixed human neutrophil substrates from the IFA: Granulocyte Mosaic 13 (Cat. No. FA 1201–1003-13 HZ, EUROIMMUN, Lübeck, Germany). H042 samples prepared in IgG-free HC serum matrix were processed according to the manufacturer's instructions, and fluorescence patterns were visualized using a fluorescence microscope and interpreted according to characteristic ANCA staining patterns.

2.9 | Preliminary Stability Evaluation

A preliminary assessment of the analytical stability of H042 as a candidate quality control material was performed using HC serum matrix under accelerated thermal and repeated freeze–thaw conditions. H042 was diluted in HC serum matrix and stored under three conditions: 4°C (control), 37°C for 7 days (accelerated stability), and ten freeze–thaw cycles at –80°C. Reactivity was measured using the EUROIMMUN MPO-ANCA IgG assay. Stability was expressed as residual reactivity relative to the 4°C control.

2.10 | Chemiluminescence Immunoassay (CLIA)

MPO-specific IgG detection was performed using commercial MPO-ANCA IgG CLIA systems according to the manufacturers' instructions. The evaluated CLIA systems included iFlash-MPO IgG (IVDR) (Cat. No. C89001G, Shenzhen YHLO Biotech Co. Ltd., Shenzhen, China), QUANTA Flash MPO Reagents (Cat. No. 701133, Werfen, Bedford, MA, USA), and Anti-MPO ChLIA (IgG) (Cat. No. WRE-CL-1103-0100-G, EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany), and MPO Antibody Assay Kit (Cat. No. K-2403-100GB, Guangzhou Kangrun Biotech Co. Ltd., Guangzhou, China). Simulated quality control samples were prepared by spiking H042 into IgG-free HC serum matrix, and signals were interpreted according to manufacturer-defined cut-off values.

2.11 | Multiplex Flow Immunoassay

Simulated quality control samples prepared by spiking H042 into IgG-free HC serum matrix were analyzed using BioPlex 2200 Vasculitis Reagent Pack (Cat. No. 6651850, Bio-Rad Laboratories Inc., Hercules, CA, USA). Results were interpreted according to manufacturer-defined CO values.

2.12 | Multiplex Flow Cytometry

Simulated quality control samples prepared by spiking H042 into IgG-free HC serum matrix were analyzed using the

Autoimmune Vasculitis Antibody Profile Detection Kit (Cat. No. 341301HN, Hunan Weigong Biotechnology Co. Ltd., Hunan, China) based on multiplex flow cytometry. Results were interpreted according to manufacturer-defined CO values.

2.13 | Statistical Analysis

Statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). The quantitative response relationship between H042 concentration and MPO-ANCA ELISA signals was evaluated using second-order polynomial regression to characterize the analytical response behavior within the tested concentration range. The fitted regression equation and coefficient of determination (R^2) were used for evaluation of the concentration-response relationship. For recombinant MPO-binding ELISA, dose–response curves were fitted using a four-parameter logistic (4PL) nonlinear regression model, and EC50 values were calculated.

2.14 | Use of Artificial Intelligence

During manuscript preparation, ChatGPT (OpenAI) was used solely for language editing, including grammar checking and improving sentence structure and readability. ChatGPT was not involved in study design, experimental procedures, data analysis, interpretation of results, or generation of scientific conclusions. All scientific content was reviewed and verified by the authors.

3 | Results

3.1 | Generation of a Human Anti-MPO scFv Phage Display Library and Identification of H042

A human anti-MPO scFv phage display library was constructed using PBMCs obtained from MPO-ANCA-positive patients. Total RNA showed high integrity with clear 28S and 18S rRNA bands, an A260/A280 ratio of 1.99, and a concentration of 1159 ng/μL (Figure 1A and Table S1). VH and VL fragments were successfully amplified from cDNA and assembled into full-length scFv constructs by overlap extension PCR (Figure 1B,C). PCR amplification of immunoglobulin variable region genes confirmed successful recovery of diverse heavy- and light-chain gene fragments for library construction (Figure S1).

A human scFv phage display library with a capacity of 1.72×10^9 CFU was successfully established. Colony PCR and Sanger sequencing of randomly selected clones demonstrated high insertion efficiency and sequence diversity, supporting the quality of the constructed library. Following four consecutive rounds of biopanning against recombinant human MPO, progressive enrichment of MPO-binding clones was observed. ELISA screening identified 347 MPO-reactive clones, among which 102 clones exhibited strong MPO-binding activity. Sequence analysis and functional screening further narrowed the candidates, and H042 was selected for subsequent recombinant expression and characterization. Collectively, these findings demonstrate the successful construction of a human

anti-MPO antibody library and identification of the H042 clone for further evaluation.

3.2 | Recombinant Expression and Characterization of H042

The selected H042 clone was converted into a full-length human IgG1 monoclonal antibody and expressed in HEK293F cells. Following Protein A affinity purification, the molecular integrity and MPO-binding activity of recombinant H042 were evaluated. Under non-reducing conditions, purified H042 showed a predominant band at approximately 150kDa, consistent with the expected molecular size of intact IgG. Under reducing conditions, distinct heavy-chain (~50kDa) and light-chain (~25kDa) bands were observed, confirming correct antibody assembly and high purity (Figure 2A,B). The MPO-binding activity of H042

was evaluated using recombinant MPO-based ELISA. H042 demonstrated concentration-dependent binding to MPO with good reproducibility (Figure 2C). The binding curve was fitted using a 4PL model, and the calculated EC50 value was 2.12ng/mL (0.014nM), indicating strong MPO recognition capability. Collectively, these findings confirm that the recombinant H042 maintains intact IgG structural integrity and specific binding capacity toward MPO following expression and purification.

3.3 | Antigen Specificity and Immunological Characterization of H042

The antigen specificity of recombinant H042 was characterized using two independent commercial antigen-specific immunoassay platforms. H042 exhibited robust reactivity against MPO, yet no measurable cross-reactivity with proteinase 3 (PR3) or

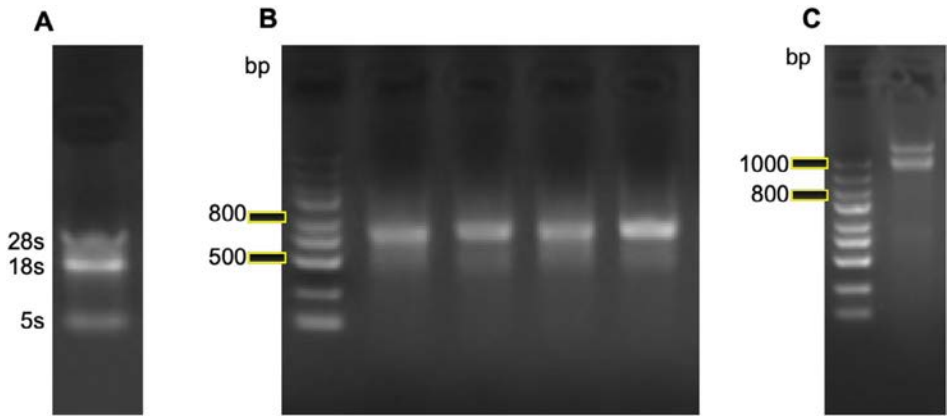


FIGURE 1 | Construction of the human anti-MPO scFv phage display antibody library. (A) Agarose gel electrophoresis analysis of total RNA extracted from PBMCs of MPO-ANCA-positive patients, showing clear 28S and 18S rRNA bands. (B) PCR amplification of immunoglobulin variable region genes (VH and VL) from cDNA. (C) Assembly of full-length scFv constructs by overlap extension PCR. MPO-ANCA, myeloperoxidase anti-neutrophil cytoplasmic antibody; PBMCs, peripheral blood mononuclear cells; scFv, single-chain variable fragment; VH, immunoglobulin heavy chain variable region; VL, immunoglobulin light chain variable region.

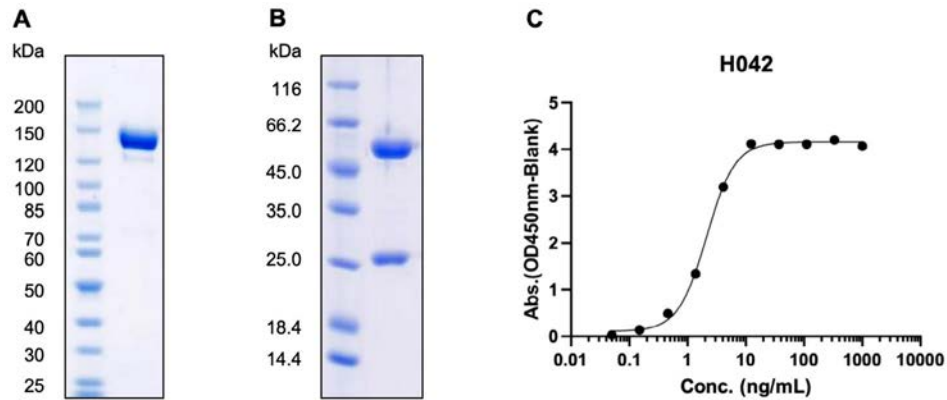


FIGURE 2 | Molecular integrity and antigen-binding activity of recombinant MPO-ANCA monoclonal antibody H042. (A) SDS-PAGE analysis under non-reducing conditions showing a predominant band at approximately 150kDa, consistent with intact IgG. (B) SDS-PAGE analysis under reducing conditions showing distinct heavy-chain (~50kDa) and light-chain (~25kDa) bands, confirming the integrity and purity of recombinant H042. (C) ELISA dose-response curve of H042 binding to recombinant human MPO antigen. The binding curve was fitted using a four-parameter logistic (4PL) model, yielding an EC50 value of 2.12ng/mL (0.014nM). 4PL, four-parameter logistic; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; MPO, myeloperoxidase.

glomerular basement membrane (GBM) antigens was detected on either the Zeus or Tellgen platforms (Table 1). We further investigated the immunological profile of H042 via IIFA employing ethanol-fixed human neutrophil substrates. H042 yielded a perinuclear ANCA (pANCA) staining pattern typical of MPO-ANCA, matching the signature fluorescent profile seen in confirmed MPO-ANCA-positive clinical specimens (Figure 3A). Collectively, these data verify that H042 bears high specificity for MPO and recapitulates the canonical immunological features of endogenous MPO-ANCA autoantibodies.

3.4 | Quantitative Response Characteristics of H042 in a Commercial MPO-ANCA ELISA System

To evaluate the quantitative response characteristics of H042 as a candidate quality control material, serially diluted H042 samples were analyzed using a commercial MPO-ANCA IgG ELISA system (EUROIMMUN). Serial dilution of H042 generated a predictable concentration-dependent response in the commercial MPO-ANCA ELISA system. The measured signals were well fitted by a second-order polynomial regression model

(Figure 3B). The quantifiable working range was 22.9–1855 ng/mL, with a coefficient of determination (R^2) of 0.993 (Figure 3B and Table S2). Collectively, these findings demonstrate that H042 exhibited predictable quantitative response characteristics within the analytical range, supporting its suitability as a recombinant candidate quality control material for MPO-ANCA assay evaluation.

3.5 | Preliminary Stability Assessment of H042 as a Candidate Quality Control Material

The preliminary analytical stability of H042 was assessed under stress conditions, including accelerated thermal exposure and repeated freeze–thaw cycles. As shown in Table 2, H042 retained substantial immunoreactivity under the evaluated stress conditions. After incubation at 37°C for 7 days, the relative signals remained 98.5% and 97.3% compared with the 4°C control. Following ten freeze–thaw cycles at –80°C, residual activities remained 94.1% and 89.1%, respectively. The HC serum matrix showed low baseline reactivity (9.83 RU/mL) and was not subjected to stress testing due to limited analytical robustness.

TABLE 1 | Antigen specificity of H042 across commercial assay systems.

Platform	Antigen	Cut-off (CO) value	Signal (H042)	Interpretation
Zeus	MPO	100 RU/mL	> 1000	Positive
	PR3		6	Negative
	GBM		1	Negative
Tellgen	MPO	5 U/mL	17.60	Positive
	PR3		0.01	Negative
	GBM		1.94	Negative

Note: Signal values represent raw assay outputs obtained according to the manufacturer’s instructions. CO values represent assay-specific diagnostic thresholds provided by each manufacturer. Results above the corresponding CO values were considered positive, whereas results below the CO values were considered negative. “RU/mL” and “U/mL” are manufacturer-defined arbitrary units and are not directly comparable across different assay platforms. Abbreviations: CO, cut-off; GBM, glomerular basement membrane; MPO, myeloperoxidase; PR3, proteinase 3; RU, relative units; U, units.

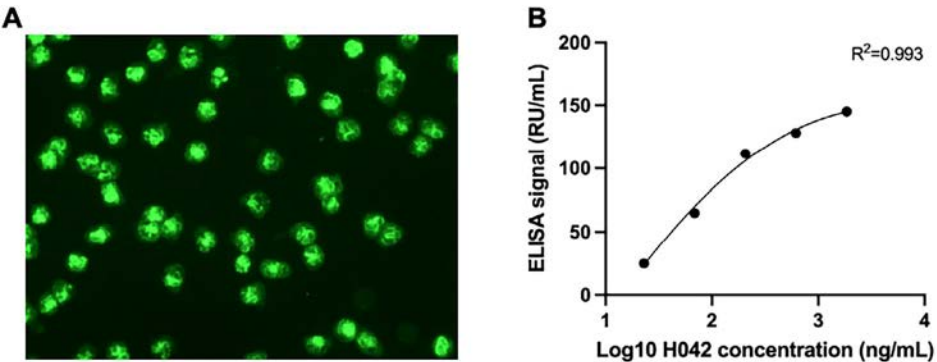


FIGURE 3 | Functional characterization of H042 as a candidate MPO-ANCA quality control material. (A) H042 was evaluated by IIFA using ethanol-fixed human neutrophil substrates according to the manufacturer’s instructions. Following incubation with fluorescein-labeled anti-human IgG secondary antibody, H042 showed a typical pANCA staining pattern under fluorescence microscopy. (B) Quantitative response characteristics of H042 were evaluated using a commercial MPO-ANCA IgG ELISA system (EUROIMMUN). Serially diluted H042 samples were analyzed, and detected signals were expressed as RU/mL. The relationship between H042 concentration and ELISA signal intensity was fitted using a second-order polynomial regression model, demonstrating a concentration-dependent response relationship ($R^2 = 0.993$). ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IIFA, indirect immunofluorescence assay; MPO-ANCA, myeloperoxidase anti-neutrophil cytoplasmic antibody; pANCA, perinuclear anti-neutrophil cytoplasmic antibody; RU/mL, relative units per milliliter.

TABLE 2 | Stability evaluation of the H042 candidate quality control material.

Sample condition	4°C control (RU/mL)	37°C, 7 days (RU/mL)	Relative signal (%)	Freeze-thaw ×10 (RU/mL)	Relative signal (%)
Neat (undiluted sample)	164.37	161.95	98.50	154.75	94.10
10-fold dilution	151.52	147.49	97.30	135.02	89.10
Serum matrix dilution	9.83	NA	NA	NA	NA

Note: Signal values are expressed in relative units (RU/mL) obtained using the EUROIMMUN MPO-ANCA assay system. Relative signal (%) was calculated relative to the 4°C control group. “NA” indicates not applicable due to low baseline signal intensity. Abbreviation: RU, relative units.

TABLE 3 | Cross-platform performance of H042 in commercial MPO-ANCA assay systems.

Platform	Assay system	Neat	10× dilution	Cut-off (CO) value
ELISA	ORGENTEC	64.51	> 100	20
	Werfen	122.74	114.35	20
	EUROIMMUN	164.37	151.52	20
	AESKU	308.75	4297.41	12
CLIA	YHLO	206.10	183.30	20
	Werfen	> 739.80	477.10	20
	EUROIMMUN	200.99	50.34	20
	Kangrun	32.46	213.9	1.5
Multiplex flow immunoassay	Bio-Rad	> 8.00	31.90	1
Multiplex Flow Cytometry	Weigong	787.58	724.42	20
Flow Cytometry-Based Multiplex Immunoassay	Zeus	> 1000	193	100

Note: Signal values are presented as manufacturer-reported assay outputs according to the corresponding assay instructions. CO values represent platform-specific diagnostic thresholds, and results above the corresponding CO values were interpreted as positive. “>” and “<” indicate values exceeding or below the analytical measurement range of the respective assay. All measurements were performed using the same reagent lot for each platform to ensure intra-assay consistency. Abbreviations: CLIA, chemiluminescence immunoassay; CO, cut-off; ELISA, enzyme-linked immunosorbent assay.

These findings provide preliminary evidence that H042 retains analytical reactivity under the evaluated stress conditions and support further investigation of its stability as a recombinant candidate quality control material for MPO-ANCA assay evaluation.

3.6 | Cross-Platform Performance of H042

H042 was further evaluated across multiple commercial MPO-ANCA assay platforms, including ELISA (four commercially available kits), CLIA (four commercially available kits), Multiplex flow immunoassay, Multiplex Flow Cytometry, and Flow Cytometry-Based Multiplex Immunoassay (three commercially available kits). As shown in Table 3, H042 produced positive signals above the predefined cutoff thresholds on every platform tested. Serial dilution testing confirmed sustained detectability of H042 across the evaluated assay formats, although platform-dependent and non-proportional changes in signal intensity were observed after dilution. Despite these differences, H042 yielded uniformly positive qualitative results on all platforms, verifying its broad compatibility with diverse MPO-ANCA immunoassay workflows. Taken together, these data confirm that H042 exhibits robust, consistent detectability

across mainstream commercial MPO-ANCA testing platforms, further underscoring its potential as a recombinant candidate quality control material for MPO-ANCA assay performance assessment.

4 | Discussion

In this study, we developed a fully human recombinant MPO-ANCA monoclonal antibody (H042) from a patient-derived scFv phage display library and systematically evaluated its performance as a candidate quality control material. H042 exhibited high antigen specificity, stable molecular integrity, broad cross-platform detectability, and robust stability under accelerated storage and repeated freeze-thaw conditions. These findings demonstrate that recombinant monoclonal antibody-based materials represent a feasible and promising approach for MPO-ANCA assay evaluation and laboratory quality assurance.

The need for reliable quality control materials remains pressing, as MPO-ANCA testing continues to be affected by considerable inter-assay variability [6, 7, 10]. This variability stems from differences in antigen preparation (e.g., native vs. recombinant MPO, purification protocols), assay design (e.g., direct ELISA,

capture ELISA, chemiluminescence immunoassay), and calibration strategies [14]. However, even after calibration with these certified reference materials, notable discrepancies persist across different platforms, suggesting that complete harmonization of MPO-ANCA testing systems remains an ongoing challenge [6, 10, 15]. Improved assay harmonization may enhance inter-laboratory consistency and support reliable longitudinal monitoring of MPO-ANCA levels.

Conventional quality control materials, primarily derived from pooled patient sera, retain the polyclonal characteristics of clinical samples, which is advantageous for representing natural antibody diversity. However, their use is limited by donor dependence, batch-to-batch variability, finite availability, and potential matrix effects. These limitations introduce significant uncertainty in value assignment and reduce reproducibility in external quality assessment (EQA) programs [16]. In contrast, recombinant monoclonal antibody-based materials offer unparalleled advantages in scalability, consistency, and controllability, providing a promising alternative for standardized quality control strategies. Our study directly supports this concept by demonstrating a reproducible, non-serum-derived source of MPO-specific antibody.

A key finding of our study is that H042 demonstrated consistent detectability across multiple commercial MPO-ANCA testing platforms, including IIFA, ELISA, CLIA, and multiplex flow immunoassay. We evaluated mainstream commercially available kits from distinct manufacturers within each methodological category. Despite fundamental disparities in analytical design, reagent formulation, and calibration algorithms across different brands and platforms, H042 yielded consistent qualitative positive results. Such uniform qualitative performance spanning different manufacturers under identical assay modalities represents an indispensable property for an ideal quality control material. Variable signal intensities observed after dilution on certain platforms may reflect platform-specific analytical characteristics, including nonlinear dose-response behavior, matrix effects, or differences in proprietary calibration protocols. Comparable concentration-dependent signal discrepancies have been widely documented in automated immunoassays, which corroborates the intrinsic methodological variability inherent to commercial MPO-ANCA detection systems. These findings further highlight the need for well-characterized and reproducible quality control materials to support harmonization of MPO-ANCA testing across different assay systems.

Compared with the IRMM-certified ERM-DA476/IFCC [9], H042 provides a complementary approach by enabling scalable and reproducible production independent of clinical sample availability. The lack of standardization in MPO-ANCA testing remains a major challenge due to differences in antigen presentation, assay formats, and calibration systems, leading to persistent inter-assay variability even with existing reference materials [10, 17]. Our study shows that H042, as a well-defined antibody, may help bridge this gap. Furthermore, H042 exhibited high antigen specificity, with no detectable cross-reactivity to PR3 or GBM antigens and produced the expected p-ANCA staining pattern in IIFA, confirming its immunological fidelity. Compared with previously reported chimeric

human-mouse antibodies [11], H042 was derived from a fully human scFv phage display library constructed from MPO-ANCA-positive patients. This approach may improve biological relevance by capturing the natural human antibody repertoire and potentially reducing artificial epitope bias that can occur with genetically engineered chimeric constructs.

Several limitations warrant acknowledgment. First, while we tested a panel of mainstream commercial MPO-ANCA assay kits/platforms, other commercially available products were excluded from our evaluation. Further studies covering a broader spectrum of commercial assays and manufacturers are required to evaluate the applicability of H042 across additional MPO-ANCA testing platforms. Second, formal commutability assessment, which is essential for establishing a reference material [17], was not performed. Thus, H042 is currently proposed as a candidate quality control material rather than a certified reference material. Third, as a monoclonal antibody, H042 does not fully represent the epitope diversity of polyclonal patient-derived antibodies. The use of a single clone may not detect all assay-specific reactivity patterns. Future studies combining multiple monoclonal clones targeting different epitopes, or engineering antibody panels, could improve clinical representativeness. Therefore, H042 should be considered an auxiliary tool for assay harmonization rather than a comprehensive solution to inter-assay variability. Finally, the accelerated thermal and repeated freeze-thaw experiments performed in this study represent only a preliminary assessment of the analytical stability of H042 and should not be interpreted as comprehensive stability verification. Real-time and long-term stability studies under defined storage and transportation conditions, together with full metrological traceability and evaluation in EQA programs, are required before further development toward a standardized reference material.

In summary, this study provides a recombinant antibody-based framework for the development of standardized MPO-ANCA quality control materials and demonstrates the feasibility of monoclonal antibody-based strategies for assay harmonization and laboratory quality assurance in MPO-ANCA testing. This approach may also be extended to other clinically relevant autoantibodies.

Author Contributions

Y.L. and X.Z. conceived the study. M.G. and Y.Y. performed the experiments, analyzed the data, and drafted the manuscript. Y.L. and X.Z. revised the manuscript and supervised this study. All authors have read and approved the submitted manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author Y.L.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** PCR amplification of antibody variable region genes used for phage display library construction. **Table S1:** RNA extraction and quality assessment of PBMC samples from MPO-ANCA-positive patients. **Table S2:** Polynomial regression analysis of the quantitative response curve of H042 in the MPO-ANCA ELISA system.



EDITORIAL

Beyond Stability: Rethinking Housekeeping Genes as Contributors to Rheumatoid Arthritis Pathogenesis

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1 | Introduction: The Housekeeping Gene Concept Under Scrutiny

The concept of housekeeping genes has evolved considerably since the term first appeared in the 1970s, when it was used primarily to describe genes encoding transfer RNA and ribosomal RNA. Over the subsequent five decades, housekeeping genes became defined as constitutive genes required for the maintenance of basic cellular functions, expressed in all cells of an organism under normal and pathophysiological conditions [1–3]. Their products participate in fundamental processes such as protein synthesis, energy metabolism, and cytoskeletal organization, making them essential for cell survival regardless of tissue type, developmental stage, or cell cycle state [4]. This presumed universality and stability led to their widespread adoption as reference controls for normalizing gene expression data in quantitative PCR and transcriptomic studies. It holds an implicit assumption that these genes resist pathological perturbations impacting tissue-specific genes [5]. In routine experimental practice, a limited set of genes, such as GAPDH, ACTB, and 18S rRNA, has been widely adopted as internal controls. However, these genes were chosen largely for experimental convenience rather than for evidence of constitutive expression, and studies have shown that they can vary considerably across tissues [6–8]. With the advent of RNA-seq, systematic identification of housekeeping genes has become possible with unprecedented accuracy. In a landmark study using data from 16 normal human tissues, Eisenberg and Levanon (2013) applied three stringent criteria: expression in all tissues, low variance across tissues, and no exceptional expression in any single tissue

to define 3804 high-confidence human housekeeping genes [9] (Table S1). This systematically defined set, rather than the limited set of conventional internal controls, serves as the foundation for our analysis.

However, this long-held view is increasingly being questioned. Recent advances in high-throughput sequencing have revealed that the expression patterns of housekeeping genes are more complex than previously appreciated. Studies in cancer biology have documented aberrant expression of several housekeeping genes in malignant tissues, suggesting that their dysregulation may contribute to disease progression [10–12]. In the context of autoimmune diseases, emerging evidence points to the involvement of housekeeping genes in inflammatory pathways [13], yet systematic investigations have remained scarce. Rheumatoid arthritis (RA), with its complex molecular architecture characterized by the collective dysregulation of hundreds of genes [14], provides an ideal context to examine whether these fundamental genes might play previously unrecognized roles in disease pathogenesis. The global burden of rheumatic diseases, exacerbated by the COVID-19 pandemic and limited access to specialist care in low-income countries, further underscores the need for deeper molecular insights [15].

2 | Consistent Transcriptional Perturbations of Housekeeping Genes in RA

To address this question systematically, we analyzed 15 independent transcriptome datasets from the Rheumatoid

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Arthritis Bioinformatics Center (RABC) [16], encompassing baseline RA patients and healthy controls (Figure S1, Tables S2 and S3, Supporting Information). These datasets were carefully curated to exclude treatment effects and ensure tissue comparability, resulting in a robust collection of gene expression profiles spanning diverse patient populations and experimental platforms.

Differential expression analysis between RA patients and healthy controls revealed a striking and unexpected pattern. When we applied cumulative hypergeometric tests to assess whether housekeeping genes were overrepresented among differentially expressed genes (DEGs), significant enrichment was observed in 11 of the 15 datasets (Table S4). This enrichment was not marginal: the overlap between DEGs and housekeeping genes consistently exceeded what would be expected by chance alone (Table 1). To quantify this effect across studies, we calculated relative enrichment factors for each dataset. Meta-analysis of these 15 studies using a random effects model yielded a pooled logarithmic relative enrichment estimate of 0.48 (95% confidence interval: 0.17–0.80), which deviated significantly from null model expectations ($p=0.0012$) (Figures 1 and S2–S4). The consistency of this signal across independent datasets, despite differences in patient characteristics, sample sizes, and microarray platforms, argues strongly against the possibility of technical artifact [17].

3 | Expression Instability: A Hallmark of Disease State

The designation “housekeeping gene” carries with it an implicit expectation of expression stability, yet our analysis challenges this very foundation. When we compared expression variability between RA patients and healthy controls, a clear pattern emerged. In two-thirds of the datasets examined, housekeeping genes exhibited significantly greater variance, standard deviation, and coefficient of variation in patient samples compared with controls (Figures S5–S7). This increased fluctuation was not observed for non-housekeeping genes to the same degree, suggesting that the disease state imposes a generalized transcriptional instability that disproportionately affects genes traditionally considered constitutive.

This observation raises fundamental questions about the nature of housekeeping gene regulation in disease. The increased variability may reflect disruption of the epigenetic mechanisms that normally maintain stable expression. Indeed, when we examined matched DNA methylation and transcriptome data from RA patients, housekeeping genes demonstrated significantly stronger correlations between promoter methylation status and expression levels compared with non-housekeeping genes (Table S5, Figures S8–S17). This association implicates epigenetic dysregulation as a potential mechanism underlying housekeeping gene

TABLE 1 | Summary of housekeeping gene enrichment findings across 15 rheumatoid arthritis transcriptome datasets.

Finding	Proportion	Significance
Datasets with significant HKG enrichment among DEGs	11/15 (73.3%)	$p < 0.05$ (cumulative hypergeometric test)
Datasets with positive $\ln(\text{Re})$ values	12/15 (80%)	Range: -0.51 to 0.88
Pooled effect size (random effects model)	$\ln(\text{Re}) = 0.48$	95% CI: 0.17 – 0.80 , $p = 0.0012$
Datasets with increased HKG expression variability in RA	10/15 (66.7%)	$p < 0.05$ (Wilcoxon rank sum test)

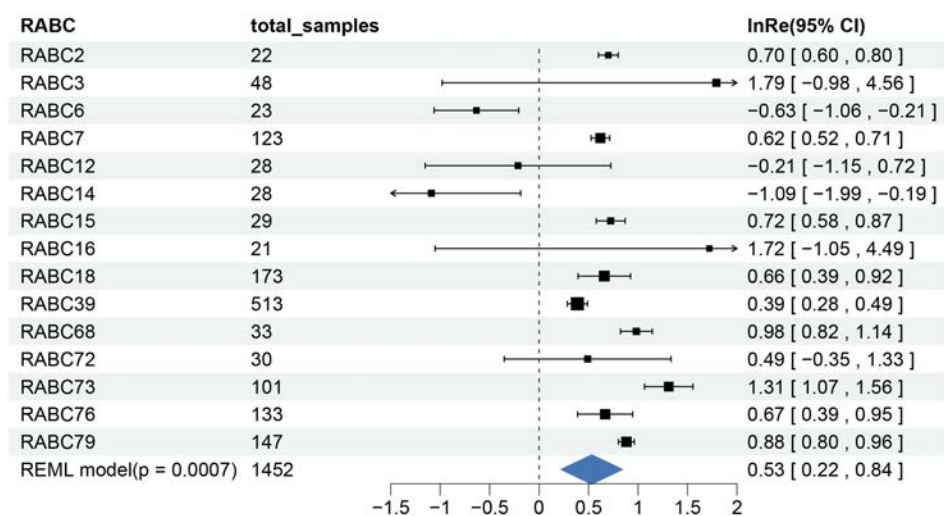


FIGURE 1 | Meta-analysis forest plot showing relative enrichment of housekeeping genes among differentially expressed genes in 15 independent rheumatoid arthritis datasets. Black squares represent point estimates with horizontal lines indicating 95% confidence intervals. The diamond shows the pooled random-effects estimate ($\ln[\text{Re}] = 0.48$, 95% CI: 0.17 – 0.80).

perturbations in RA, consistent with the well-documented role of DNA methylation changes in autoimmune pathogenesis [18].

4 | Broader Implications: Pioneering Discoveries on Housekeeping Genes in Autoimmune Disease

Our findings in RA align with emerging evidence from other autoimmune and inflammatory conditions. Importantly, our research team has been at the forefront of systematically investigating housekeeping gene dysregulation in autoimmune diseases. In a recent study published by our group, we investigated housekeeping genes in psoriasis and identified 34 housekeeping genes associated with disease pathogenesis through integrated multi-cohort and single-cell transcriptomic analysis [12]. Using weighted gene co-expression network analysis combined with differential expression methods, we demonstrated that the co-expression relationships between six genes (*APOL2*, *DCUN1D3*, *UBE2F*, *HIGD1A*, *PPIF*, and *STAT3*) and known psoriasis-related genes differed significantly between psoriasis patients and healthy individuals. Single-cell transcriptomic profiling further revealed that these housekeeping genes were differentially expressed primarily in basal, spinous, supraspinous, and proliferating keratinocytes. Ligand-receptor interaction analysis demonstrated significant activation of the interleukin-17, interleukin-6, and midkine pathways within keratinocyte subpopulations, which led to the upregulation of *STAT3*, *EIF5A*, and *RAN*, thereby promoting keratinocyte hyperproliferation and enhancing immune reactivity [19].

Our team has systematically revealed for the first time that housekeeping genes, long considered mere bystanders in disease processes, exhibit consistent and reproducible dysregulation across multiple autoimmune conditions. The convergence of evidence from RA and psoriasis suggests a broader paradigm that housekeeping genes may function as dual regulators in disease contexts, participating in pathogenic mechanisms while retaining their essential cellular functions. The interleukin-17 pathway, central to both psoriasis and RA pathogenesis, appears to intersect with housekeeping gene regulation through mechanisms involving metabolic reprogramming and epigenetic modification [20]. The mechanistic target of rapamycin pathway, which integrates signals from growth factors, nutrients, and immune stimuli, has been shown to regulate both housekeeping and inflammatory gene expression, providing a potential link between basic cellular metabolism and immune activation in RA [21]. These mechanistic insights are complemented by recent advances in understanding trained immunity, where metabolic and epigenetic reprogramming of innate immune cells shapes chronic inflammatory responses [22, 23].

5 | Epigenetic and Genetic Evidence for Housekeeping Gene Involvement in RA Pathogenesis

The evidence presented here challenges the conventional partitioning of genes into “housekeeping” and “tissue-specific” categories in disease research. Our finding that housekeeping genes are recurrently and significantly enriched among RA-associated DEGs suggests that these fundamental cellular components may

represent previously overlooked contributors to autoimmune pathogenesis. The increased expression variability observed in patient samples and the strong correlation with DNA methylation changes point toward epigenetic dysregulation as a plausible mechanism.

To explore whether the observed expression variations are epigenetically or genetically driven, we performed mQTL-SMR and eQTL-SMR analyses. These identified hundreds of housekeeping genes whose promoter methylation or expression showed causal associations with RA risk, with substantial overlap between the two layers (Tables S6 and S7). Mediation analysis on genes sharing the same causal variant further supported direct epigenetic-transcriptional cascades for RNPEP and PET100 (Table S8). For RNPEP, methylation appears to promote expression, which in turn increases RA risk, with approximately 77.5% of the methylation effect mediated through expression. This suggests that epigenetic activation of RNPEP encoding aminopeptidase B, a zinc-dependent metallopeptidase involved in peptide processing [24], may represent a novel RA risk mechanism. Conversely, PET100 showed a protective mediation pattern where methylation suppresses its expression, and lower expression is associated with reduced RA risk, with a mediation proportion of about 50.5%. Given PET100's role in mitochondrial respiratory chain assembly [25, 26], this finding points to a potential link between mitochondrial dysfunction and RA [27], with epigenetic silencing of PET100 possibly acting as a compensatory protective mechanism. Collectively, these findings indicate that housekeeping gene dysregulation in RA involves both genetic and epigenetic influences, with DNA methylation acting as a mediator in the pathway from genetic variation to altered expression and disease risk.

6 | Beyond Stability: Clinical Correlates and Translational Opportunities

To explore the clinical relevance of housekeeping gene expression fluctuations in RA, we examined whether their expression tracks with disease activity in a large patient cohort. Using four validated activity measures and well-established clinical thresholds, nearly one-third of housekeeping genes showed expression patterns that consistently paralleled disease activity (Tables S9 and S10). These findings suggest that housekeeping gene fluctuations are not random but reflect the clinical state of RA patients. A smaller subset of housekeeping genes was also observed to distinguish seropositive from seronegative RA (Tables S11 and S12), raising the possibility that certain genes within this conventional class may contribute to subtype-specific pathways. While these observations are preliminary and require further validation, they underscore the potential clinical relevance of housekeeping gene dysregulation in RA and point toward avenues for future biomarker and stratification research.

From a practical perspective, these observations have immediate implications for experimental design. The use of housekeeping genes as normalization controls in RA transcriptomic studies may require careful reevaluation, as their expression stability cannot be assumed a priori in diseased tissues. More fundamentally, our findings invite a conceptual shift in how we think about gene function in disease. Rather than viewing

housekeeping genes as passive bystanders, we might consider them as integral components of disease-associated transcriptional networks, whose perturbations could propagate effects throughout the system [28].

Future research directions about housekeeping genes are numerous. Functional studies examining the consequences of housekeeping gene knockdown or overexpression in relevant cell types could establish causal relationships with inflammatory phenotypes. Longitudinal studies tracking housekeeping gene expression patterns during disease progression and in response to therapy might reveal whether these changes are reversible and whether they correlate with clinical outcomes. The development of computational approaches that account for housekeeping gene variability could improve the accuracy of differential expression analyses in RA and other complex diseases. Furthermore, integrating housekeeping gene signatures with existing clinical and molecular data may enhance risk stratification and guide personalized treatment decisions, particularly in resource-limited settings where comprehensive immunomonitoring is not feasible.

7 | Conclusion

The unexpected involvement of housekeeping genes in RA pathogenesis, together with our team's pioneering work in psoriasis, invites us to look beyond the usual suspects in autoimmune research. Housekeeping genes, long relegated to methodological footnotes, may yet prove to be important characters in the story of disease. The recognition that housekeeping genes can actively participate in autoimmune pathogenesis opens new avenues for understanding disease mechanisms and may ultimately lead to novel therapeutic strategies targeting these fundamental cellular components. As we refine our understanding of the molecular architecture of rheumatic diseases, it is time to give housekeeping genes the attention they deserve.

Author Contributions

Wenhua Lv and Hao Tang contributed equally as first authors, responsible for study conceptualization, data analysis, and manuscript drafting. Ruijie Zhang, Mingming Zhang, and Yongshuai Jiang contributed equally as corresponding authors, providing oversight of the research, critical revision of the manuscript, and final approval of the submitted version.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from public domain resources. Additional detailed results and information can be found in [Supporting Information](#) methods, figures, and tables.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** Supplementary Materials and Method **Figure S1:** The flow chart of dataset inclusion and exclusion. **Figure S2:** Sensitivity analysis result by removing one dataset each time. A meta-analysis was re-performed by removing each of the datasets and a new estimate and confidence interval of integrated effect size was plotted. The p values of new meta-analyses were placed on the last column. **Figure S3:** Integration of effect size (lnRe) by a subgroup analysis for blood sample dataset. The point estimate and 95% confidence interval of effect size for each single was represented by black rectangles and line segment respectively. The point estimate of integrated effect size was represented by a dark blue diamond. **Figure S4:** Integration of effect size (lnRe) based on the results of another standard error estimation method. The point estimate and 95% confidence interval of effect size for each single was represented by black rectangles and line segment respectively. The point estimate of integrated effect size was represented by a dark blue diamond. **Figure S5:** Comparison of the stability of gene expression levels between RA samples and healthy controls (sd). The vertical axis represents the standard deviation of gene expression levels. A p value of Wilcoxon rank sum test is placed on the top of each boxplot. **Figure S6:** Comparison of the stability of gene expression levels between RA samples and healthy controls (variance). The vertical axis represents the variance of gene expression levels. A p value of Wilcoxon rank sum test is placed on the top of each

boxplot. **Figure S7:** Comparison of the stability of gene expression levels between RA samples and healthy controls (cv). The vertical axis represents the coefficient of variation of gene expression levels. A p value of Wilcoxon rank sum test is placed on the top of each boxplot. **Figure S8:** Housekeeping genes have higher Pearson correlation coefficient than non-housekeeping genes in RA patients (Random sampling from the 1st to the 10th). One asterisk (*) means that the p -value is less than 0.05, and two asterisks (**), three asterisks (***), and four asterisks (****) indicate that the p -value is less than 0.01, 0.001, and 0.0001, respectively. The sign of 'ns' means 'not significant'. **Figure S9:** Housekeeping genes have higher Pearson correlation coefficient than non-housekeeping genes in RA patients (Random sampling from the 11th to the 20th). **Figure S10:** Housekeeping genes have higher Pearson correlation coefficient than non-housekeeping genes in RA patients (Random sampling from the 21st to the 30th). **Figure S11:** Housekeeping genes have higher Pearson correlation coefficient than non-housekeeping genes in RA patients (Random sampling from the 31st to the 40th). **Figure S12:** Housekeeping genes have higher Pearson correlation coefficient than non-housekeeping genes in RA patients (Random sampling from the 41st to the 50th). **Figure S13:** Housekeeping genes have higher Pearson correlation coefficient than non-housekeeping genes in RA patients (Random sampling from the 51st to the 60th). **Figure S14:** Housekeeping genes have higher Pearson correlation coefficient than non-housekeeping genes in RA patients (Random sampling from the 61st to the 70th). **Figure S15:** Housekeeping genes have higher Pearson correlation coefficient than non-housekeeping genes in RA patients (Random sampling from the 71st to the 80th). **Figure S16:** Housekeeping genes have higher Pearson correlation coefficient than non-housekeeping genes in RA patients (Random sampling from the 81st to the 90th). **Figure S17:** Housekeeping genes have higher Pearson correlation coefficient than non-housekeeping genes in RA patients (Random sampling from the 91st to the 100th). **Table S1:** Housekeeping gene list. **Table S2:** Detailed information of datasets included in this research. **Table S3:** Clinical information for 15 datasets. **Table S4:** Enrichment result based on cumulative hypergeometric tests. **Table S5:** Comparison of Pearson correlation coefficients between housekeeping genes and non-housekeeping genes for RA patients. **Table S6:** Significant results of mQTL-SMR analysis. **Table S7:** Significant results of eQTL-SMR analysis. **Table S8:** Mediation analysis results for RNPEP and PET100. **Table S9:** Reliable housekeeping genes showing significant correlation between gene expression with disease activity (regression analysis). **Table S10:** Reliable housekeeping genes showing significant correlation between gene expression with disease activity (ANOVA). **Table S11:** Reliable housekeeping genes showing significant correlation between gene expression with serum levels (regression analysis). **Table S12:** Reliable housekeeping genes showing significant correlation between gene expression with serum levels (t-test).



EDITORIAL

Pain Phenotyping in Rehabilitation—Between Conceptual Promise and Translational Limitations

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1 | The Promise of Pain Phenotyping

Pain phenotyping has been widely promoted as a pathway toward personalized rehabilitation, particularly as a means of informing assessment, prognosis, and rehabilitation decision-making—but its clinical utility in real-world rehabilitation practice remains uncertain. Pain phenotyping refers to the systematic characterization of patients according to clinically relevant patterns of pain-related features, which may include symptom characteristics, sensory manifestations, functional limitations, psychological and behavioral factors, and other dimensions considered relevant to the expression of pain. In this context, phenotyping seeks to characterize meaningful patterns of heterogeneity among individuals rather than simply assigning a diagnostic label [1].

The rationale for pain phenotyping emerges from contemporary pain science, which recognizes chronic pain as a heterogeneous and multidimensional experience that cannot always be adequately captured by a single diagnostic category. From this perspective, patients with the same clinical diagnosis may differ substantially in sensory symptoms, function, psychological and behavioral characteristics, and contextual factors, potentially resulting in different clinical trajectories and responses to rehabilitation [1].

Conceptual frameworks from contemporary pain science—including the distinction between nociceptive, neuropathic, and nociplastic pain, as well as multidimensional approaches to characterizing pain—have expanded our understanding of heterogeneity in chronic pain. Pain phenotyping builds on this perspective by seeking to characterize clinically meaningful patterns of this heterogeneity, with the potential to

inform individualized rehabilitation assessment and decision-making [1].

In this editorial, we specifically consider pain phenotyping in the context of rehabilitation for chronic musculoskeletal pain, focusing on its potential to inform clinical assessment, prognostic reasoning, and the selection and adaptation of rehabilitation interventions. An implicit assumption underlying phenotyping is that identifying clinically coherent subgroups should help predict treatment response, clinical trajectories, and the risk of chronicity [2, 3].

For rehabilitation, this heterogeneity provides a potential rationale for moving beyond diagnosis-based approaches toward more individualized clinical reasoning. A phenotype may help clinicians organize relevant patient characteristics, identify potentially modifiable factors, formulate hypotheses about prognosis, and consider whether different rehabilitation strategies may be appropriate for different patient profiles. However, these potential applications represent a translational hypothesis rather than an established clinical benefit. The key question is therefore not whether clinically distinct phenotypes can be identified, but whether their identification adds meaningful information to rehabilitation assessment and decision-making beyond existing clinical approaches. However, this hypothesis still lacks robust validation, particularly in independent populations and real-world clinical settings [2, 3].

Importantly, pain phenotyping does not necessarily depend on a single domain or classification system. Multidomain approaches have been proposed to characterize patients across complementary dimensions, including pain characteristics, sensory features, physical functioning, psychological and behavioral

factors, and other clinically relevant domains [2, 3]. Similarly, the ACTION–American Pain Society Pain Taxonomy (AAPT) provides a multidimensional framework for chronic pain classification that considers pain-related characteristics alongside biological, psychological, and social dimensions [4]. Such frameworks reinforce the concept that clinically meaningful pain phenotyping may require an integrated characterization of multiple domains rather than reliance on pain intensity or a single mechanistic classification [4].

In rehabilitation, such multidimensional phenotyping may provide a more comprehensive representation of patient heterogeneity than approaches based solely on pain mechanisms or diagnostic categories. The potential value of this approach, however, depends on whether combining multiple domains improves the identification of clinically meaningful profiles and provides additional information that can be translated into individualized rehabilitation decisions.

2 | Translational Limitations of Pain Phenotyping

The potential value of pain phenotyping in rehabilitation therefore depends on whether these theoretically meaningful patterns can be translated into reliable, reproducible, and clinically actionable information. Despite strong theoretical appeal and substantial research efforts, important limitations remain regarding its validity, stability, generalizability, and ability to improve rehabilitation decision-making. These limitations extend beyond the absence of a universally accepted classification system. They include issues related to construct validity, external validity, reproducibility, temporal stability, and clinical utility—dimensions that are essential for a phenotype to move beyond a descriptive category and meaningfully inform rehabilitation decision-making [1, 2].

First, there is no unified operational taxonomy; subsequently, there are multiple classification models that are often not equivalent [1]. Moreover, patients with chronic musculoskeletal pain frequently present mixed profiles, combining nociceptive, nociplastic, and psychosocial characteristics in varying degrees. This overlap, along with the dynamic nature of pain over time, limits the usefulness of rigid classification systems [2]. Thus, the challenge is not merely to label or classify phenotypes, but to demonstrate that these categories can reliably discriminate prognoses, clinical trajectories, and responses to rehabilitation interventions in a way that meaningfully informs rehabilitation decision-making [2]. Beyond these structural limitations, a more fundamental issue emerges: the clinical interpretability of phenotypes.

Quantitative sensory testing (QST) also represents an important component of pain phenotyping, particularly for characterizing sensory profiles and investigating alterations in pain processing. In particular, the nociplastic pain criteria proposed by Kosek et al. incorporate both self-reported features and clinically elicited evoked pain hypersensitivity through bedside sensory testing, such as mechanical or thermal allodynia [1]. Although QST-based phenotyping remains underreported and underutilized in routine clinical practice, it may provide complementary information to self-reported and clinical measures and

potentially contribute to a more comprehensive characterization of patients [5].

A further challenge concerns the nature of the instruments used in phenotyping. Many phenotyping approaches rely substantially on self-reported measures and indirect indicators of underlying mechanisms, which limits construct validity and the precision of inferences regarding underlying neurophysiological processes. Although these tools are valuable, they do not directly measure neurophysiological mechanisms, increasing uncertainty in clinical interpretation [1, 6].

This distinction between concept and inference is critical. A phenotype may be conceptually plausible, but this does not imply that available instruments can directly capture the mechanism it is intended to represent. Accordingly, terms such as nociplastic pain, central sensitization, or elevated psychosocial profile should be interpreted as probabilistic clinical constructs rather than direct representations of underlying neurobiology [1, 6].

It is also important to note that many phenotypes are derived from cluster analyses conducted in specific samples, raising concerns about external validity and reproducibility across different clinical contexts [3]. In addition, phenotypes derived from exploratory analyses may partially reflect unobserved heterogeneity or sample-dependent statistical artifacts. This issue is particularly relevant when clustering models are applied without external validation, as they may be prone to overfitting and show reduced generalizability in independent populations [3].

Finally, there is still limited evidence that phenotype-guided rehabilitation interventions result in better patient-relevant outcomes compared with usual rehabilitation care [7]. While the rationale for targeted interventions is plausible, robust clinical trials supporting this approach remain scarce. Therefore, the translational value of phenotyping should not be judged solely by its ability to group patients with similar characteristics but also by its capacity to improve clinical prediction and generate measurable benefits in patient-relevant outcomes [2].

3 | The Risk of Labeling in a Dynamic System

A critical consideration is that pain phenotypes may evolve over time and may overlap within the same individual. Such phenotypic drift or coexistence does not necessarily represent a limitation of phenotyping itself. Rather, identifying overlapping or mixed profiles may provide clinically relevant information about the multiple dimensions contributing to an individual's pain experience and may help clinicians develop and adapt individualized rehabilitation strategies over time. The potential concern arises when phenotypic categories are interpreted as fixed identities rather than as dynamic and probabilistic representations of the patient's current clinical presentation. Recognizing such overlap may instead help clinicians identify the different dimensions of a patient's presentation and consider how these may contribute to functional limitations, symptom persistence, and rehabilitation needs. The challenge is therefore not to eliminate overlap, but to determine whether recognizing these multidimensional profiles improves clinical reasoning and patient-centered rehabilitation [1, 2, 6].

Collectively, these considerations highlight the risk that clinical decisions may become overly guided by phenotypic labels, potentially oversimplifying clinical reasoning and neglecting patient-specific complexity. Phenotyping should therefore avoid transforming analytical categories into fixed clinical identities. Rather than replacing clinical reasoning, it should support clinicians in recognizing probable patterns, revising hypotheses over time, and adapting decisions according to patient evolution.

4 | Phenotyping and Rehabilitation Decision-Making

Although pain phenotyping provides an attractive conceptual framework for personalized rehabilitation, there is still limited evidence that phenotype-guided rehabilitation strategies lead to better outcomes compared with usual rehabilitation care [2]. This distinction is crucial in differentiating statistical significance from clinical utility. A model may identify statistically distinct subgroups while still failing to provide actionable information for selecting rehabilitation interventions or accurately estimating prognosis in rehabilitation practice.

At its current stage, phenotyping should be understood as a probabilistic and exploratory tool—useful for supporting rehabilitation decision-making and hypothesis generation, but not as a prescriptive instrument capable of determining the optimal rehabilitation intervention for each individual. In practice, this means that phenotyping can help structure clinical assessment, broaden the identification of modifiable factors, and encourage a more individualized approach. However, its application should remain integrated within a multidimensional assessment that is continuously updated based on the patient's response to treatment.

For example, classifying a patient as having a “nociplastic phenotype” does not, by itself, determine whether exercise, education, or multimodal rehabilitation should be prioritized.

5 | Future Directions

Future research should prioritize longitudinal designs to investigate the stability of phenotypes over time, the integration of multimodal assessments—including behavioral, functional, and potentially biological dimensions—and critically, the testing of phenotype-guided rehabilitation interventions in adequately powered randomized controlled trials.

Temporal stability deserves particular attention. If phenotypes lack longitudinal stability, their usefulness as stratification tools is limited—especially when used to predict chronicity risk, functional trajectories, or response to rehabilitation interventions.

Bridging the gap between conceptual advances and clinical applicability remains a central challenge for both researchers and clinicians. Progress in this area is essential for translating the promise of personalized rehabilitation into meaningful benefits for individuals with chronic musculoskeletal pain.

Additionally, future studies should clearly distinguish between explanatory, predictive, and prescriptive models. This distinction may help prevent exploratory findings from being prematurely applied as clinical rules before their external validity, reproducibility, and impact on outcomes have been adequately established. Future research may also benefit from adaptive and dynamic modeling approaches capable of capturing within-person variability over time.

6 | Concluding Remarks

Pain phenotyping represents an important framework for understanding heterogeneity in chronic musculoskeletal pain and potentially informing personalized rehabilitation. However, the current challenge lies not only in identifying phenotypes but in recognizing their limitations and avoiding their oversimplified application in clinical practice. Bridging the gap between concept and applicability is essential to ensure that the promise of personalized rehabilitation translates into real patient benefits—rather than increased labeling or diagnostic overlap.

Finally, phenotyping will become clinically relevant to rehabilitation when it demonstrates the ability to predict trajectories, anticipate the risk of chronicity, and guide rehabilitation interventions with superiority over usual rehabilitation care.

Author Contributions

André Pontes-Silva, Almir Vieira Dibai-Filho, and Cid André Fidelis-de-Paula-Gomes: conceptualization, investigation, methodology, validation, visualization, writing (original draft, review, and editing).

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The authors have nothing to report.

Consent

The authors have nothing to report.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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LETTER TO THE EDITOR

Beyond Pressure Pain Thresholds: Making Exercise-Induced Hypoalgesia Actionable in Knee Osteoarthritis

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Dear Editor,

We read with great interest the double-blind randomized trial by Toomey and colleagues, which examined whether targeted pre-exercise education can enhance exercise-induced hypoalgesia in knee osteoarthritis [1]. Its findings are valuable because they separate a change in belief from a group-level change in experimental and patient-reported pain. For rehabilitation practice, however, the decisive question is whether the physiological response measured in an individual patient can guide a safe decision about the next exercise session.

Both groups showed an average increase in knee pressure pain thresholds after the exercise task, whereas the intervention did not demonstrate an advantage in resting pain or stair-evoked pain. This pattern does not establish that pressure pain thresholds lack clinical value. Rather, it leaves unanswered whether a participant with a threshold increase also experienced a symptom change that would justify reassurance or progression of the prescribed exercise. Paired participant-level plots or rank associations between knee threshold changes and changes in resting and movement-evoked pain could provide a clinically interpretable account of data already collected. Until such concordance is shown, a laboratory threshold change should not be regarded as a surrogate for patient-perceived relief or readiness to increase exercise load.

Successful translation also depends on whether educational content gives patients a practical response pathway when pain occurs during activity. The report should clarify whether the positive-education script included prespecified monitoring of symptom exacerbation and principles for maintaining, modifying, reducing, or reviewing the prescribed load. This is not only an intervention-fidelity issue. A systematic review of therapeutic-exercise trials found incomplete reporting of harms and exercise intensity in knee osteoarthritis [2].

EULAR recommends individualizing exercise dosage and progression within multimodal osteoarthritis care, while French rheumatology and rehabilitation guidance emphasizes person-centered education and adherence support [3, 4]. An explicit account of how pre-exercise expectations, maximum contraction pain, rating of perceived exertion, and immediate symptoms informed subsequent exercise decisions would therefore help transform a belief-oriented message into an implementable pathway for routine care.

Toomey and colleagues have addressed an important translational problem. Aligning experimental pain measures with patient symptoms and a transparent response-guided exercise pathway could clarify when pre-exercise education becomes useful in knee osteoarthritis rehabilitation.

Author Contributions

Jingjing Yang: writing – original draft preparation, writing – reviewing and editing. Yi Yue: project administration. Mingming Li: supervision.

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Ethics Statement

The authors have nothing to report.

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The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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LETTER TO THE EDITOR

Comment on: “Association Between Chikungunya Infection and Rheumatoid Arthritis in Taiwan—A Cross-Sectional Study”

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To the Editor,

We have read with interest the article by Yen et al. and appreciate the authors' effort to explore a clinically relevant question [1]. While the study is underpowered for causal inference, its most valuable contribution lies not in the association itself, but in the striking discordance between commercial ELISA kits and the neutralization assay (Cohen's $\kappa=0$). This finding carries direct implications for clinical practice.

In routine care, a positive CHIKV ELISA often influences diagnostic decisions, sometimes leading to reclassification of the disease or changes in therapy [2]. Yet this study shows that in RA patients, ELISA positivity does not reliably reflect true neutralizing antibodies. IgM and IgG differ: RF and autoantibodies cause false-positive IgM via nonspecific binding of conjugated secondary antibodies, whereas heterophile antibodies and polyclonal hypergammaglobulinaemia affect IgG differently. Timing also matters: RT-PCR is best during the first 5–7 days, IgM appears thereafter and can persist for months, and IgG/neutralizing antibodies develop later and indicate past exposure. Thus, acute post-travel febrile arthralgia with positive IgM may suggest recent infection but is prone to RF interference; in long-standing RA, positive IgG may reflect remote exposure but not neutralization. High negative but zero positive agreement means a negative ELISA helps exclude exposure, while a positive result has limited positive predictive value and should not be interpreted alone.

We therefore contend that commercial CHIKV ELISAs should not be used as stand-alone diagnostic tests in RA patients. A negative ELISA can help exclude exposure. However, a positive IgM or IgG requires confirmation by neutralization, or

interpretation with RT-PCR, paired acute/convalescent titres, and clinical/travel history before any diagnostic or therapeutic change. If clinical suspicion is strong, for instance in cases of acute febrile arthralgia after returning from an endemic area, the neutralization test should be the preferred confirmatory method [3]. Where neutralization is unavailable, positive ELISA results must be interpreted cautiously and never used alone to guide therapy.

The two neutralization-positive RA patients described had links to endemic regions (southern China and Thailand). This reminds us that imported CHIKV infections do occur in Taiwan, and their chronic joint manifestations can closely mimic RA [4]. Indeed, a recent meta-analysis reported a combined RA incidence of 13.7% following CHIKV infection, underscoring the clinical relevance of this overlap [5]. Nevertheless, the cross-sectional design precludes any conclusion about causality; we cannot determine whether CHIKV triggered RA in these individuals or whether the coexistence is coincidental. The authors are prudent to avoid overinterpretation.

In our view, the main contribution of this work is to highlight a serious diagnostic uncertainty that affects day-to-day management. Clinicians should therefore: (a) use CHIKV ELISAs in a reflexive/confirmatory workflow—negative ELISA helps exclude exposure, but positive IgM or IgG in RA patients requires neutralization testing or, if unavailable, interpretation with RT-PCR, paired titres, and clinical/travel history before management changes; (b) never alter diagnosis or therapy based on ELISA alone; and (c) consider CHIKV only when travel history and presentation are highly suggestive, seeking confirmatory neutralization if feasible.

Larger prospective studies in endemic regions, with paired ELISA and neutralization data, are urgently needed to establish evidence-based algorithms. Until then, this study serves as a valuable reminder that current serological tools are inadequate, and clinical judgment must remain paramount.

Author Contributions

Haoxuan Yan: conceptualization, methodology, project administration, supervision, writing – review and editing. **Licheng Lv:** methodology, project administration, supervision, writing – original draft, writing – review and editing.

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Conflicts of Interest

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Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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LETTER TO THE EDITOR

Distinct Clinical Implications of Anti-Topoisomerase I Antibody Positivity and Antibody Titers in Systemic Sclerosis

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Dear Editor,

Anti-topoisomerase I (anti-Scl-70) antibodies are among the most clinically relevant autoantibodies in systemic sclerosis (SSc), strongly associated with diffuse cutaneous disease and interstitial lung disease (ILD) [1–4]. Although antibody positivity is widely used for disease phenotyping and prognostication [5, 6], whether quantitative antibody titers provide additional information beyond antibody status remains uncertain. Longitudinal studies suggest that antibody titers may parallel skin disease activity [7], but their relationship with objectively quantified pulmonary fibrosis and ILD phenotypes remains poorly defined [7, 8]. Distinguishing the clinical implications of antibody positivity from quantitative antibody titers is important because these measures may reflect different biological processes and could refine risk stratification in SSc.

We therefore conducted a prospective cross-sectional study to evaluate the associations of anti-topoisomerase I antibody positivity and quantitative antibody titers with skin and lung involvement in patients with systemic sclerosis. Consecutive adults fulfilling the 2013 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria [9] for SSc attending a tertiary referral center between January 2024 and June 2025 were enrolled. Anti-topoisomerase I antibody titers were quantified using ELISA in antibody-positive patients. Skin involvement was assessed using the modified Rodnan skin score (mRSS), while pulmonary involvement was evaluated by pulmonary function testing and high-resolution computed tomography (HRCT), with ILD extent quantified using Goh's staging system [10].

Among 100 patients with SSc (mean age 41 ± 10.8 years; 90% women), 56 (56%) were positive for anti-topoisomerase I antibodies. Baseline demographic characteristics and disease duration were comparable between antibody-positive and antibody-negative patients. However, antibody positivity identified a distinctly more severe disease phenotype, with higher frequencies of diffuse cutaneous disease, fibrotic ILD, and UIP, as summarized in Table 1. Diffuse cutaneous SSc was significantly more frequent in antibody-positive patients than in antibody-negative patients (69.6% vs. 38.6%; $p=0.0038$), whereas limited cutaneous disease predominated among antibody-negative individuals. Consistent with this observation, skin involvement was significantly greater

TABLE 1 | Clinical phenotype according to anti-topoisomerase I antibody status in systemic sclerosis.

Characteristic	Anti-Scl-70 positive (n = 56)	Anti-Scl-70 negative (n = 44)	p-value
Diffuse cutaneous SSc	69.6%	38.6%	0.0038
mRSS	21.6 ± 8.7	15.8 ± 8.8	0.0014
UIP	28.6%	6.8%	0.009
Fibrotic ILD	85.7%	65.9%	0.03
Median HRCT involvement	20%	10%	0.004

in antibody-positive patients, with a higher mean modified Rodnan skin score (21.6 ± 8.7 vs. 15.8 ± 8.8 ; $p = 0.0014$).

Pulmonary involvement also differed significantly according to antibody status. Although ILD was common throughout the cohort, antibody-positive patients had a significantly higher prevalence of fibrotic lung disease. Usual interstitial pneumonia was observed more than fourfold more frequently in antibody-positive patients than in antibody-negative patients (28.6% vs. 6.8%; $p = 0.009$). Likewise, fibrotic ILD (UIP plus fibrotic NSIP) was significantly more common among antibody-positive patients (85.7% vs. 65.9%; $p = 0.03$). Median total HRCT lung involvement was also greater in the antibody-positive group (20% vs. 10%; $p = 0.004$), whereas the extent of established fibrotic change alone did not differ significantly. Antibody-positive patients also walked shorter distances during the six-minute walk test, although pulmonary arterial hypertension and forced vital capacity did not differ significantly between groups.

A different pattern emerged when quantitative antibody titers were analyzed within the antibody-positive subgroup. Higher anti-topoisomerase I titers correlated significantly with skin involvement (Spearman's $\rho = 0.35$, $p < 0.001$), with an even stronger relationship among patients with diffuse cutaneous SSc ($\rho = 0.40$, $p = 0.013$) as shown in Figure 1. Importantly, this association remained significant after adjustment for disease duration and disease subtype in multivariable linear regression ($\beta = 0.022$ per U/mL; $p = 0.008$), supporting an independent relationship between antibody titers and skin fibrosis severity. In contrast, antibody titers showed no significant correlation with HRCT fibrosis extent, fibrotic ILD subtype, or pulmonary function indices, including forced vital capacity. Thus, while antibody positivity identified patients with fibrotic lung phenotypes, increasing antibody titers primarily reflected greater cutaneous involvement without indicating the burden of established pulmonary fibrosis.

Our findings demonstrate that anti-topoisomerase I antibody positivity and quantitative antibody titers should not be regarded as interchangeable biomarkers in SSc. Antibody positivity identified a diffuse cutaneous and fibrotic pulmonary

phenotype, whereas quantitative antibody titers reflected skin fibrosis without mirroring pulmonary fibrosis burden. These observations extend previous studies linking anti-topoisomerase I positivity with pulmonary fibrosis [2, 3, 8] by demonstrating a significantly higher prevalence of UIP and fibrotic ILD in antibody-positive patients. Clinically, antibody positivity may help identify patients requiring early HRCT evaluation and closer longitudinal pulmonary surveillance, whereas quantitative titers may be more informative for assessing cutaneous disease severity.

In contrast, quantitative antibody titers demonstrated a clear relationship with cutaneous disease severity but not with radiological fibrosis or pulmonary function. These observations are concordant with the longitudinal study by Hu et al. [7], who reported that changes in anti-topoisomerase I antibody levels paralleled changes in the modified Rodnan skin score. Our study extends those findings by incorporating detailed HRCT phenotyping and demonstrating that, despite predicting skin fibrosis, antibody titers do not reflect the extent of pulmonary fibrosis or the radiological pattern of ILD. This suggests that quantitative antibody measurement and qualitative antibody positivity capture different aspects of disease biology.

A plausible biological explanation for this dissociation is that skin fibrosis is more closely linked to ongoing immune activation and fibroblast stimulation, processes that may parallel active autoantibody production. In contrast, pulmonary fibrosis detected on HRCT represents cumulative structural injury resulting from multiple mechanisms, including chronic inflammation, aberrant tissue repair, disease duration and treatment exposure. Consequently, a single antibody measurement is unlikely to correlate closely with the extent of established fibrotic damage within the lung. This concept may explain why antibody positivity identifies patients predisposed to fibrotic lung disease, whereas antibody magnitude reflects the activity of cutaneous fibrosis rather than irreversible pulmonary injury.

To our knowledge, few prospective studies have simultaneously evaluated qualitative anti-topoisomerase I antibody status and quantitative antibody titers against detailed HRCT phenotypes and skin involvement within the same cohort.

Our study has several limitations. The cross-sectional design precludes assessment of temporal changes in antibody titers and disease progression. As this was a single-center study conducted at a tertiary referral hospital, referral bias likely contributed to the high prevalence of ILD and may limit generalisability. In addition, antibody titers were measured at a single time point, and the influence of previous immunosuppressive therapy on antibody concentrations could not be fully evaluated. Nevertheless, prospective recruitment, systematic HRCT assessment using Goh's staging system, standardized measurement of antibody titers, and comprehensive phenotyping strengthen the validity of our observations.

Our findings suggest that anti-topoisomerase I positivity should be interpreted as a marker of fibrotic lung phenotype, whereas antibody titers should be interpreted as a marker of skin disease severity rather than pulmonary fibrosis burden.

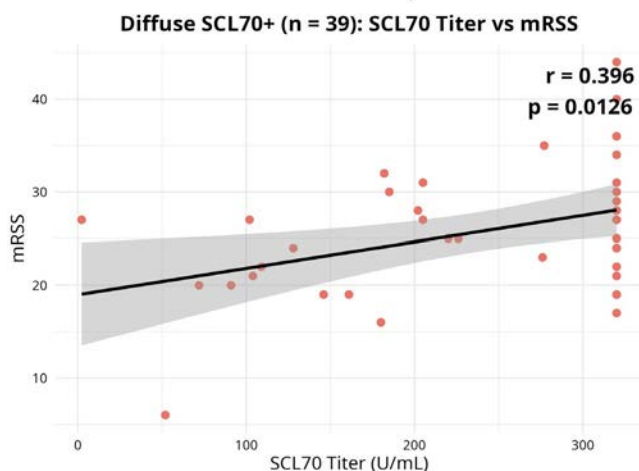


FIGURE 1 | Association between anti-topoisomerase I antibody titers and modified Rodnan skin score in diffuse cutaneous systemic sclerosis ($n = 39$).

Author Contributions

R.D.: investigation, data curation, formal analysis, documentation, writing, review and editing. S.M.: formal analysis, investigation (SCL-70 antibody titer analysis), writing, review and editing. Y.K.: investigation (SCL-70 antibody titer analysis), writing, review and editing. S.K.S.: conceptualization, investigation, methodology, clinico-radiological correlation, supervision, writing, review and editing.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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LETTER TO THE EDITOR

Clarifying Registration, Numerical Consistency, and Comparative Claims in an Acute Gout Trial

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Dear Editor,

We read with great interest the randomized, double-blind Phase II study by Xue et al. [1] evaluating SSGJ-613 for acute gout. The investigators address an important therapeutic gap and report rapid pain improvement together with fewer subsequent flares over 12 weeks. We congratulate the authors on completing a multicenter, double-dummy trial. Several reporting and interpretive issues, however, merit clarification before these findings are translated into practice.

First, the trial-registration information appears discordant with the published protocol. The article cites [ClinicalTrials.gov](https://clinicaltrials.gov) identifier NCT06169891 and identifies the study as SSGJ-613-AG-II [1]. Yet NCT06169891 describes a Phase III study (SSGJ-613-AG-III-01) with planned enrollment of 500 participants and two groups: SSGJ-613 200mg and compound betamethasone [2]. By contrast, NCT05588908 describes a Phase Ib/II program whose Phase II portion includes the three groups reported in the article—SSGJ-613 200mg, SSGJ-613 300mg, and compound betamethasone—and therefore appears to match the published study more closely [3]. We ask the authors and journal to confirm the correct registration and issue a correction if necessary. Explicitly linking the article to the matching registry record is essential for assessing prespecified outcomes, amendments, and selective reporting.

Second, the abstract reports a 12-week new-flare rate of 54.8% in the comparator group, whereas the Results section reports 16 of 31 participants (51.6%) [1]. This discrepancy should be reconciled, and any related risk differences, confidence intervals, and p values should be checked.

Finally, the primary 72-h pain outcome showed no statistically significant differences versus compound betamethasone

($p=0.4532$ and $p=0.0848$) [1]. Because the study used no formal sample-size calculation and specified no noninferiority or equivalence margin, these p values do not establish that SSGJ-613 was “comparable” to the active comparator. The relevant question requires prespecified margins and confidence intervals for between-group effects; failure to detect superiority is not evidence of equivalence [4]. Presenting adjusted treatment contrasts with 95% confidence intervals and tempering equivalence-like language would better reflect the exploratory design. The same caution applies to safety comparisons based on 59 SSGJ-613 recipients followed for only 12 weeks.

Clarifying these points would strengthen the article's transparency while preserving its valuable contribution as an early efficacy and safety signal for a novel nonsteroidal treatment of acute gout.

Author Contributions

Yi Zou drafted the manuscript. Guang Hong Ye critically revised the manuscript for important intellectual content. Both authors read and approved the final version and agree to be accountable for all aspects of the work.

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The authors have nothing to report.

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Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article because no new data were created or analyzed.

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REVIEW

Effects of Yoga on Rheumatoid Arthritis: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Yoga, a holistic mind–body practice used to improve physical function and psychological well-being in chronic inflammatory conditions such as rheumatoid arthritis (RA). This systematic review and meta-analysis aimed to assess the effectiveness of yoga in reducing psychological function and inflammation in individuals with RA.

Methods: A thorough literature search was performed in MEDLINE (via PubMed) for studies published between January 1994 and June 2025. Randomized controlled trials (RCT) investigating the impact of yoga on RA were selected. Data extraction was carried out independently by two reviewers. The outcomes evaluated included both subjective and objective indicators of Disease Activity Score (DAS), Health assessment questionnaire (HAQ-DI) and inflammatory markers.

Result: Eight RCT involving 651 participants were eligible for this study. Yoga interventions significantly improved psychological outcomes, with reductions in DAS 28 (SMD = −0.44; 95% CI = [−0.56 to −0.31]; $p < 0.0001$) compared to the control group. However, no significant changes were found in HAQ-DI (SMD = −0.23; 95% CI = [−0.81 to 0.36]; $p = 0.30$). Regarding inflammatory biomarkers, yoga significantly reduced serum Interleukin-6 (IL-6) levels (SMD = −0.65; 95% CI = [−1.17 to −0.13]; $p = 0.03$), while changes in Tumor Necrosis Factor- α (TNF- α) were not statistically significant (SMD = −0.71; 95% CI = [−1.74 to 0.33]; $p = 0.10$).

Conclusion: This meta-analysis indicates that yoga significantly improves mental health by reducing stress, anxiety, depression & lowers inflammation through reduced IL-6 levels. However, its effect on physical health and TNF- α was not consistent, suggesting more research is needed. Further large-scale studies are needed to validate and standardize its use.

1 | Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease primarily affecting the synovial joints and characterized by persistent inflammation that can lead to joint destruction, disability, and increased mortality. It commonly begins in small, symmetric joints and may progress if untreated [1]. RA is influenced by genetic and environmental factors, such as smoking, occupational, and air pollutants. Diagnosis is challenging due to the absence of a definitive laboratory test, particularly in early stages [2]. Epidemiological studies indicate that RA affects approximately 0.5%–1.0% of the global population, with

a prevalence of around 0.8% in most populations. Women are affected approximately three times more frequently than men, highlighting a marked sex-related predisposition to the disease. The burden of RA varies across geographic regions and ethnic groups, but it remains one of the most common chronic inflammatory arthritides worldwide. In India, the prevalence has been reported to be approximately 0.75%, which is comparable to rates observed in several Western populations [3].

Although disease-modifying antirheumatic drugs (DMARDs) remain the cornerstone of rheumatoid arthritis management, their long-term use may be associated with adverse effects and does

not completely alleviate symptoms or prevent functional impairment in all patients. Consequently, there is increasing interest in complementary non-pharmacological interventions that may improve physical and psychological outcomes while supporting conventional treatment [4].

Non-pharmacological interventions such as physical and occupational therapy play a crucial role in controlling disease progression and improving patient outcomes. Regular physical activity is vital for maintaining joint mobility and strengthening surrounding muscles. Low-impact exercises like swimming and yoga are particularly beneficial, as they build muscle strength without straining the joints [2]. Despite its benefits, physical activity remains underutilized among RA patients. Yoga, a holistic mind-body practice, integrates physical postures, breathing, and meditation to promote physical, mental, and emotional well-being. It influences both neurological and immune pathways, offering a comprehensive approach to managing RA [5].

Despite advances in pharmacological management, many patients continue to experience persistent symptoms, psychological distress, functional limitations, and reduced quality of life. Consequently, complementary interventions that can be integrated with standard medical care have attracted increasing attention. Yoga, a holistic mind-body practice incorporating physical postures, breathing techniques, meditation, and relaxation, has emerged as a promising adjunctive therapy because of its potential to improve physical function, psychological well-being, autonomic regulation, and inflammatory responses.

Previous systematic reviews have reported potential benefits of yoga for rheumatoid arthritis, including improvements in disease activity, functional disability, pain, and inflammatory biomarkers. However, these reviews were largely qualitative or included a limited number of randomized controlled trials, preventing robust quantitative estimates of treatment effects. Moreover, several recent randomized controlled trials evaluating psychological outcomes and inflammatory biomarkers have been published since these reviews and have not been incorporated into an updated quantitative synthesis. Therefore, an updated systematic review and meta-analysis including only randomized controlled trials was undertaken to provide a more comprehensive and precise evaluation of the effects of yoga on disease activity, psychological outcomes, functional disability, and inflammatory biomarkers in individuals with rheumatoid arthritis.

2 | Materials and Methods

The systematic review and meta-analysis were conducted according to the PRISMA guidelines [6].

2.1 | Search Strategy

A thorough literature search was conducted independently by two reviewers across Medline (via PubMed) databases, using the keywords: Yoga “[MeSH Terms] AND”, rheumatoid arthritis “[MeSH Terms] and” Yoga “[Title/Abstract] AND” rheumatoid arthritis [Title/Abstract]. The literature search was conducted

in MEDLINE via PubMed. MEDLINE was selected because it is a comprehensive biomedical database with extensive coverage of clinical and complementary medicine literature. We acknowledge that inclusion of additional databases such as Embase, Scopus, Web of Science, and Cochrane Library could improve comprehensiveness. The search covered all available studies from January 1994 to June 2025, with an additional check to include the most recent publications. Any disagreements were resolved through discussion with a third reviewer until agreement was achieved.

2.2 | Eligibility Criteria

2.2.1 | Study Selection and Inclusion Criteria

This systematic review and meta-analysis examined randomized controlled trials investigating yoga interventions in RA patients. Studies were included if they: (1) involved adult participants diagnosed with RA, (2) implemented yoga as the primary intervention, (3) included control groups, (4) measured psychological outcomes using validated instruments: Disease Activity Score (DAS 28 questionnaire), Health Assessment Questionnaire (HAQ-DI), and/or inflammatory biomarkers (IL-6, TNF- α), and (5) provided sufficient data for meta-analysis.

2.2.1.1 | Patients. Eligible studies included comparisons between yoga and non-yoga interventions or yoga combined with standard medication versus standard medication alone. Standard medication refers to conventional rheumatoid arthritis management, including disease-modifying antirheumatic drugs (DMARDs) such as methotrexate, sulfasalazine, hydroxychloroquine, and leflunomide, with NSAIDs and corticosteroids used when clinically indicated. Participants were selected based on physician diagnosis or the American College of Rheumatology (ACR) classification criteria [7]. Studies involving RA affecting the knees, hands, or feet were considered, with no limitations on age, sex, comorbidities, or diagnostic methods.

2.2.1.2 | Interventions. Only randomized controlled trials (RCTs) that evaluated yoga as the primary intervention were included. Variations in yoga style, duration, or frequency were not restricted.

2.2.1.3 | Comparisons. Eligible studies included comparisons between yoga and non-yoga interventions or yoga combined with standard medication versus standard medication without yoga.

2.2.1.4 | Outcomes. Studies were included based on the outcomes of interest rather than the specific measurement instruments used. Validated tools assessing psychological outcomes, quality of life, disease activity, inflammation, or functional disability were considered eligible. The measurement tools listed in the manuscript were examples of commonly reported instruments and were not intended to exclude studies using other validated measures.

To be eligible for inclusion, RCTs needed to evaluate at least one of the specified outcomes, including the primary outcome of disease activity assessed by DAS28, health assessment by HAQ-DI,

and secondary outcomes such as inflammatory markers, specifically IL-6 and TNF- α levels.

In this meta-analysis, we focused specifically on evaluating the effectiveness of yoga in RA patients by synthesizing outcomes related to DAS28, HAQ-DI, and inflammatory cytokines based on the available evidence from RCTs.

2.2.2 | Exclusion Criteria

Articles were excluded from the meta-analysis based on the following criteria:

Language: Studies not published in English.

Accessibility: Studies without full-text availability.

Study Design: Exclusion applied to observational studies, cohort studies, crossover trials, case reports, case series, non-randomized controlled trials, and preclinical research such as in vitro and animal studies.

2.3 | Data Extraction and Analysis

Data extraction was independently performed by two reviewers. Any discrepancies were resolved through discussion and consensus. When consensus could not be reached, a third reviewer was consulted to make the final decision.

Heterogeneity was assessed using I^2 statistics, τ^2 , and Q tests. Standardized Mean Differences (SMD) with 95% confidence intervals were calculated for continuous outcomes. Statistical significance was set at $p < 0.05$.

2.4 | Certainty of Evidence

The certainty of evidence for each outcome was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. Evidence was evaluated across the domains of risk of bias, inconsistency, indirectness, imprecision, and publication bias and rated as high, moderate, low, or very low certainty.

2.5 | Study Risk of Bias Assessment

The risk of bias in the included studies was evaluated using the Cochrane Risk of Bias tool, which covers six key domains: selection, performance, detection, attrition, reporting, and other biases. Two independent reviewers assessed each study across these areas (Table 2).

2.6 | Quality Assessment

Study quality was evaluated using standard risk of bias assessment tools for randomized controlled trials, examining randomization, allocation concealment, blinding, and outcome reporting.

2.7 | Statistical Methods

A meta-analysis was conducted using R software (version 4.2.2), calculating standardized mean differences (SMD) with 95% confidence intervals (CI) to estimate effect sizes. Effect sizes were interpreted following Cohen's guidelines as small (0.2–0.5), medium (0.5–0.8), and large (> 0.8). Heterogeneity was assessed using I^2 statistics, prediction intervals, and Cochran's Q test. Due to high heterogeneity, a random-effects model was applied for more reliable results [8].

3 | Results

3.1 | Literature Search Results

A thorough literature search (Figure 1) from 1994 to 2025 identified eight randomized controlled trials that fulfilled the inclusion criteria and were incorporated into the final systematic review and meta-analysis. A total of 74 articles were excluded due to irrelevant, review, or elimination during title and abstract screening. According to the inclusion and exclusion criteria, eight studies were included for meta-analysis.

3.2 | Details of the Included Studies

The characteristics of each study are detailed in Table 1. All included studies were published between 1994 and 2022. Of the eight studies included (total $n = 651$), six were conducted in India, one in Los Angeles, and one in the United Arab Emirates. The patient in the study group received yoga therapy; the duration of intervention is between 6 weeks to 12 weeks, where the patient in the control group received DMARDs. No serious adverse events were reported. The review systematically evaluated the impact of yoga therapy on psychological, health assessment, and inflammatory markers. Specifically, the studies assessed DAS 28 scores in all eight trials [9–16], HAQ-DI in four trials [10, 13, 15, 16], IL-6 in four trials [9, 12–14], and TNF- α levels in three trials [9, 13, 14].

3.3 | Details of Yoga Intervention

The intervention duration varied: five studies implemented an 8-week yoga program [9–13], two studies followed a 12-week protocol [14, 15], and one study had a 6-week intervention period [16]. All studies involved patients with rheumatoid arthritis (RA). The control group received DMARDs. The yoga included a combination of asanas (physical postures), pranayama (breathing techniques), dhyana (meditation), savasana (relaxation), and interactive sessions on stress management, nutrition, and lifestyle modification [9–16].

3.4 | Risk of Bias of the Included RCTs

Cochrane risk of bias assessments for the eight included randomized controlled trials are summarized in Table 2. Most studies demonstrated a low risk of bias for random sequence generation, allocation concealment, performance bias,

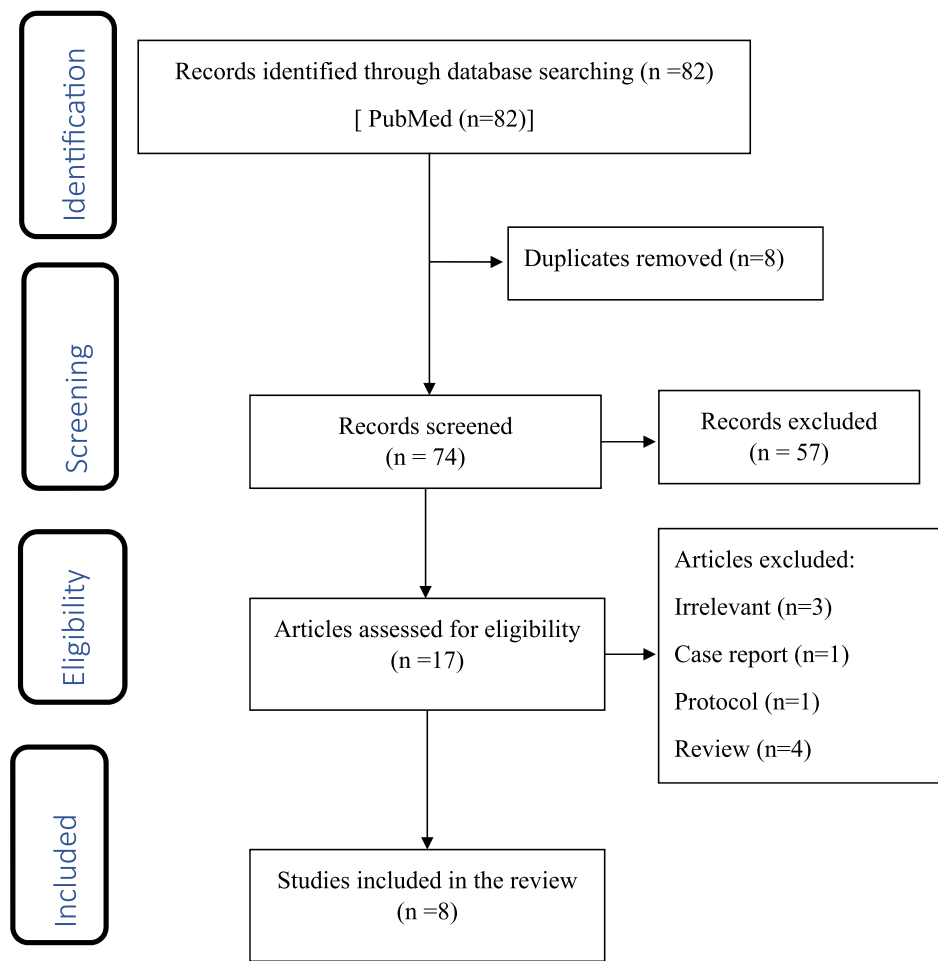


FIGURE 1 | Details of the systematic review process.

selective reporting, and other potential sources of bias [10–14]. Two studies [9, 11] showed an unclear risk for attrition bias, while another study presented an unclear risk for detection and attrition bias [15]. Another study showed an unclear risk across several domains, including random sequence generation, allocation concealment, performance bias, and detection bias [16]. Overall, the majority of the trials exhibited low risk across most bias domains, indicating acceptable methodological quality.

The GRADE assessment indicated moderate-certainty evidence for DAS28, low-certainty evidence for HAQ-DI and IL-6, and very low-certainty evidence for TNF- α (Table 3). The certainty of evidence was primarily downgraded because of concerns regarding risk of bias associated with the lack of blinding in yoga interventions, heterogeneity among studies, and imprecision due to small sample sizes.

3.5 | Meta Analysis

3.5.1 | Psychological Function (DAS 28 Questionnaire)

A meta-analysis of 8 studies with 651 participants (326 intervention, 325 control) examined the impact of Yoga interventions on DAS 28 scores among rheumatoid arthritis patients. The

random effects model showed a statistically significant reduction in DAS 28 scores (SMD: -0.44 ; 95% CI: $[-0.56$ to $-0.31]$; $p < 0.0001$) with no heterogeneity between studies ($I^2 = 0.0\%$; $p = 0.86$). The findings suggest that Yoga interventions demonstrated a moderate and consistent beneficial effect in reducing depression, anxiety, and stress symptoms as measured by DAS 28 in rheumatoid arthritis patients across all studies (Figure S1).

3.5.2 | HAQ-Di

A meta-analysis of 4 studies with 215 participants (108 intervention, 107 control) examined the impact of interventions on the outcome measure. The random effects model showed no statistically significant effect (SMD: -0.23 ; 95% CI: -0.81 to 0.36 ; $p = 0.30$) with low heterogeneity between studies ($I^2 = 29.3\%$; $p = 0.24$). The findings suggest that the interventions did not demonstrate a significant effect on the outcome measure, with relatively consistent results across the included studies (Figure S2). Although DAS28 was pre-specified as a primary outcome in the eligibility criteria, an insufficient number of included studies reported DAS28 as a quantifiable endpoint suitable for pooling; therefore, a separate meta-analysis for this outcome could not be conducted. The available DAS28 data were narratively described where reported by individual studies.

TABLE 1 | The summary of the studies included in the review.

Author, year of publication, country	Age, gender, sample size	Study design	Intervention details for experimental group	Intervention details for control group	Outcome variables	Results
Gautam S et al. (2020, India)	18–60 years, Both, E = 33, C = 33	RCT	YBLI included daily 120 min sessions of asanas, pranayama, and dhyana held five times a week for 8 weeks with DMARDs	DMARDs for the duration of 8 weeks	DAS28, inflammatory markers (IL-6, IL-17, TGF- β , TNF α), BDNF, β endorphin, sirtuin 1	DAS28 ($p < 0.001$), IL 6 ($p = 0.001$), IL 17 ($p = 0.962$), TGF- β ($p = 0.049$) TNF α ($p = 0.006$), BDNF ($p < 0.001$), β endorphin ($p = 0.015$), sirtuin 1 ($p < 0.001$).
Gautam S et al. (2021, India)	18–60 years, Both, E = 35, C = 35	RCT	YBLI included daily 120 min sessions of asanas, pranayama, and dhyana held five times a week for 8 weeks with DMARDs	DMARDs for the duration of 8 weeks	DAS28, HAQ-DI, cortisol, Melatonin, Serotonin	DAS28 ($p < 0.001$), HAQ-DI ($p < 0.001$), cortisol ($p = 0.03$), Melatonin ($p < 0.001$), Serotonin ($p < 0.001$)
Gautam S et al. (2022, India)	18 years above, Both, E = 70, C = 70	RCT	YBLI included daily 120 min sessions of asanas, pranayama, and dhyana held five times a week for 8 weeks	DMARDs for the duration of 8 weeks	DAS28, sHLA-G	DAS28 ($p < 0.001$), sHLA-G ($p = 0.018$)
Gautam S et al. (2023, India)	Both, E = 32, C = 32	RCT	YBLI included daily 2-h sessions of asanas, pranayama, and dhyana held five times a week for 8 weeks	DMARDs for the duration of 8 weeks	DAS28, inflammatory markers (IL-6, IL-17 and TGF- β)	DAS28 ($p = 0.008$), IL-6 ($p = 0.001$), IL-17 ($p = 0.750$) and TGF- β ($p = 0.015$)
Gautam S et al. (2019, India)	18–60 years, Both, E = 36, C = 36	RCT	Yoga based mind body based intervention program lasted for approximately 120 min per day and 5 sessions per week for 8 weeks	DMARDs for the duration of 8 weeks	DASS 28, Spro-inflammatory & anti-inflammatory cytokines (IL-6, IL-17A, TNF and TGF-g) immunomodulatory marker-soluble HLA-G; biomarkers of neuroplasticity- BDNF, serotonin, endorphins; longevity (SIRT1), HAQ-DI	DAS28 ($p < 0.0001$), IL 6 ($p = 0.002$), IL 17A ($p < 0.0001$), TNF ($p < 0.0001$), TGF ($p = 0.003$), HLA-G ($p = 0.011$), BDNF ($p < 0.0001$), Serotonin ($p < 0.0001$), Sirtuin ($p < 0.0001$), β endorphin ($p < 0.0001$), HAQ-DI ($p = 0.001$)
Ganesan S et al. (2020, India)	Between 30 and 60 years, Both, E = 83, C = 83	RCT	yoga therapy for 12 weeks (30 min, 3 times/week)	Standard Medical care	DAS 28, IL6, TNF α , cortisol	DAS 28 ($p = 0.000$), IL 6 ($p = 0.139$), TNF α ($p = 0.098$), Cortisol ($p = 0.006$)

(Continues)

TABLE 1 | (Continued)

Author, year of publication, country	Age, gender, sample size	Study design	Intervention details for experimental group	Intervention details for control group	Outcome variables	Results
Evan S et al. (2013, Los Angeles)	16–35 years, Both, E = 11, C = 15	RCT	Yoga (90 min each; two weekly sessions; 6 weeks)	Usual-care (normal physical exercise)	DAS-28, HAQ-DI	DAS-28 ($p = 0.18$), HAQ-DI ($p = 0.10$)
Badsha et al. (Dubai, 2009)	Above 18 years, BOTH, E = 26, C = 21	RCT	Yoga (60 min each; two weekly sessions; 6 weeks) + medication (DMARDs)	DMARDs for the duration of 6 weeks	DAS-28, HAQ-DI	DAS-28 ($p = 0.021$), HAQ-DI ($p = 0.0015$)

Abbreviations: BDNF, Brain-Derived Neurotrophic Factor; DAS28-ESR, disease activity score-28–erythrocyte sedimentation rate; DMARDs, disease modifying anti-rheumatic drugs; HAQ-DI, Health Assessment Questionnaire; IL, interleukin; RA, Rheumatoid Arthritis; RCT, Randomized Controlled Trial; s HLA-G, soluble Human Leukocyte Antigen-G; TGF, Transforming Growth Factor; TNF α , Tumor Necrosis Factor alpha; YBLI, yoga-based lifestyle intervention.

TABLE 2 | Risk of bias assessment of the included randomized controlled trials.

Author, year of publication	Selection bias				Performance bias (blinding participants & personnel)	Detection bias (blinding outcome assessment)	Attrition bias (incomplete outcome data)
	Random sequence generation	Allocation concealment	Reporting bias (selective reporting)	Other bias (other sources of bias)			
Gautam S et al. (2020)	Low	Low	Low	Low	Low	unclear	Low
Gautam S et al. (2021)	Low	Low	Low	Low	Low	Low	Low
Gautam S et al. (2022)	Low	Low	Low	Low	Low	Unclear	Low
Gautam S et al. (2023)	Low	Low	Low	Low	Low	Low	Low
Gautam S et al. (2019)	Low	Low	Low	Low	Low	Low	Low
Ganesan S et al. (2020)	Low	Low	Low	Low	Low	Low	Low
Evan S et al. (2013)	Low	Low	Low	Low	Unclear	Low	Unclear
Badsha et al. (2009)	Unclear	Unclear	Low	Unclear	Unclear	Low	Unclear

Note: Risk of bias was assessed using the Cochrane Collaboration modified tool for assessing risk of bias for randomized controlled trials, Part-I & II.

TABLE 3 | GRADE summary of findings.

Outcome	No. of studies (participants)	Relative effect (SMD, 95% CI)	Certainty of evidence (GRADE)	Reasons for downgrading	Interpretation
DAS 28	8 (651)	-0.44 (-0.56 to -0.31)	Moderate (⊕⊕⊕○)	Downgraded one level for risk of bias due to lack of participant/personnel blinding in several studies	Yoga probably reduces disease activity compared with control
HAQ-DI	4 (215)	-0.23 (-0.81 to 0.36)	Low (⊕⊕○○)	Downgraded for risk of bias and imprecision (small sample size and confidence interval crossing the line of no effect)	The effect of yoga on functional disability remains uncertain
IL-6	4 (368)	-0.65 (-1.17 to -0.13)	Low (⊕⊕○○)	Downgraded for risk of bias and inconsistency ($I^2 = 62\%$)	Yoga may reduce IL-6 levels, although confidence in the estimate is limited due to heterogeneity
TNF- α	3 (304)	-0.71 (-1.74 to 0.33)	Very Low (⊕○○○)	Downgraded for risk of bias, inconsistency ($I^2 = 76\%$), and imprecision (wide confidence interval crossing no effect)	The effect of yoga on TNF- α is highly uncertain

Abbreviations: CI, Confidence Interval; DAS28, Disease Activity Score in 28 joints; GRADE, Grading of Recommendations Assessment, Development and Evaluation; HAQ-DI, Health Assessment Questionnaire-Disability Index; IL-6, Interleukin-6; SMD, Standardized Mean Difference; TNF- α , Tumor Necrosis Factor-alpha.

3.5.3 | Inflammatory Biomarkers

3.5.3.1 | Interleukin-6 (IL-6). A meta-analysis of 4 studies with 368 participants (184 intervention, 184 control) examined the impact of interventions on IL-6 levels. The random effects model showed a statistically significant reduction in IL-6 levels (SMD: -0.65; 95% CI: -1.17 to -0.13; $p = 0.03$) with substantial heterogeneity between studies ($I^2 = 61.6\%$; $p = 0.05$). The findings suggest that the interventions demonstrated a moderate beneficial effect in reducing IL-6, an inflammatory cytokine, though with considerable variation in effect sizes across the included studies (Figure S3).

3.5.3.2 | TNF Alpha. A meta-analysis of 3 studies with 304 participants (152 intervention, 152 control) examined the impact of interventions on the outcome measure. The random effects model showed no statistically significant effect (SMD: -0.71; 95% CI: -1.74 to 0.33; $p = 0.10$) with substantial heterogeneity between studies ($I^2 = 76.2\%$; $p = 0.01$). The findings suggest that while there was a trend toward benefit, the interventions did not demonstrate a statistically significant effect on the outcome measure, with considerable variation in effect sizes across the included studies limiting reliable conclusions (Figure S4).

4 | Discussion

This systematic review and meta-analysis examined the effects of yoga in patients with rheumatoid arthritis and demonstrated potential benefits in psychological outcomes and certain inflammatory parameters. The findings demonstrate statistically significant improvements in DAS 28 and IL-6 levels, while HAQ-DI and TNF- α did not reach statistical significance. Primary outcomes evaluated psychological well-being (DAS 28) and functional disability (HAQ-DI), while secondary outcomes assessed inflammatory cytokines (IL-6, TNF- α) (Table 4). These results suggest that Yoga may be a promising non-pharmacological intervention for the management of RA.

To the best of our knowledge, a few systematic reviews and meta-analyses conducted several years ago evaluated the impact of various forms of yoga on inflammation, immune function, and RA. These studies suggested that yoga may serve as a beneficial complementary therapy for managing chronic inflammatory conditions, positively influencing both biological and psychosocial outcomes in individuals with RA [17, 18]. However, the limited availability of high-quality randomized trials at that time restricted the strength and generalizability of these conclusions.

Emerging evidence suggests that yoga exerts beneficial effects on RA by modulating the psycho-neuro-immune axis, thereby reducing systemic inflammation and disease activity. Yoga has been shown to significantly reduce the DAS28 scores, which is a composite index of RA disease activity [13]. This reduction corresponds with a decline in pro-inflammatory cytokines, particularly IL-6, IL-17A, and a concurrent upregulation of the anti-inflammatory cytokine TGF- β , indicating restoration of immune homeostasis [13, 19]. IL-6 is known to promote Th17 differentiation and inflammation through the JAK-STAT signaling pathway, while simultaneously inhibiting T-reg cell development in the presence of TGF- β , thus disturbing immune tolerance [20].

TABLE 4 | Summary of pooled meta-analysis results evaluating the effects of yoga in patients with rheumatoid arthritis.

Outcome	No. of studies	Participants (yoga/control)	Effect size (SMD/MD)	95% CI	p	I ²	Interpretation
DAS 28	8	326/325	-0.44	-0.56 to -0.31	<0.00001	0%	Yoga significantly improved psychological well-being compared with the control group. The effect size was small to moderate, with no observed heterogeneity, indicating consistent findings across the included studies
HAQ-DI	4	108/107	-0.23	-0.81 to 0.36	0.43	29%	No statistically significant improvement in functional disability was observed following yoga intervention compared with the control group. The heterogeneity was low ($I^2 = 29\%$), indicating relatively consistent findings across the included studies
IL-6	4	184/184	-0.65	-1.17 to -0.13	0.01	62%	Yoga significantly reduced IL-6 levels compared with the control group. The pooled effect was of moderate magnitude, although moderate heterogeneity was observed across the included studies.
TNF- α	3	152/152	-0.71	-1.74 to 0.33	0.18	76%	No statistically significant reduction in TNF- α levels was observed following yoga intervention compared with the control group. Considerable heterogeneity ($I^2 = 76\%$) indicates substantial variability among the included studies

Abbreviations: CI, Confidence Interval; HAQ-DI, Health Assessment Questionnaire-Disability Index; I², heterogeneity statistic; IL-6, Interleukin-6; SMD, Standardized Mean Difference; TNF- α , Tumor Necrosis Factor-alpha.

Yoga helps correct this imbalance by increasing vagal tone and reducing Hypothalamic Pituitary Adrenal axis (HPA) hyperactivity, thereby reducing IL-6 and IL-17A levels [21].

The effect of yoga on physical functioning, as assessed by HAQ-DI, was inconclusive, likely due to the short duration and nature of interventions. Functional improvements in RA typically require prolonged and structured rehabilitation, as supported by previous evidence indicating that exercise interventions of at least 12 weeks are generally required to produce meaningful changes in physical function in RA patients [17, 18]. Although changes in TNF- α levels were not statistically significant, observed trends suggest potential immunomodulatory effects. Variability in findings may be attributed to differences in sample sizes, baseline disease activity, and assessment methods. The divergence between DAS 28 improvement and the absence of significant functional ability change (HAQ-DI) may reflect the differing responsiveness of psychological and physical outcomes to short-term yoga interventions; psychological measures are generally more sensitive to brief mind-body practices, while structural improvements in physical function require sustained, longer-duration programs. Further large-scale, standardized studies are recommended to clarify these outcomes.

Despite some limitations, this meta-analysis has several strengths. Including only randomized controlled trials helps improve the reliability of the results. A thorough literature search and strong methodology further support the findings.

The certainty of evidence varied across outcomes according to the GRADE framework. Moderate-certainty evidence supported the beneficial effect of yoga on disease activity (DAS28), whereas the evidence for HAQ-DI and IL-6 was rated as low certainty because of methodological limitations and heterogeneity. The certainty of evidence for TNF- α was very low owing to substantial heterogeneity and imprecision, indicating that further well-designed randomized controlled trials are likely to influence the current estimates.

However, a few limitations should be noted. The quality of the included studies varied, with concerns related to allocation concealment, blinding, detection, and attrition bias in some trials. In particular, performance bias should be interpreted with caution because blinding of participants and personnel is inherently difficult in yoga-based interventions. Unlike pharmacological trials, participants are aware of receiving yoga, and instructors cannot be blinded to the intervention. Consequently, some degree of performance bias is unavoidable in non-pharmacological interventions and may have influenced subjective outcomes, such as psychological well-being and self-reported functional status. Studies had small sample sizes, which may reduce the generalizability of the results. Future studies should focus on identifying the long-term effects of yoga and studying how these interventions interact with standard RA treatments. Additionally, the review protocol was not prospectively registered (e.g., in PROSPERO), which may have increased the risk of selective reporting and reduced methodological transparency. Large, well-designed trials using standardized yoga interventions, either alone or alongside conventional treatments, are needed to confirm these findings.

5 | Conclusion

In conclusion, this meta-analysis offers encouraging evidence supporting the effectiveness of Yoga in improving psychological outcomes (DAS 28) and reducing IL-6 levels in individuals with rheumatoid arthritis. However, yoga did not significantly improve physical function (HAQ-DI) or reduce TNF- α levels, indicating that its benefits are not uniform across all outcome domains. However, these results should be viewed considering certain study limitations. Further well-designed research is needed to determine the best treatment approaches and assess long-term safety. Yoga may serve as a promising complementary approach to support the management of RA within integrative medicine.

Author Contributions

Abinaya Suresh: Conceptualization, methodology, literature search, study selection, data extraction, interpretation of results and manuscript drafting. Shobana Ramaswamy: Conceptualization, supervision, interpretation of results and revision of the manuscript. Santhi Silambanan: Conceptualization, supervision. Mahesh Kumar Kuppasamy: Methodology, supervision, interpretation of results, critical review, and revision of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** The effect of yoga on Disease Activity Score (DAS) 28 with control group. **Figure S2:** The effect of yoga on Health Assessment Questionnaire (HAQ-DI) with control group. **Figure S3:** The effect of yoga on Interleukin (IL) 6 with control group. **Figure S4:** The effect of yoga on Tumor Necrosis Factor (TNF) alpha with control group.



EDITORIAL

Medium- To High-Dose Aspirin in Kawasaki Disease: Time to Revisit an Old Tradition

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Kawasaki disease (KD) is an acute systemic vasculitis of childhood and remains the leading cause of acquired heart disease in children. The major concern in KD is not the fever or mucocutaneous inflammation alone, but the risk of development of coronary artery lesions (CALs), including coronary artery dilatation and aneurysm formation. The incidence of KD has also increased in developing nations in recent decades, driven by greater awareness among clinicians and improvements in diagnosis. The pathophysiology includes medium-vessel vasculitis, predominantly involving the coronary arteries, with immune activation and endothelial dysfunction. Timely treatment with intravenous immunoglobulin (IVIG) has changed the natural history of the disease and remains the cornerstone of therapy [1].

Aspirin has been used as an adjunctive agent in KD since the 1970s. Traditionally, it was given in moderate to high doses (30–100 mg/kg/day) during the acute febrile phase for its presumed anti-inflammatory effect, and then continued in low doses (3–5 mg/kg) during the convalescent phase for its antiplatelet effect. North American practice has generally favored high-dose aspirin, usually 80–100 mg/kg/day, while Japanese guidelines and several Asian centres have used a moderate dose of 30–50 mg/kg/day during the acute phase [2].

This dosing strategy, however, has more historical support than strong trial-based support [3]. Much of the early use of aspirin was based on clinical tradition and retrospective observations rather than on properly controlled trials. The early report by Kato and colleagues in 1979 established aspirin's important role in KD treatment by suggesting fewer CALs with aspirin-containing regimens compared with corticosteroid regimens [4]. However, this study was non-randomized and had important limitations, including possible selection bias and uneven group

allocation. Therefore, it could not settle the question of the best aspirin dose.

In recent decades, the therapeutic paradigm of moderate to high dose aspirin during the acute phase has been subjected to questions regarding its utility as it has been associated with potential adverse effects, including hepatotoxicity, gastrointestinal bleeding, and rarely Reye's syndrome. The debate over the optimal aspirin dosage in KD has intensified recently, and numerous systematic reviews and meta-analyses have examined its efficacy [5–10]. The meta-analyses have assessed the effect of aspirin on the incidence of CALs, efficacy and safety, interactions with IVIG, and differences in population-specific responses.

The most important outcome of KD treatment is, without question, the prevention of CALs. If high-dose aspirin is to be justified, it should show a clear advantage in reducing CALs. The available literature does not consistently show such a benefit [5–10]. A meta-analysis from China, which included 6 studies and 11 103 children, showed no significant difference in CAL incidence between low-dose and high-dose aspirin groups: RR = 0.85 (95% CI: 0.63–1.14, $p = 0.28$), indicating low-dose aspirin is not inferior to high-dose aspirin, although the meta-analysis had moderate heterogeneity [5]. Various other meta-analyses have also corroborated similar findings. Similarly, Chiang et al. found no significant difference in CAL incidence between the low/no-dose and high-dose aspirin groups in a meta-analysis of 9 cohort studies [6]. Jia et al., which included 8 retrospective cohort studies and reported on 12 176 patients, also found a similar outcome [7].

Liu et al.'s study, which included 9 retrospective studies with 12 258 patients, also concluded that low-dose aspirin (3–5 mg/kg) is not inferior to high-dose aspirin for CAL prevention [10]. In

a recent meta-analysis of 12 studies involving 68495 children, no significant difference in coronary outcomes was observed between the no/low-dose and high-dose aspirin groups [9]. In a network meta-analysis, Huang et al. analyzed the more detailed dose-response of aspirin [10]. There was no significant difference among the low (3–5 mg/kg), medium (30–50 mg/kg), and high-dose aspirin (80–100 mg/kg) groups in the incidence of CALs. These meta-analyses involving large patient populations suggest that aspirin does not significantly affect the incidence of CALs in KD.

Similarly, meta-analyses have examined the effects of different aspirin dosages on IVIG resistance in KD. A meta-analysis from China found no significant difference in IVIG resistance between the high- and low-dose aspirin groups [5]. Findings from other meta-analyses have also supported this outcome [6–10]. Although Huang et al. showed a trend toward lower IVIG resistance in those with aspirin vs. those without, the null value overlaps the confidence interval, suggesting no significant effect [10]. IVIG exerts its immunomodulatory effect in KD through multiple pathways, including decreasing the production of inflammatory cytokines, blocking Fc receptors, and inhibiting complement activity, effects that are not influenced by aspirin [11]. So, it is possible that the association is just by chance in the context of multiple comparisons. Additionally, all meta-analyses have determined the role of aspirin on the duration of hospitalization. The meta-analysis by Zheng found no significant difference in hospitalization duration between the high-dose and low-dose aspirin groups [5]. These studies, although not perfect, point in the same direction: low-dose aspirin does not appear inferior to high-dose aspirin for coronary outcomes when IVIG is used appropriately.

Hospital stay has also been assessed in several studies, but it is a weak outcome for judging aspirin efficacy. The duration of hospitalization is influenced by local protocols, clinician preferences, availability of echocardiography, parental concerns, affordability, and discharge policies. Therefore, even if one study shows a small difference in hospital stay, it does not necessarily prove that one aspirin dose is clinically superior.

Safety is an important part of this discussion. Aspirin is familiar to pediatricians, but high-dose aspirin is not completely

harmless. Gastrointestinal irritation, gastrointestinal bleeding, transaminitis, and, rarely, Reye syndrome are recognized concerns. Huang et al. reported that in the aspirin group, 5.3% of patients experienced various gastrointestinal side effects, including abdominal discomfort and upper or lower gastrointestinal bleeding [10]. Increased adverse events have also been mentioned by Safar et al. However, specific adverse effects have not been documented [9]. Overall, the review concludes that aspirin has been associated with mild to severe adverse effects at higher doses. Besides gastrointestinal-related adverse effects, aspirin is also associated with hepatotoxicity. Although the meta-analysis by Zheng et al. reported that 0.9% of patients experienced hepatotoxicity in one trial, as evidenced by elevated transaminase levels, it did not specify which group developed this complication [5]. Similarly, Huang et al. reported a similar observation, but the incidence rate-dose relationship has not been quantified [8]. Furthermore, given the limited data across studies on aspirin hepatotoxicity, comprehensive liver function monitoring is needed, and future studies are essential. Rey's syndrome, although rare, is a potentially life-threatening complication and has been documented in a limited number of meta-analyses and remains a major concern while treating KD [10]. The meta-analysis from China has demonstrated that many studies have reported this important complication [5]. Besides, complications such as hemolysis in G6PD deficiency and exacerbation of asthma have also been demonstrated [10].

Recent evidence from a multicenter randomized noninferiority trial involving 134 children with KD demonstrated that IVIG alone was noninferior to IVIG plus high-dose aspirin (80–100 mg/kg/day) for preventing CAL, with no significant differences in coronary outcomes between groups [12]. Furthermore, the ongoing large-scale DAPI trial is expected to provide definitive evidence regarding the optimal aspirin dose and may finally resolve this longstanding controversy [13].

Taken together, the overall evidence suggests that the conventional use of moderate to high doses of aspirin is not beneficial for reducing the incidence of CALs, the duration of hospitalization, or IVIG resistance in KD. However, current meta-analyses supporting the role of low dose aspirin in the acute stage of KD have predominantly included retrospective studies, thereby introducing multiple sources of bias (Table 1). Additionally,

TABLE 1 | Characteristics of systematic reviews evaluating aspirin dosing.

Study	Number of studies (n)	Type of review	Enrolled patients (n)	Study design
Huang et al. (2021) [10]	6	Network meta-analysis	1944	Retrospective cohorts
Chiang et al. (2021) [6]	9	Meta-analysis	12 182	Retrospective cohorts
Jia et al. (2020) [7]	8	Meta-analysis	12 716	Retrospective cohorts
Liu et al. (2022) [8]	9	Meta-analysis	12 258	Retrospective cohorts
Safar et al. (2024) [9]	12	Meta-analysis	68 495	Mixed designs
Zheng et al. (2019) [5]	6	Meta-analysis	Not mentioned	Mixed designs
Lei et al. (2022) [14]	56	Network meta-analysis	Not mentioned	Randomized controlled trials

heterogeneity in treatment protocols, outcome measures, and lack of individual patient data limits the strength of currently available evidence. Future studies with adequately powered non-inferiority randomized trials, standardized treatment protocols, and outcome measures including incidence of coronary artery lesions, time to defervescence, and duration of hospitalization would help clarify the current uncertainty.

The debate should therefore not be framed as a simple question of aspirin versus no aspirin. Low-dose aspirin still has a practical and accepted role as antiplatelet therapy, particularly after defervescence and in children with coronary involvement. The more relevant question is whether anti-inflammatory doses of aspirin are still required routinely in the IVIG era. Current evidence suggests that routine high-dose aspirin for all children with acute KD may not be necessary. Further, a more rational approach would be to focus on early diagnosis, timely IVIG, careful echocardiographic follow-up, and risk-based intensification of treatment in children at high risk of IVIG resistance or coronary complications.

In conclusion, high-dose aspirin in acute KD is a long-standing practice, but long-standing practice should not be confused with strong evidence. The available literature does not show a clear additional benefit of high-dose aspirin over low-dose aspirin in preventing CALs, reducing IVIG resistance, or shortening hospital stay. Considering its potential adverse effects, it is reasonable to reconsider the role of routine medium to high-dose aspirin in the acute phase of KD. Low-dose aspirin for its antiplatelet effect, especially in children with coronary involvement, remains clinically relevant. In the IVIG era, high-dose aspirin appears to be more a matter of habit than evidence. It may now be time to separate the proven antiplatelet role of aspirin from its unproven anti-inflammatory role in acute KD.

Author Contributions

Rakesh Kumar Pilania: conceptualization, methodology, data curation, formal analysis, writing – review and editing, interpretation of results. **C. V. Gaythri:** literature search, data extraction. **Pratap Kumar Patra:** conceptualization, methodology, investigation, data curation, formal analysis, writing – original draft, interpretation of results.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CLINICAL IMAGE

Anterior Chest Wall Prominence in a Patient With Chronic Inflammatory Back Pain

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1 | Riddle/Mystery Summary

A 60-year-old woman presented with a five-year history of inflammatory thoracolumbar pain that partially improved with naproxen, progressive painful swelling of the anterior chest wall, and bilateral heel pain. Examination showed a prominent erythematous swelling over the right sternoclavicular region and tenderness of the manubriosternal and sternoclavicular joints (Figure 1). She had no characteristic cutaneous manifestations, personal or family history of psoriasis, or other clinical features of spondyloarthritis.

Complete blood count and inflammatory markers were normal; ANA, ENA, rheumatoid factor, anti-CCP antibodies, and HLA-B27 were negative. Previous computed tomography showed sclerotic vertebral and sternoclavicular lesions suspicious for metastases; PET was therefore performed as part of an oncologic evaluation. PET showed increased uptake at the sternoclavicular joint, without lesions suspicious for a primary malignancy or metastatic disease. Bone scintigraphy showed increased tracer uptake in the manubrium, sternoclavicular joints, and thoracic spine (Figure 2). The five-year clinical course, normal inflammatory markers, and characteristic lesion distribution made infection and malignancy less likely.

What condition is illustrated in these images?

2 | Answer

These findings are consistent with SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis, and osteitis), within the



FIGURE 1 | Hard, erythematous prominences on the anterior chest wall overlying the sternoclavicular and manubriosternal joints.

spectrum that includes chronic recurrent multifocal osteomyelitis (CRMO) [1].

SAPHO syndrome is a rare autoinflammatory disorder characterized by sterile osteitis and hyperostosis, most commonly involving the anterior chest wall, particularly the sternoclavicular and manubriosternal joints. Characteristic skin manifestations may support the diagnosis but can be absent; spinal involvement and peripheral enthesitis may also occur [2]. Imaging typically demonstrates osteosclerosis, hyperostosis, and increased radionuclide uptake at affected sites [3]. Recognition of this clinical-imaging pattern can prevent unnecessary invasive diagnostic procedures.

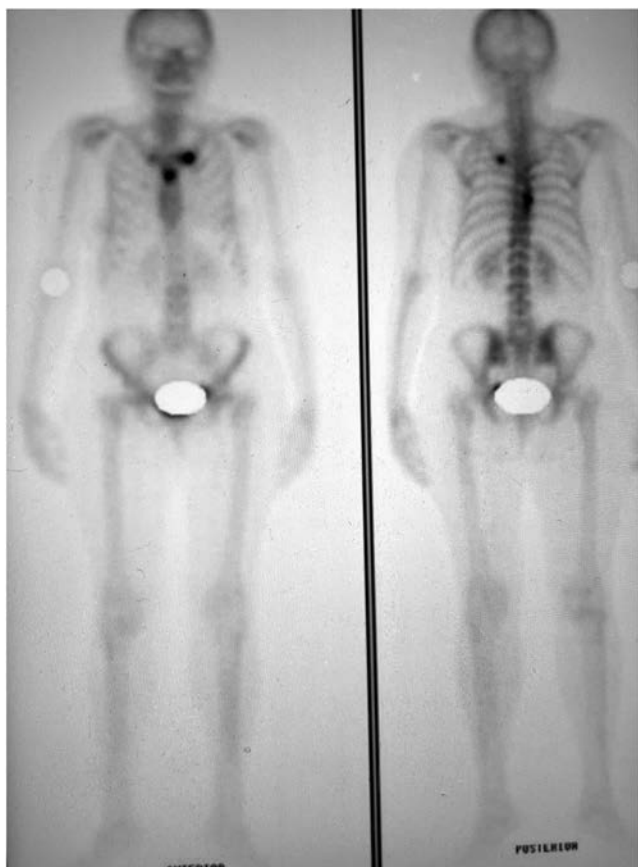


FIGURE 2 | Anterior and posterior bone scintigraphy projections showing increased tracer uptake in the manubrium, bilateral sternoclavicular joints (predominantly on the left), and posterior elements of the mid-thoracic vertebrae (predominantly on the right).

Author Contributions

Mauricio Restrepo-Escobar cared for the patient. Both authors analyzed the clinical findings and images, reviewed the literature, drafted and revised the manuscript, and approved the final version.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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LETTER TO THE EDITOR

Discordant Internal and External Validation Performance in a Multicenter Nomogram for Overlap Syndrome in Systemic Lupus Erythematosus

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Dear Editor,

We read with interest the study by Li et al. [1] describing a multicenter nomogram to predict overlap syndrome (OS), the co-occurrence of systemic sclerosis, rheumatoid arthritis, and Sjögren's syndrome—in patients with systemic lupus erythematosus (SLE). We raise two related methodological concerns about the reported model performance and its reporting completeness, which we believe merit clarification before this nomogram is adopted in clinical practice.

First, the 95% confidence intervals for discrimination in the internal and external validation cohorts do not overlap: the internal validation area under the curve (AUC) was 0.877 (95% CI 0.843–0.911), while the external validation AUC was 0.760 (95% CI 0.730–0.790). The upper bound of the external interval (0.790) falls below the lower bound of the internal interval (0.843), indicating a decline in discrimination that is unlikely to reflect chance alone once the model is applied outside its derivation setting. This finding is difficult to reconcile with the authors' conclusion that the model shows "good predictive performance" suitable for clinical use across settings, since an AUC of 0.760 represents meaningfully weaker discrimination than the internal figures suggest.

Second, neither the abstract nor the reported results specify the number of patients who developed OS in the development, internal validation, or external validation cohorts. Fourteen candidate predictors were retained in the final multivariable model (anti-SSA and anti-SSB antibodies, proteinuria, occult blood in urine, age, eosinophil ratio, hematocrit, platelet count, direct and indirect bilirubin, rheumatoid factor, immunoglobulin A, prothrombin time, and ferritin). Without the outcome event

counts, readers cannot verify whether the events-per-variable ratio met recommended thresholds for stable coefficient estimation [2], a consideration directly relevant to interpreting the internal-to-external decline in discrimination noted above. The Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) statement explicitly recommends reporting the number of participants and outcome events for each analysis to allow this assessment [3].

We recognize that some of these details may appear in the full-text tables. However, given their direct bearing on the model's central performance claim, we suggest that explicit reporting of outcome event counts per cohort, together with a statement of adherence to TRIPOD reporting recommendations, would substantially strengthen readers' ability to judge whether this nomogram is ready for the clinical application the authors propose.

We congratulate the authors on assembling this large multicenter cohort and hope these comments contribute constructively to the interpretation of their findings.

Author Contributions

Conceptualization, methodology: H.L.N., E.C.-D. Writing – original draft: H.L.N. Writing – review and editing: H.L.N., E.C.-D.

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Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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LETTER TO THE EDITOR

Cases Report: Prominent Distal Pitting Edema Resembling Remitting Seronegative Symmetrical Synovitis With Pitting Edema in the Giant Cell Arteritis–Polymyalgia Rheumatica Spectrum: Diagnostic Value of Multimodal Imaging in Two Cases

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Dear Editor,

Polymyalgia rheumatica (PMR) is characterized by pain and stiffness in the shoulder and pelvic girdles and increased inflammatory markers. PMR remains a clinical diagnosis and can be difficult to establish without disease-specific diagnostic tests; approximately one-third of patients given a provisional PMR diagnosis are subsequently reclassified as having another condition [1]. Conversely, PMR may not be considered initially when prominent peripheral edema dominates the clinical presentation. Herein, we describe two patients with prominent distal pitting edema to illustrate the diagnostic value of multimodal imaging in atypical PMR and giant cell arteritis (GCA) presentations.

The first patient, a 91-year-old woman with a history of hypertension, dyslipidemia, and bronchial asthma, was referred to our hospital for further evaluation of progressive edema of all four extremities and persistently elevated inflammatory markers. Her symptoms had begun 1 month earlier. Although she had been evaluated previously, the underlying cause remained undetermined, and her edema continued to worsen. On admission, her body temperature was 37.7°C. Physical examination revealed pitting edema of all four extremities (Figure 1A,B). Tenderness was noted on both shoulders, elbows, hands, and hips. Laboratory tests showed elevated C-reactive protein (CRP; 7.08 mg/dL), erythrocyte sedimentation rate (ESR; 120 mm/h), matrix metalloproteinase-3 (MMP-3; 234.7 ng/mL), and vascular endothelial growth factor (VEGF; 3213.5 pg/mL) levels.

Rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) antibodies, antinuclear antibodies (ANA), myeloperoxidase–antineutrophil cytoplasmic antibodies (MPO-ANCA), and proteinase 3–antineutrophil cytoplasmic antibodies (PR3-ANCA) were negative. Thyroid function was normal, and blood cultures were negative. No new medications had been initiated before symptom onset. Moreover, venous ultrasonography showed no evidence of thrombosis. Non-contrast computed tomography (CT) demonstrated subcutaneous edema of the extremities and bursal fluid accumulation around the left shoulder joint. Fluorodeoxyglucose positron-emission tomography/CT (FDG-PET/CT) revealed increased FDG uptake at several periarticular and bursal sites (shoulders, wrists, lumbar interspinous regions, hips, ischial tuberosities, knees, and ankles), in a distribution consistent with PMR (Figure 1C–G). No evidence of malignancy was identified. PMR was diagnosed based on clinical presentation, increased inflammatory markers, and characteristic imaging findings. Oral prednisolone (15 mg/day) was initiated, leading to pain improvement on the following day and normalization of CRP level. She was discharged on hospital Day 15.

The second patient, an 81-year-old man with a history of Type 2 diabetes mellitus and chronic obstructive pulmonary disease, developed shoulder pain 3 months before admission, followed by pitting edema of the dorsal aspects of both hands and feet and persistently elevated inflammatory markers 2 months later. Initially, remitting seronegative symmetrical synovitis with

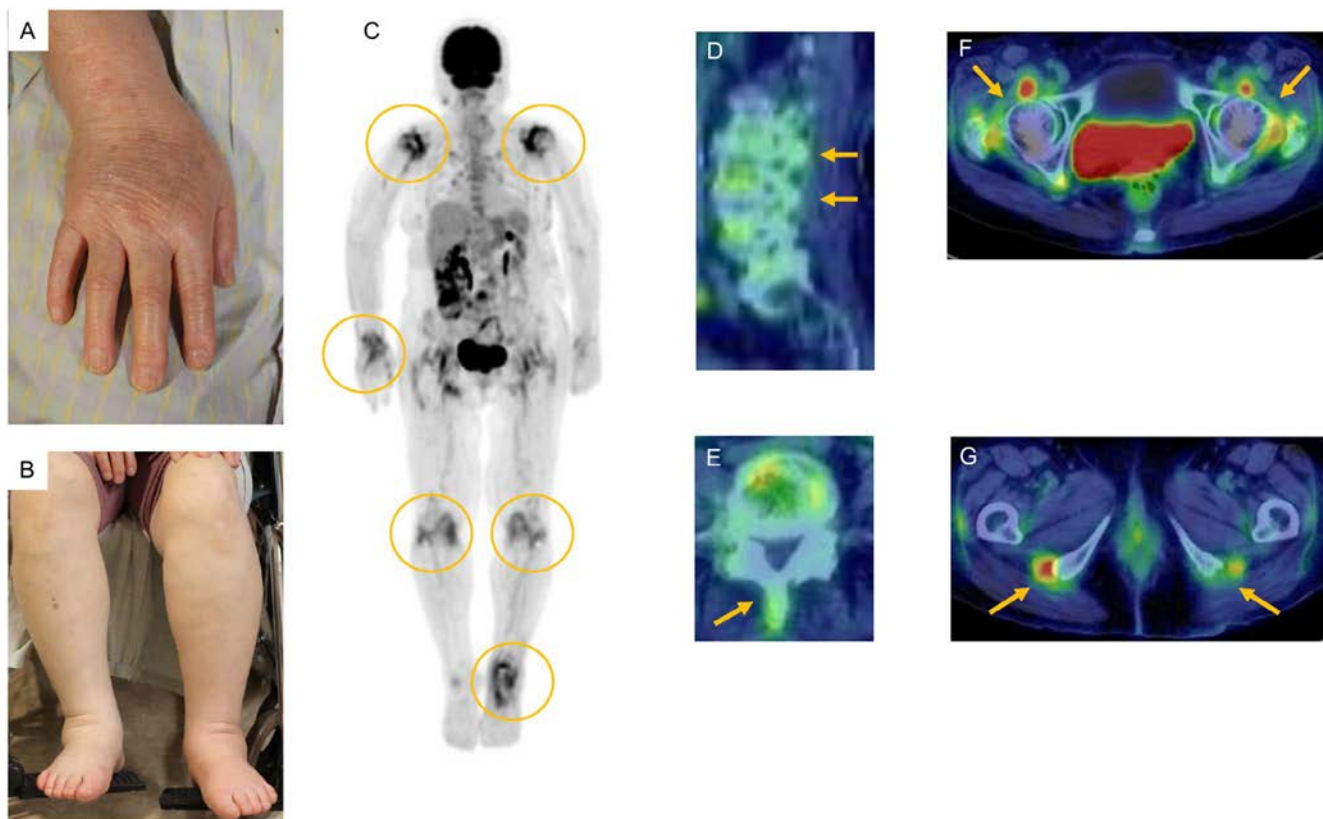


FIGURE 1 | (A, B) Patient's presentation at initial examination. Pitting edema involved all four extremities and was more pronounced in the right upper and left lower extremities. (C) Whole-body maximum-intensity-projection FDG-PET image showing increased FDG uptake in both shoulders and hip regions. (D, E) Sagittal and axial FDG-PET/CT images showing increased FDG uptake in the lumbar interspinous region. (F, G) Axial FDG-PET/CT images showing increased FDG uptake in greater trochanteric regions (F) and ischial tuberosities (G). Arrows indicate sites of increased FDG uptake. CT, computed tomography; FDG-PET, fluorodeoxyglucose positron-emission tomography.

pitting edema (RS3PE) syndrome was suspected. He was referred to our hospital for further evaluation. On admission, he was afebrile and hemodynamically stable. Physical examination revealed pitting edema of the dorsal aspects of both hands and feet, and pain and swelling involving the fingers, wrists, shoulders, and toes. Laboratory tests showed increased CRP (8.46 mg/dL), ESR (78 mm/h), MMP-3 (293.4 ng/mL), and VEGF levels (850.7 pg/mL). RF, anti-CCP antibodies, ANA, MPO-ANCA, and PR3-ANCA were negative. Thyroid function was normal, and blood cultures yielded no growth. Ultrasonography demonstrated dorsal subcutaneous edema. Magnetic resonance imaging (MRI) of the right hand showed dorsal subcutaneous edema on short tau inversion recovery images and extensor and flexor tenosynovitis on contrast-enhanced T1-weighted images (Figure 2A,B). Although these findings were compatible with RS3PE syndrome, shoulder ultrasonography revealed bilateral biceps tenosynovitis, raising the possibility of PMR. Contrast-enhanced CT revealed multifocal bursitis around both shoulders and ischial tuberosities, with additional enhancement around the pubic symphysis, further supporting PMR diagnosis. During hospitalization, the patient developed right temporomandibular joint (TMJ) pain and mild tenderness over temporal arteries, with slight prominence of the right artery. Temporal artery ultrasonography showed findings suggestive of arteritis. Contrast-enhanced MRI revealed bilateral superficial temporal artery wall enhancement, consistent with temporal arteritis (Figure 2C). Bilateral TMJ enhancement (more prominent on

the right) was also observed and consistent with TMJ arthritis (Figure 2D). Ophthalmologic examination demonstrated no abnormalities. Based on these findings, PMR with concomitant GCA was diagnosed. Oral prednisolone (30 mg/day) was initiated, resulting in rapid improvement of hand and foot edema and joint pain, together with normalization of CRP level. He was discharged on hospital Day 18.

Distal pitting edema is an uncommon PMR manifestation (1.1%–12%) [2–4]. Although it has been less well characterized in GCA, one population-based cohort reported this finding in 10% of patients [5]. Extensive tenosynovial inflammation is thought to cause diffuse swelling and consequent pitting edema [3, 5]. RS3PE syndrome was originally described as acute-onset, seronegative symmetrical synovitis in older adults, accompanied by marked pitting edema of the hands or feet [6]. In a prospective study comparing 21 patients with PMR-associated distal pitting edema and 23 with pure RS3PE, similar age at onset, acute-phase reactant levels, peripheral synovitis, and glucocorticoid responsiveness were found; MRI also suggested shared involvement of extra-articular synovial structures [7]. Therefore, PMR with pitting edema has been proposed as an intermediate phenotype between typical proximal PMR and pure RS3PE with prominent distal manifestations.

In both patients, prominent pitting edema initially suggested RS3PE syndrome. In Case 1, FDG-PET/CT revealed uptake at

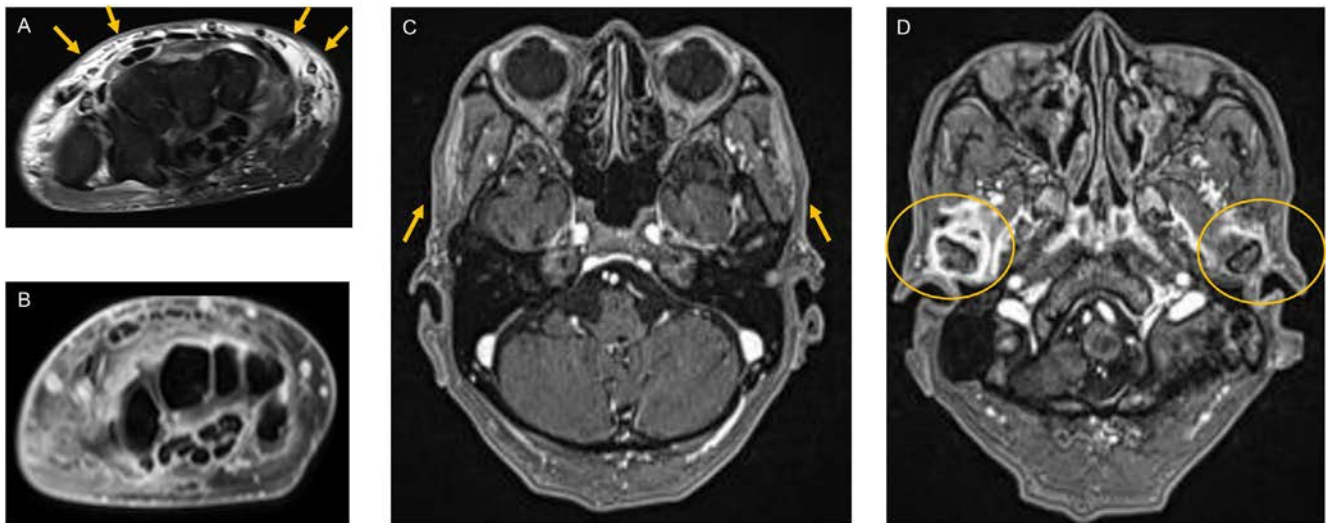


FIGURE 2 | (A) Short tau inversion recovery image of the right hand showing marked dorsal subcutaneous edema (arrows). (B) Contrast-enhanced T1-weighted image of the right hand showing extensor and flexor tenosynovitis. (C) Contrast-enhanced T1-weighted image showing bilateral superficial temporal artery wall enhancement, consistent with temporal arteritis. (D) Contrast-enhanced T1-weighted image showing enhancement around temporomandibular joints (more prominent on the right), consistent with temporomandibular joint arthritis.

characteristic PMR sites. FDG uptake at two or more ischial tuberosities, greater trochanters, and lumbar spinous processes identified active PMR with a sensitivity and specificity of 85.7% and 88.2%, respectively [8]. The presence of this characteristic uptake pattern supported the diagnosis in Case 1. Although serum VEGF levels were elevated in both patients, this finding provided limited diagnostic discrimination because VEGF can also be elevated in PMR, GCA, and RA [9]. In Case 2, hand MRI initially suggested RS3PE syndrome. However, shoulder ultrasonography and contrast-enhanced CT demonstrated multifocal bursitis in a distribution consistent with PMR. Furthermore, the patient developed new-onset TMJ pain during hospitalization, a known GCA manifestation [10, 11]. Although temporal artery tenderness and prominence were subtle, the overall clinical presentation and contrast-enhanced MRI findings led to a GCA diagnosis.

Thus, prominent distal pitting edema may represent a peripheral phenotype within the GCA–PMR spectrum. Recognizing this presentation and integrating multiple imaging modalities may facilitate identification of the underlying disease and appropriate treatment selection.

Author Contributions

H.Y. was involved in the clinical care of the Patient 1, whereas M.W., H.Y., K.K., and H.K. were involved in the clinical care of the Patient 2. M.W. and H.Y. contributed to the interpretation of the clinical courses of both cases. M.W. drafted the manuscript, and the other authors critically revised it for important intellectual content. M.W. and H.Y. were responsible for the selection and preparation of the clinical images. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work, ensuring that any questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All listed authors meet the ICMJE criteria for authorship.

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Ethics Statement

All procedures were conducted in accordance with the ethical principles enshrined in the Declaration of Helsinki.

Consent

Written informed consent was obtained from the patients for the publication of this case report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available from the corresponding author upon request.

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EDITORIAL

From Vasospasm to Precision Microvascular Medicine: Rethinking the Evaluation and Management of Raynaud's Phenomenon

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Raynaud's phenomenon (RP) has long been regarded as a benign vasospastic disorder characterized by episodic digital color changes triggered by cold exposure or emotional stress. However, accumulating evidence has fundamentally changed this perception. In patients with connective tissue diseases, particularly systemic sclerosis (SSc), RP represents far more than transient vasospasm. It is often the earliest manifestation of progressive microvascular injury and serves as a window into the pathobiology of systemic vascular disease. The challenge for clinicians is no longer merely recognizing RP, but identifying which patients are at risk for irreversible vascular damage, digital ulceration, and systemic complications.

Recent advances presented at the American College of Rheumatology Convergence and reflected in the updated British Society for Rheumatology (BSR) recommendations illustrate a paradigm shift from symptom-oriented management toward precision assessment of the microcirculation. One of the most significant developments has been the increasing role of nailfold capillaroscopy. Once considered a specialized research technique, capillaroscopy is rapidly becoming an essential component of routine rheumatology practice [1]. It enables direct visualization of the microvasculature, allowing clinicians to distinguish primary RP from secondary RP associated with systemic sclerosis and other connective tissue diseases. Beyond diagnosis, capillaroscopic findings such as giant capillaries, capillary loss, abnormal angiogenesis, and microhemorrhages provide valuable prognostic information regarding disease progression and digital vascular complications. The challenge is therefore no longer whether capillaroscopy should be performed, but how it can be implemented efficiently in busy

clinical settings. Technological advances are making this transition increasingly feasible. Table 1 is comparison of Nailfold Microvascular Assessment Techniques in Systemic Sclerosis. Clinical interpretation of imaging modalities. Nailfold videocapillaroscopy currently provides the most established and standardized approach for assessing SSc-associated nailfold microangiopathy. Dermatoscopy and smartphone-based imaging offer advantages in portability and accessibility, but their broader clinical application is limited by differences in image quality, acquisition protocols, and device performance. AI-assisted analysis represents a promising approach for automated recognition and quantification of capillary abnormalities; however, current evidence should be distinguished from future applications involving individual-level prediction of disease progression or vascular complications. Accordingly, these technologies should presently be viewed as complementary approaches rather than interchangeable alternatives, with their clinical utility ultimately requiring prospective validation and demonstration of improved patient outcomes (Table 2).

Conventional videocapillaroscopy remains the reference standard but requires dedicated equipment, expertise, and considerable examination time [2]. In contrast, portable dermatoscopes and smartphone-based imaging systems have emerged as practical alternatives that substantially reduce costs while improving accessibility. Although these devices currently have lower image resolution and limited quantitative capabilities, they may be sufficient for clinical screening when combined with standardized interpretation algorithms. This democratization of capillaroscopy has the potential to extend microvascular assessment beyond tertiary referral centers to community

TABLE 1 | Comparison of nailfold capillaroscopic imaging techniques.

Technique	Image resolution	Cost	Portability	Training required	Reproducibility	Current clinical role
Conventional video-capillaroscopy	High	High (dedicated equipment)	Low	Substantial	High, with standardized protocol	Reference standard for specialist diagnostic and prognostic assessment
Dermatoscopy	Moderate	Low-moderate	Moderate	Moderate	Moderate; reasonable agreement with videocapillaroscopy for pattern recognition	Accessible alternative for screening in general rheumatology or primary care
Smartphone-based/portable imaging	Lower	Low	High	Minimal-moderate	Variable; improves with standardized acquisition protocols	Emerging screening/monitoring adjunct; not yet a substitute for videocapillaroscopy

rheumatology practices. Artificial intelligence (AI) may further accelerate this transformation. Machine learning algorithms are increasingly capable of recognizing scleroderma-specific capillary patterns, identifying giant capillaries, hemorrhages, abnormal morphology, and capillary density automatically. Automated image analysis offers several advantages, including improved reproducibility, reduced observer variability, and shortened interpretation time [3]. Importantly, AI is unlikely to replace expert clinicians but rather to function as a clinical decision support tool that facilitates early identification of patients requiring specialist evaluation. As prospective validation studies mature, AI-assisted capillaroscopy may become an integral component of precision rheumatology. Equally important is the growing recognition that vascular disease should not be assessed solely through imaging. Clinical trials in systemic sclerosis have increasingly emphasized patient-reported outcomes (PROs), acknowledging that symptom burden often differs substantially from physician assessment. Raynaud's attacks affect not only pain and hand function but also emotional well-being, occupational performance, and quality of life. Instruments such as the Assessment of Systemic Sclerosis-associated Raynaud's Phenomenon (ASRAP) questionnaire represent important progress toward standardized measurement of patient experience [4]. Integrating objective capillaroscopic findings with validated PROs provides a more comprehensive understanding of disease activity and therapeutic response than either measure alone. Digital ulcers remain one of the most devastating complications of systemic sclerosis-associated RP. They are associated with chronic pain, infection, impaired hand function, work disability, and reduced quality of life. Importantly, digital ulcers reflect progressive obliterative vasculopathy rather than isolated ischemic episodes. Their management therefore requires a multidisciplinary approach that extends beyond vasodilator therapy. Recent BSR guidelines appropriately recommend calcium channel blockers as first-line therapy for RP, with phosphodiesterase-5 inhibitors and intravenous prostanoids reserved for refractory disease [5]. Bosentan remains an important option for prevention of recurrent digital ulcers, while surgical sympathectomy and botulinum toxin injection may be considered in carefully selected patients with severe refractory ischemia. These recommendations reinforce the importance of individualized, stepwise treatment strategies rather than a one-size-fits-all approach. Perhaps the most important conceptual advance is the recognition that persistent or refractory digital ischemia should prompt clinicians to search for alternative pathogenic mechanisms. Not all digital ulcers result solely from vasospasm or structural vasculopathy. Superimposed inflammatory vasculitis, antiphospholipid syndrome, vaso-occlusive disease, calcinosis, or infection may significantly influence management decisions. Cases presented in recent educational sessions demonstrate that targeted immunosuppression or anticoagulation may be required when inflammatory or thrombotic mechanisms coexist. Such observations highlight the importance of individualized etiologic assessment before escalating vasodilator therapy alone. Future research should move beyond isolated biomarkers toward integrated digital vascular phenotyping. Combining capillaroscopic imaging, thermal imaging, patient-reported outcomes, laboratory biomarkers, wearable technologies, and artificial intelligence offers an opportunity to construct comprehensive digital vascular profiles. Such multidimensional assessment could facilitate earlier diagnosis, improve

TABLE 2 | Comparison of Nailfold microvascular assessment techniques in systemic sclerosis.

Technique	Main characteristics	Advantages	Limitations	Reproducibility/ Standardization	Current clinical role
Nailfold videocapillaroscopy (NVC)	High-magnification digital imaging of nailfold capillaries; allows visualization and documentation of capillary morphology and density	High-resolution imaging; enables detailed assessment of capillary enlargement, giant capillaries, hemorrhages, capillary loss, and neoangiogenesis; images can be stored for follow-up and quantitative analysis	Requires dedicated equipment and trained personnel; acquisition and interpretation may vary among operators; relatively higher cost and limited availability in some settings	Relatively well established, but affected by acquisition technique, image quality, scoring systems, and observer expertise	Established clinical assessment tool, particularly for evaluation of suspected SSC and characterization of SSC-associated microangiopathy
Dermatoscopy	Portable optical imaging using a dermatoscope, generally with lower magnification than conventional NVC	Simple, rapid, portable, relatively inexpensive; may facilitate assessment in routine outpatient practice	Lower magnification and potentially lower image resolution; less standardized acquisition; quantitative assessment may be more difficult; operator and device variability	Variable; depends on device, magnification, image quality, and examiner experience	Clinical adjunct/ screening approach where conventional NVC is unavailable or impractical
Smartphone-based imaging	Smartphone camera combined with magnification or optical attachments for nailfold imaging	Highly portable; inexpensive; potentially scalable; facilitates image sharing, telemedicine, longitudinal monitoring, and remote assessment	Device heterogeneity; variable optical quality, illumination, magnification, and positioning; lack of standardized acquisition protocols; diagnostic and prognostic performance requires further validation	Currently variable and incompletely standardized; cross-device comparability remains an important limitation	Emerging technology with potential for screening, monitoring, and tele-rheumatology; not yet a replacement for standardized NVC
AI-assisted capillaroscopy	Automated or semi-automated image analysis using machine-learning/ deep-learning algorithms	Potential to reduce observer dependence; automated detection and quantification of capillary abnormalities; may facilitate large-scale image analysis and longitudinal monitoring	Dataset size and representativeness; annotation quality; device and image heterogeneity; algorithm generalizability; external validation; limited prospective evidence demonstrating improved clinical outcomes	Investigational; requires standardized datasets, external validation, and prospective clinical validation	Emerging research tool; automated classification is promising, whereas prediction of SSC development, digital ulcers, or internal-organ involvement remains investigational

risk stratification, predict digital ulcer development, and guide personalized therapeutic interventions. In parallel, longitudinal observational studies are needed to determine how dynamic microvascular changes correlate with clinical outcomes and treatment response. The emergence of digital health technologies further expands these possibilities. Smartphone applications capable of capturing serial ulcer photographs or monitoring digital perfusion remotely may facilitate decentralized clinical trials and continuous patient monitoring. Integration of cloud-based image analysis with AI-assisted interpretation may allow community physicians to obtain expert-level support without requiring referral to specialized centers. These innovations align closely with the broader movement toward precision medicine and tele-rheumatology.

In conclusion, Raynaud's phenomenon should no longer be viewed simply as episodic vasospasm. Instead, it represents an accessible clinical model of systemic microvascular disease and an opportunity for early intervention before irreversible tissue injury occurs. Advances in capillaroscopy, artificial intelligence, patient-reported outcomes, and evidence-based therapeutic strategies are collectively reshaping the clinical management of RP and systemic sclerosis. The future lies not merely in treating vasospasm but in integrating multimodal vascular assessment with precision therapeutics to improve long-term outcomes. As these technologies become increasingly accessible, the transition from descriptive vascular assessment to quantitative precision microvascular medicine appears not only possible but inevitable.

1 | Conclusion

Raynaud's phenomenon is an important clinical manifestation of systemic sclerosis-associated vascular disease, but its interpretation requires careful distinction between primary Raynaud's phenomenon, SSc-spectrum disease, and established systemic sclerosis. Nailfold capillaroscopy provides valuable objective information on microvascular structure and can contribute to the characterization and risk stratification of patients with suspected or established systemic sclerosis. However, capillaroscopic abnormalities should not be interpreted as a stand-alone predictor of individual clinical outcomes. Their prognostic significance is best considered in conjunction with clinical manifestations, disease characteristics, and SSc-specific autoantibodies. Patient-reported outcomes provide complementary information on symptom burden, functional impact, quality of life, and treatment response that cannot be fully captured by vascular imaging alone. Similarly, digital ulcers should be regarded as multifactorial vascular complications in which structural vasculopathy may coexist with vasospasm, thrombosis, calcinosis, trauma, infection, and other inflammatory or thrombotic mechanisms. Emerging technologies, including dermatoscopy, smartphone-based imaging, and artificial intelligence-assisted image analysis, may improve accessibility, quantitative assessment, longitudinal monitoring, and integration of microvascular data. Nevertheless, these approaches remain at different stages of validation. In particular, automated recognition of capillary abnormalities should be distinguished from prediction of clinically meaningful outcomes such as progression to systemic sclerosis, development of digital ulcers, or

internal-organ involvement. Larger and more representative datasets, standardized image acquisition and scoring systems, external validation, and prospective studies demonstrating clinical utility are needed before these technologies can be incorporated routinely into clinical decision-making. The future of SSc-associated vascular assessment is therefore likely to involve complementary integration of clinical evaluation, nailfold imaging, patient-reported outcomes, serological characteristics, and emerging digital technologies. Rather than replacing established clinical assessment, these approaches may progressively enhance individualized risk assessment and longitudinal monitoring. A transition toward more quantitative and multimodal microvascular assessment is promising, but its clinical value should ultimately be determined by prospective validation and demonstrable improvements in patient care.

Author Contributions

Chieh Chen: resources, data curation, writing – review and editing, visualization, supervision, project administration. **Jeffrey Liao:** data curation, writing – review and editing. **Su-Boon Yong:** resources, data curation, writing – review and editing, project administration. All authors have read and agreed to the final version of the manuscript. No generative artificial intelligence or artificial intelligence-assisted technology was used in the writing process, and the authors bear full responsibility for the content of their manuscripts.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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LETTER TO THE EDITOR

Case Report: Successful Treatment of Extensive Calcinosis With Intralesional Sodium Thiosulfate in Juvenile Dermatomyositis

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Dear Editor,

Calcinosis is a major source of morbidity in juvenile dermatomyositis (JDM), affecting up to approximately 40% of patients [1]. Despite corticosteroids, immunomodulatory therapies, intravenous immunoglobulin, and rehabilitation, calcinosis may develop and, once established, lacks a consistently effective treatment [1, 2]. Sodium thiosulfate (STS) has been explored for dermatomyositis-associated calcinosis, primarily through intravenous or topical administration [2–5]. Here, we describe clinical and radiographic improvement in a child with extensive JDM-associated calcinosis after a single session of intralesional STS administered as part of multimodal therapy.

A 5-year-old Nigerian girl presented in the summer of 2024, designated Week 0, for a second opinion regarding JDM complicated by severe calcinosis, pain, and progressive loss of ambulation. Based on the limited available history, she had been diagnosed approximately 1 year earlier in her home country and had received corticosteroid monotherapy with irregular follow-up and variable adherence. At presentation, vital signs and growth parameters were normal, with height and weight at the 75th and 50th percentiles for age, respectively. She appeared chronically ill, with marked muscle wasting and fixed flexion contractures of the hips, knees, and ankles, without arthritis. Extensive calcinosis involving the thighs and calves was firm and painful to palpation. Severe contractures limited manual muscle testing, the Childhood Myositis Assessment Scale, and the Childhood Health Assessment Questionnaire because of her young age and restricted range of motion. She had no head lag

and retained upper-extremity function but required assistance with sitting, standing, and walking. Skin examination showed no rash or ulceration. Cardiorespiratory, gastrointestinal, and neurologic examinations were otherwise unremarkable.

Laboratory evaluation showed a normal complete blood count and normal inflammatory markers, including erythrocyte sedimentation rate and C-reactive protein. Muscle enzyme levels were normal except for mildly elevated creatine phosphokinase at 341 U/L (reference range, 30–200 U/L). Antinuclear antibody was positive at low titer; myositis-specific and myositis-associated autoantibodies, anti-double-stranded DNA antibody, anti-extractable nuclear antigen antibodies, and antiphospholipid antibodies were negative. Radiographs and magnetic resonance imaging demonstrated extensive soft-tissue calcifications involving the posterior thighs, popliteal fossae, and anterior right calf (Figure 1). Magnetic resonance imaging showed no evidence of active myositis, and chest radiography was normal. The normal inflammatory markers and absence of MRI evidence of active myositis at visit do not exclude previously treated, active, or currently inactive JDM and were interpreted in the context of her prior diagnosis, marked muscle wasting, fixed contractures, and extensive calcinosis. The clinical and laboratory pattern of weakness and elevated CK raise a strong argument for a rigorous diagnosis of JDM and was considered most consistent with JDM-associated dystrophic calcinosis. Normal inflammatory markers in partially treated JDM are relatively common [6, 7]. Other categories of calcinosis, including metastatic, idiopathic, iatrogenic, and calciphylaxis-related calcification, were

considered less likely based on the clinical context and available laboratory findings.

At week 1, she received intravenous methylprednisolone, 250 mg/day (16 mg/kg/dose), for 3 consecutive days, followed by oral prednisolone, 30 mg/day (2 mg/kg/day), for 1 month, with gradual tapering to 5 mg/day by week 12. She was also started on intravenous immunoglobulin, 2 g/kg per dose, administered every 2 weeks during the first month and monthly thereafter, together with mycophenolate mofetil, 250 mg/day (350 mg/m² per dose), calcium, and vitamin D supplementation. The elevated creatine kinase was suggestive of ongoing active disease along with the activity of the patient's calcinosis on exam. Given her progressive functional loss and the limited treatment options for established calcinosis, the decision to add STS was made after multidisciplinary discussion and shared decision-making with the family.

At week 4, she underwent outpatient ultrasound-guided intral-
esional STS administration under deep sedation. Ultrasound
guidance allowed the medication to be injected inside the areas
of dense calcification by accessing them from various angles
through non-calcified regions or where the calcification was

thin. The calcific deposits were visible on imaging and suf-
ficiently accessible for targeted injection. A total of 3 g, cor-
responding to 12 mL of a 25% STS solution diluted with normal
saline, was injected in 0.5-mL aliquots circumferentially within
the calcific lesions. The procedure was tolerated well, and no
metabolic, renal, or urinary adverse effects were observed.
Post-treatment pain was minimal and responded to oral acet-
aminophen for a few days. She subsequently completed 1 year of
structured physical rehabilitation.

Over 2 years of follow-up, she remained on low-dose corticoste-
roids, mycophenolate mofetil, and monthly intravenous immu-
noglobulin. She demonstrated marked clinical improvement,
with resolution of contractures and normalization of range of
motion sufficient to permit independent ambulation, running,
and jumping. At the first follow-up visit 3 months after treatment
initiation, her pain had decreased and mobility had improved.
Serial imaging showed progressive radiographic regression of
calcinosis (Figure 2), and follow-up laboratory studies remained
normal.

The immunopathogenesis of dystrophic calcinosis in JDM is
complex and treatment remains challenging [1, 2]. Current

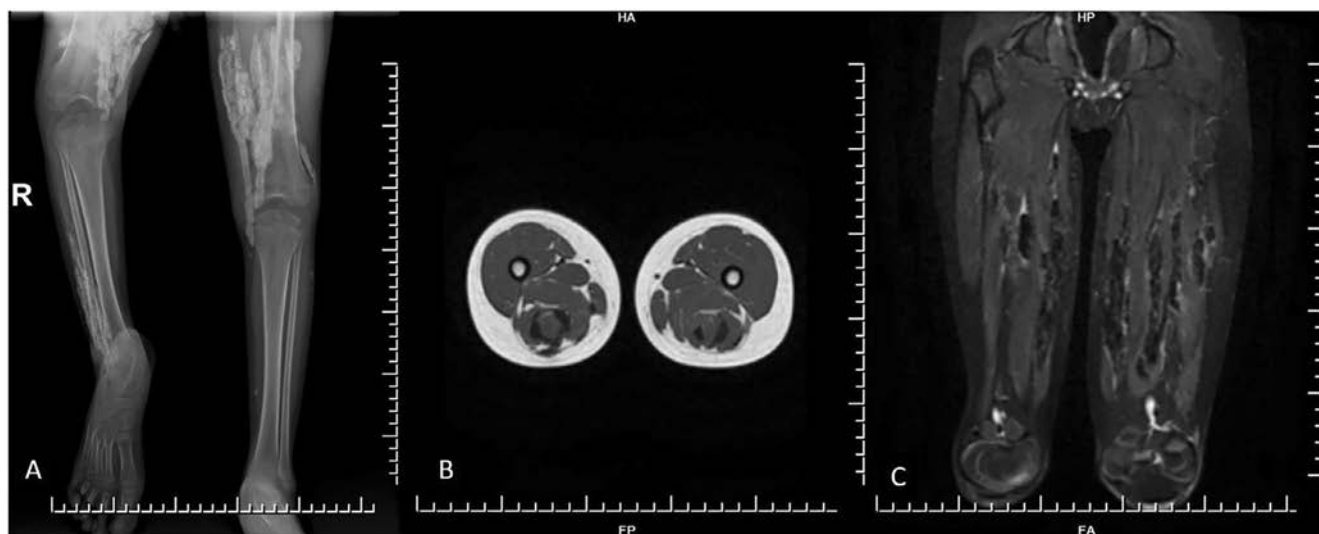


FIGURE 1 | X-ray of thighs and legs before STS injection (A) show prominent, symmetrical, dystrophic calcifications which are more abundant in bilateral posterior thigh regions. In axial T1W (B) and coronal T2W STIR (C) MRI images, calcifications in bilateral semimembranosus, semiten-
dinosus and biceps femoris muscles appear as hypointense lesions with no prominent perimuscular edema.

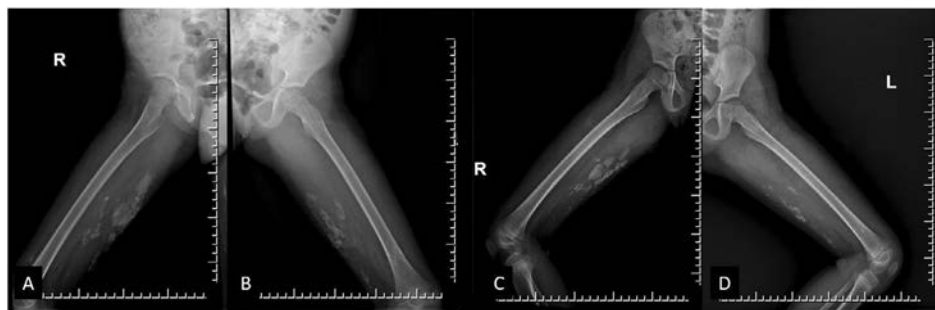


FIGURE 2 | X-rays of the thighs after 8 months (A, B) and 12 months (C, D) after STS injection show gradual and prominent resolution of calcinosis.

evidence supports a multifactorial process in which chronic inflammation, oxidative stress, mitochondrial injury, and impaired tissue clearance may promote hydroxyapatite deposition in affected soft tissues, often despite normal systemic calcium and phosphate homeostasis [1, 2, 8]. In this patient, the clinical presentation of elevated muscle enzymes and weakness that are steroid responsive and the pattern of calcinosis was considered consistent with JDM-associated dystrophic calcinosis despite normal inflammatory markers and no evidence of active myositis on both lower extremities MRI. Because the calcinosis was extensive, painful, and associated with progressive loss of ambulation, multimodal treatment was initiated after multidisciplinary discussion and with the family's approval. A reduced-intensity regimen of corticosteroids, mycophenolate mofetil, and intravenous immunoglobulin was used to minimize infection risk while addressing possible residual tissue inflammation. The subsequent improvement may reflect the combined effects of immunomodulatory therapy, rehabilitation, and intralesional STS; the specific contribution of STS cannot be determined.

STS is of interest because its potential therapeutic effects may extend beyond calcium chelation and increased calcium salt solubility to modulation of the local pro-calcific tissue environment. Experimental data suggest that thiosulfate may limit oxidative tissue injury by scavenging reactive oxygen species, reducing hydrogen peroxide, preserving endogenous antioxidant defenses, and participating in hydrogen sulfide-related sulfur signaling and protein persulfidation [8, 9]. STS also intersects with mitochondrial sulfur metabolism pathways involved in redox homeostasis. In endothelial cells, STS has been reported to inhibit mitochondrial respiration, promote a compensatory glycolytic shift, and stimulate proliferation and neovascularization [8, 9]. These findings provide biologic hypotheses for a possible local effect on chronically injured tissue, but their relevance to JDM-associated calcinosis and intralesional treatment in humans remains uncertain.

Intralesional injection can cause severe pain. In this patient, administration under deep sedation allowed targeted treatment of symptomatic lesions. No metabolic, renal, or urinary adverse effects were observed. Sodium thiosulfate has established clinical uses, including use with sodium nitrite for acute life-threatening cyanide poisoning and use to reduce cisplatin-associated ototoxicity in pediatric patients with localized, non-metastatic solid tumors [10]. It has also been used off-label for calciphylaxis and other disorders of pathologic calcification [11]. These uses provide clinical context but do not establish the safety or efficacy of intralesional STS for JDM-associated calcinosis. Targeted injection may be considered for anatomically accessible lesions when systemic exposure is undesirable, but additional clinical study is needed [4, 5, 11, 12].

This report is limited by its single-patient design and by the inability to isolate the specific contribution of intralesional STS from concurrent immunomodulatory therapy and rehabilitation. Spontaneous regression cannot be excluded. Nevertheless, calcinosis had progressed with loss of mobility before referral, pain and mobility improved within 3 months of treatment initiation, and radiographs showed continued regression during follow-up. These observations suggest that intralesional STS

may be a feasible adjunctive option, but they do not establish treatment efficacy.

Author Contributions

This report is limited by its single-patient design and by the inability to isolate the specific contribution of authors.

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Disclosure

The views expressed in this article are those of the author and do not reflect the official policy of the Department of War, or U.S. Government.

Conflicts of Interest

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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EDITORIAL

Pneumocystis jirovecii Pneumonia Prophylaxis in Rheumatic Disease: The Limits of Borrowed Thresholds

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Pneumocystis jirovecii pneumonia (PJP) is a serious opportunistic infection associated with substantial morbidity and mortality in patients with rheumatic diseases [1]. However, unlike HIV, oncology, and transplant populations, rheumatology lacks formal guidelines to inform PJP prophylaxis. As a result, prophylaxis decisions in rheumatology have often borrowed risk thresholds from other populations. Recent reviews have synthesized who is at risk and who may benefit from prophylaxis in rheumatic disease [2, 3]. Less attention has been paid to where commonly used prophylaxis thresholds originated and whether they remain valid when applied to rheumatic populations.

1 | HIV: CD4 Count Threshold

Prior to widespread use of both antiretroviral therapy (ART) and PJP prophylaxis, PJP occurred in 70%–80% of people living with HIV (PWH), with mortality rates of 20%–40% [4]. Early cohort studies in PWH demonstrated that PJP risk rises substantially when CD4 counts fall below 200 cells/mm³, while infection is unlikely to develop in PWH with CD4 counts > 250 cells/mm³ [5]. These data in PWH established the threshold for primary prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMX), and current guidelines recommend prophylaxis when CD4 counts are <200 cells/mm³ or when CD4 percentage is <15% [4, 6]. The CD4 threshold in PWH is based on a well-established relationship between a single, predictive marker of T-cell immunodeficiency and PJP risk.

2 | Oncology: Risk and Treatment Exposure Threshold

American Society of Clinical Oncology (ASCO) and Infectious Diseases Society of America (IDSA) recommendations support

PJP prophylaxis when a chemotherapy regimen is associated with a PJP risk exceeding approximately 3.5% [7]. Prolonged glucocorticoid exposure, commonly defined as prednisone $\geq$ 20 mg/day for $\geq$ 4 weeks, is frequently used as a clinical trigger for prophylaxis [7]. This risk percentage originated from a meta-analysis that determined the risk-benefit breakeven point for starting PJP prophylaxis, which found that TMP/SMX reduced PJP occurrence by 91% in non-HIV immunocompromised patients, and adverse events requiring discontinuation occurred in 3.1% of adults [8].

The threshold of $\geq$ 20 mg of prednisone used to define immunocompromise warranting PJP prophylaxis is more a matter of expert opinion rather than established through prospective studies [9]. This threshold is a pragmatic approach to guide care and is generally considered in patients who have additional causes of immunodeficiency [10].

3 | Transplant: Time and Net Immunosuppression

Universal or near-universal PJP prophylaxis is recommended for all solid organ transplant (SOT) and allogeneic HSCT recipients, reflecting a predictable period of intense immunosuppression after a transplant event [11]. Prophylaxis is typically continued for 6–12 months, when immunosuppression is greatest, with the duration of prophylaxis varying by transplant type [11]. Prophylaxis may be continued or reinitiated in the setting of additional risk factors such as acute rejection, cytomegalovirus infection, or other intensified immunosuppressive therapy [11].

Existing guidelines in populations outside of rheumatology reflect different approaches to prophylaxis in

immunocompromised populations. The decision to start PJP prophylaxis in PWH follows a clear CD4 count threshold, while oncologic and transplant patients receive prophylaxis based on length of immunosuppression and with consideration of specific medical conditions.

Rheumatic diseases do not neatly reproduce existing risk structures. Immune dysfunction reflects a dynamic combination of disease, treatment, and host susceptibility, raising the question of whether thresholds derived from other populations remain valid when separated from the populations in which they were developed.

4 | What Can and Cannot Be Applied to Rheumatology

4.1 | CD4 Count

Though CD4 counts are widely used to guide PJP prophylaxis outside of rheumatic conditions, this metric has limited reliability in autoimmune diseases. Fifty percent of autoimmune disease patients who develop PJP have CD4 counts $> 300/\mu\text{L}$, with a median of $302/\mu\text{L}$ compared to $19/\mu\text{L}$ in PWH [12]. Using CD4 counts $< 200/\mu\text{L}$ as a marker of increased PJP risk in non-HIV patients, a threshold identified in 73% of non-HIV cases, would miss approximately one-quarter of rheumatic disease cases [13]. Thus, although lymphocyte depletion remains biologically relevant, the HIV-derived CD4 threshold does not function reliably as a stand-alone prophylaxis threshold in rheumatic disease.

4.2 | Glucocorticoid Exposure

Glucocorticoid exposure is a well-established risk factor for PJP in patients with rheumatic diseases, with risk increasing with dose and duration. However, whether a specific glucocorticoid dose can serve as a universal threshold for prophylaxis is less clear. Although prednisone $\geq 20\text{ mg/day}$ for at least four weeks is commonly used as a clinical trigger for risk assessment, rheumatology-specific studies have evaluated thresholds ranging from 15 to 30 mg/day , without identifying a single cutoff that reliably distinguishes patients who should receive prophylaxis [2].

The limitations of a fixed threshold are apparent when comparing rheumatic diseases. Patients with ANCA-associated vasculitis (AAV) and those with giant cell arteritis (GCA) may both receive prolonged high-dose glucocorticoids. PJP occurs substantially more frequently in AAV, particularly during induction therapy, while remaining rare in GCA [1, 14, 15]. Conversely, clinically meaningful PJP risk can occur below the conventional prednisone 20 mg/day threshold when glucocorticoids are combined with additional risk factors. Patients receiving low- or medium-dose glucocorticoids have demonstrated increased risk in the presence of factors such as lymphopenia, glucocorticoid pulse therapy, or concomitant immunosuppression [16].

Thus, glucocorticoid exposure clearly translates as a risk factor for PJP, but a specific dose threshold does not necessarily translate as a decision threshold for prophylaxis. Glucocorticoid dose

and duration may be better used as triggers for assessment of the patient's broader disease, treatment, and host-related risk rather than as independent indications for prophylaxis.

4.3 | Time-Based Immunosuppression

The duration of PJP prophylaxis in transplant recipients can be guided by time-based criteria, and post-transplant SOT recipients commonly receive prophylaxis for at least 6–12 months [11]. However, rheumatologic immunosuppression fluctuates, and prophylaxis duration should instead be individualized based on corticosteroid dose and degree of immunosuppression rather than by a fixed time interval. Recent observational data suggest that tapering prednisone to approximately 12.5 mg/day may represent a reasonable point at which discontinuation can be considered, but this has not been prospectively validated as a stopping threshold [17]. Other factors to consider include concomitant immunosuppressive use and induction therapy [18].

4.4 | What Translates to Rheumatology: Net Immunosuppression

In rheumatology, PJP risk is better understood through the patient's net immunosuppression rather than a single threshold. Rather than starting prophylaxis based on thresholds such as CD4 count or prednisone dosage $\geq 20\text{ mg/day}$, clinicians should consider a patient's overall immunosuppressive regimen, including factors that may increase risk. This holistic view reflects the cumulative effects of underlying disease, corticosteroid exposure, concomitant immunosuppressive therapies, lymphopenia, pulmonary disease, and other host factors [3, 19, 20].

These additional risk factors are dynamic and can be cumulative. Lymphopenia, particularly with absolute lymphocyte counts $< 500\text{--}700\text{ cells}/\mu\text{L}$, has been associated with increased PJP risk [19]. Other host factors that contribute to higher PJP risk include chronic pulmonary comorbidities, especially interstitial lung disease (ILD). In patients with rheumatic disease and pre-existing pulmonary diseases or ILD, there are higher odds of developing PJP [19]. Older age is both a risk factor and a prognosticator of worse outcomes in PJP for patients with rheumatic diseases, with the median age at diagnosis in non-survivors being 74 years versus 64 years in survivors [20].

Experience from HIV, oncology, and transplant populations provides rheumatologists with valuable principles for PJP prevention, but not directly transferable thresholds. The CD4 count, corticosteroid exposure, and time since initiation of immunosuppression remain informative, but their numerical cutoffs do not reliably account for the differences in risk across rheumatic populations. Until rheumatology-specific thresholds are prospectively validated, these measures may be better understood as signals that prompt individualized assessment of a patient's net immunosuppression rather than universal rules or guidelines that determine prophylaxis. Future studies should quantify rheumatology-specific absolute risk, define the balance of prophylactic benefit and harm across disease and treatment contexts, and identify validated markers for both initiation and discontinuation of prophylaxis.

TABLE 1 | Concepts that apply or cannot be applied to PJP prophylaxis in rheumatology.

Practice outside of rheumatology	Origin	Does it translate?	Implication for rheumatology
CD4 < 200	HIV [4–6]	Poorly	Not a stand-alone threshold
Prednisone ≥ 20 mg/day for ≥ 4 weeks	Oncology [7, 8]	Risk factor: yes; Decision threshold: incompletely	Should act as a trigger for risk assessment to determine need for prophylaxis
6–12 months of prophylaxis after transplant	Transplant [11]	Poorly	Follow changing immunosuppression rather than time alone
Immune recovery to stop prophylaxis	HIV [4–6, 11]	Concept transfers; marker (CD4 count) does not	No validated rheumatology equivalent
Net state of immunosuppression	Oncology and Transplant [7, 8, 11]	Yes	Integrate disease, treatment, and host factors to determine need for prophylaxis

Author Contributions

C.W. completed a literature review and wrote the original draft. V.P. contributed to conceptualization, developed Table 1 and supervised the writing of the manuscript. All authors critically reviewed, edited, and approved the final manuscript.

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The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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ORIGINAL ARTICLE

Integrated Analysis of Gut Microbiota and Fecal Metabolites in Chinese Patients With Rheumatoid Arthritis: Associations With Disease Activity

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ABSTRACT

Aim: Rheumatoid arthritis (RA) is a chronic autoimmune disease with intestinal dysbiosis implicated through the gut–joint axis. This study aimed to delineate fecal microbiome and metabolomic signatures in RA and identify biomarkers associated with disease activity.

Method: Twenty-eight RA patients and 19 healthy controls were included in this cross-sectional study. Gut microbiota was characterized by 16S rRNA sequencing with operational taxonomic unit (OTU) and amplicon sequence variant (ASV) analysis. Fecal metabolites were profiled using untargeted metabolomics. Correlations between microbiota, metabolites, and disease activity indices were assessed.

Results: RA patients showed altered alpha diversity and distinct microbial composition versus healthy controls. Eight differential genera and three species were identified based on the combined OTU and ASV analyses. *Allisonella* was enriched in RA and positively correlated with disease activity, whereas *Bifidobacterium adolescentis* showed a negative correlation trend with autoantibody/inflammatory markers. Metabolomic analysis revealed 31 and 36 altered metabolites in both ion modes. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment indicated dysregulation of unsaturated fatty acid biosynthesis and tryptophan metabolism. Microbiota–metabolite pairs correlated with disease activity.

Conclusion: RA is characterized by distinct gut microbiota and fecal metabolite alterations associated with disease activity. Specific microbial taxa and metabolic pathways may serve as biomarkers, lending further support to the gut–joint axis in RA pathogenesis.

1 | Introduction

Rheumatoid arthritis (RA) is a prototypical chronic autoimmune disease characterized by progressive, symmetric joint destruction, and systemic inflammation [1]. Its pathogenesis involves a complex interplay between genetic predisposition and environmental triggers [2]. Environmental factors can alter the gut microbiota, increase intestinal permeability, and promote intestinal inflammation, facilitating translocation of microbial products, and inflammatory mediators into the joints [3]. These

observations have led to the concept of a “gut–joint axis” as a potential mechanistic link in the development and progression of RA [4].

Profiling the gut microbiome with its metabolic output offers a means to interrogate the gut–joint axis, because gut microbiota-derived metabolites mediate links between intestinal dysbiosis and systemic joint inflammation [5, 6]. The gut microbiota shapes host metabolic and immune responses that are dysregulated in RA [5, 7, 8]. Although integrated microbiome–metabolome

Highlights

1. Treatment-naïve RA patients show gut microbial dysbiosis with *Allisonella* enrichment (positively correlated with disease activity) and a trend toward reduced *Bifidobacterium adolescentis*, supporting disease-intrinsic alterations along the gut–joint axis.
2. Fecal metabolomics reveals dysregulated tryptophan metabolism and unsaturated fatty acid biosynthesis, with DHA depletion and elevated L-valine correlating with autoantibody titers and disease activity.
3. Integrated microbiome-metabolome analysis identifies disease activity-associated microbial-metabolic signatures as candidate biomarkers for future validation in larger cohorts.

profiling has been applied to RA, including in untreated or newly diagnosed cohorts [9, 10], the microbial taxa and metabolites identified differ substantially across cohorts, and additional well-characterized, treatment-naïve cohorts are needed to establish which signatures are reproducible. This study investigated fecal microbiome and metabolomic profiles in Chinese patients with treatment-naïve RA versus healthy controls, and sought to identify bacterial taxa and fecal metabolites associated with RA and to elucidate candidate biomarkers within the gut–joint axis.

2 | Methods

2.1 | Study Design and Participants

This cross-sectional observational study was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. A total of 28 patients with RA and 20 healthy individuals were consecutively recruited in Xuzhou, China. The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethics committee (approval numbers xyy11[2021]021 and xyy11[2023]032). Written informed consent was obtained from all participants.

Patients fulfilled the 2010 revised criteria of the American College of Rheumatology (ACR) for RA. Healthy controls had no personal or family history of rheumatic diseases. One healthy control was excluded due to diarrhea reported near the time of sample collection, resulting in a cohort of 28 patients and 19 healthy controls. Participants were aged 18 to 70 years. Exclusion criteria included: (1) metabolic diseases (e.g., diabetes and hyperlipidemia) or serious underlying diseases (e.g., acute coronary syndrome, chronic renal failure, and tumors); (2) infectious diseases (e.g., respiratory tract infection, digestive tract infection, and urinary tract infection); (3) other autoimmune diseases (e.g., systemic lupus erythematosus and inflammatory bowel disease); (4) allergic diseases; (5) major gastrointestinal surgery within 3 months; (6) use of antibiotics, probiotics, or prebiotics within 3 months; and (7) pregnancy or lactation. Individuals with chronic heavy alcohol consumption or markedly unbalanced dietary patterns (e.g., strict vegetarian or exclusively meat-based diets) were excluded at the screening stage, based on pre-enrollment verbal interviews,

and all participants were required to refrain from taking any nutritional supplements (including vitamins and fish oil) within 3 months before enrollment.

All patients were newly diagnosed with RA, defined as receiving their first formal diagnosis by a rheumatologist prior to enrollment, and were treatment-naïve to glucocorticoids or disease-modifying antirheumatic drugs. Most patients tested positive for rheumatoid factor (RF) (> 20 IU/mL) and anti-cyclic citrullinated peptide antibody (ACPA) (≥ 5 U/mL), and all were in the active phase of RA.

2.2 | Clinical Data Collection

Comprehensive clinical data were collected, including age, sex, height, weight, body mass index (BMI), disease duration, smoking status, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), RF, ACPA, Disease Activity Score 28-ESR (DAS28-ESR), Disease Activity Score 28-CRP (DAS28-CRP), Clinical Disease Activity Index (CDAI), Simplified Disease Activity Index (SDAI), and Health Assessment Questionnaire-Disability Index (HAQ-DI). Individual-level clinical and serological data, along with the processed microbiome and metabolomics datasets, are provided in the Data S1.

2.3 | Sample Collection and Processing

Stool samples were collected for 16S rRNA gene sequencing and untargeted metabolomics. For microbiome analysis, four healthy control samples were discarded due to poor quality (low read counts), resulting in 28 patient samples and 15 healthy control samples for the final microbiome dataset. For metabolomics analysis, after additional quality control filtering, 20 RA samples and 19 healthy control samples were included in the final dataset. All 20 RA participants in the metabolomic dataset had qualified microbiome data and were therefore included in the integrated microbiome–metabolite correlation analysis. The overall participant flow, from enrollment through quality control filtering to the final sample sizes for each analysis, is illustrated in Figure S1.

2.4 | DNA Extraction, 16S rRNA Sequencing, and Bioinformatics Analysis

Total DNA was extracted from approximately 0.3g stool per participant using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany). The V4 region of the 16S rRNA gene was amplified using primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 926R (5'-CCGTCAATTCMTTGTAGTTT-3'). Paired-end sequencing was performed on the Illumina NovaSeq 6000 platform (Taihe Bio-Tech Co. Ltd., Suzhou, China). After quality control and filtering, sequences with $\geq 97\%$ similarity were clustered into operational taxonomic units (OTUs) using USEARCH (version 8.1.1831; Robert C. Edgar, USA). The amplicon sequence variant (ASV) method was employed as an adjunct for higher-precision analysis. Between-group differences in overall microbial community composition were formally tested by permutational multivariate analysis of variance (PERMANOVA; Adonis) based on Bray–Curtis and Jaccard distance matrices with 999 permutations,

for both OTU- and ASV-based datasets. For the integrated microbiome-metabolite analysis, bacterial taxa involved in tryptophan metabolism (Figure 5A) were defined based on previously published literature rather than direct functional profiling, as 16S rRNA sequencing does not directly measure metabolic activity.

2.5 | Sample Processing and Untargeted Metabolomics Analysis

Stool samples (50 mg) were extracted with 80% methanol and analyzed by untargeted liquid chromatography-tandem mass spectrometry (Waters ACQUITY UPLC coupled with Thermo Q Exactive; Waters, Milford, MA, USA; Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation was performed on an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm; Waters, Milford, MA, USA) at 40°C with a flow rate of 0.3 mL/min. Mass spectrometry was performed in both positive and negative electrospray ionization modes. Differential metabolites were screened by combining a nominal $p \leq 0.05$ with a variable importance in projection (VIP) ≥ 1 from the first predictive component of the OPLS-DA model. Fold change, calculated as the ratio of mean abundance in patients with RA to that in healthy controls, was computed for all metabolites and is reported to describe the direction and magnitude of change; it was not applied as a filtering threshold. MetaboAnalyst R (version 2.0.2; Xia Lab, McGill University, Montreal, Canada) was used for KEGG pathway enrichment analysis [11–13]. Pathway data were sourced from KEGG under usage license 251677.

2.6 | Statistical Analysis

Data were analyzed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) and R (version 3.6.2; R Foundation for Statistical Computing, Vienna, Austria). Clinical data were presented as mean ± standard deviation (SD). Between-group comparisons were performed using the Wilcoxon test or unpaired *t*-test. *p* Values less than 0.05 were considered statistically significant. For the microbiome analysis, *p* values from feature-level comparisons (differential taxa and species) were adjusted for multiple comparisons using the false discovery rate (Benjamini-Hochberg method), and an FDR-adjusted *p* value (*q* value) < 0.20 was used as the threshold [14, 15]; only features consistently identified in both OTU-based and ASV-based analyses were advanced for downstream interpretation. *p* Values for community-level comparisons (PERMANOVA) are unadjusted permutation *p* values. For the metabolomic analysis, nominal *p* values were retained without adjustment for multiple comparisons, as this was an exploratory, hypothesis-generating analysis.

3 | Results

3.1 | Baseline Clinical Characteristics

Baseline characteristics are summarized in Table 1; we enrolled 28 RA patients and 19 healthy controls. The two groups showed no significant differences in age, sex, smoking status, or body mass index. All patients were newly diagnosed with

RA according to the 2010 ACR/EULAR classification criteria and were treatment-naïve to glucocorticoids or disease-modifying antirheumatic drugs. Many patients had prolonged disease duration (mean 39.55 ± 89.09 months) owing to delayed access to specialist rheumatology care. All patients were in the active phase of RA, with most in the medium-high activity range.

3.2 | Altered Gut Microbiota Diversity and Composition in Chinese Patients With RA

The Venn diagram (Figure 1A) shows 591 OTUs in controls versus 720 in RA, with 539 shared. Principal component analysis (PCA) showed partial separation between the two groups, with higher intra-group similarity in healthy controls (Figure 1B). PERMANOVA based on Bray-Curtis and Jaccard distance matrices confirmed that overall microbial community composition differed significantly between groups in both OTU- and ASV-based datasets (all $p < 0.05$; $R^2 = 0.028$ – 0.038 ; Table S1), although the effect sizes were modest, as is typical of case-control comparisons of the gut microbiome. The Shannon index was lower and the Simpson index was higher in the RA group compared with healthy controls (Figure 1C,D), suggesting reduced bacterial alpha diversity in RA. ASV-based analysis concurred (Figure S2).

At the phylum level, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* dominated both groups (Figure 2A,B). *Firmicutes* abundance was lower in RA, whereas *Bacteroidetes* was higher (Figure 2C and Figure S3A). Consequently, the *Firmicutes*-to-*Bacteroidetes* (F/B) ratio was lower in RA patients (Figure 2D and Figure S3B). The Linear discriminant analysis effect size (LEfSe) revealed significant differences spanning class to genus levels (Figure 2E), concordant in ASV analysis (Figure S3C–F). At the genus level, RA patients exhibited elevated abundances of *Allisonella* (mean relative abundance 0.054% vs. 0.020% in healthy controls; $p = 0.016$, Wilcoxon test), *Acidaminococcus*, and *Caproiciproducens*, while *Turicibacter*, *Alloprevotella*, and 12 other genera, were enriched in healthy controls. *Allisonella* was positively correlated with DAS28-ESR, *Turicibacter* exhibited a negative correlation with DAS28-ESR, ESR, and CRP, and *Alloprevotella* was negatively associated with RF and ACPA (Figure 2F).

At species level, 18 taxa differed, with 15 depleted in RA (including *B. adolescentis*, *B. plebeius*, and *D. longicatena*; Figure 2G). The robustness of these findings was supported by concordance between OTU-based and ASV-based analyses, with both methods consistently identifying reduced *B. adolescentis* in RA (Figure 2G and Figure S3G). *B. adolescentis* exhibited a negative correlation trend with ACPA, ESR, and CRP (Figure 2H and Figure S3H), though statistical significance was not achieved (see also Figure S3I).

3.3 | Fecal Metabolomic Alterations in Chinese RA Patients

To investigate fecal metabolic alterations associated with gut microbial dysbiosis, we conducted untargeted metabolomic profiling of fecal samples from 20 RA patients and 19 healthy

TABLE 1 | Baseline characteristics of healthy controls and patients with rheumatoid arthritis.

	Healthy controls (<i>n</i> = 19)	Rheumatoid arthritis (<i>n</i> = 28)	<i>p</i>
General information			
Age, mean ± SD (years)	45.74 ± 13.41	53.14 ± 12.04	0.115
Sex (females/males)	11/8	18/10	0.763
Disease duration, mean ± SD (months)		39.55 ± 89.09	—
Smoker, <i>n</i> (%)	3 (16%)	8 (29%)	0.485
BMI, mean ± SD	23.38 ± 2.95	25.63 ± 4.19	0.054
Disease activity			
HAQ-DI, mean ± SD		1.9 ± 0.41	—
CDAI, mean ± SD		38.18 ± 11.90	—
SDAI, mean ± SD		41.13 ± 13.44	—
DAS28-ESR, mean ± SD		5.38 ± 1.07	—
DAS28-CRP, mean ± SD		4.82 ± 1.01	—
Serological features			
RF, mean ± SD (IU/mL)		161.03 ± 194.98	—
ACPA, mean ± SD (U/mL)		175.23 ± 151.35	—
ESR, mean ± SD (mm/h)		48.04 ± 31.61	—
CRP, mean ± SD (mg/L)		29.55 ± 35.74	—

Note: *p* values were computed using the Wilcoxon test.

Abbreviations: ACPA, anti-cyclic citrullinated peptide antibody; BMI, body mass index; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; DAS28-CRP, Disease Activity Score 28-C-reactive protein; DAS28-ESR, Disease Activity Score 28-Erythrocyte Sedimentation Rate; ESR, erythrocyte sedimentation rate; HAQ-DI, Health Assessment Questionnaire-Disability Index; RF, rheumatoid factor; SD, standard deviation; SDAI, Simplified Disease Activity Index.

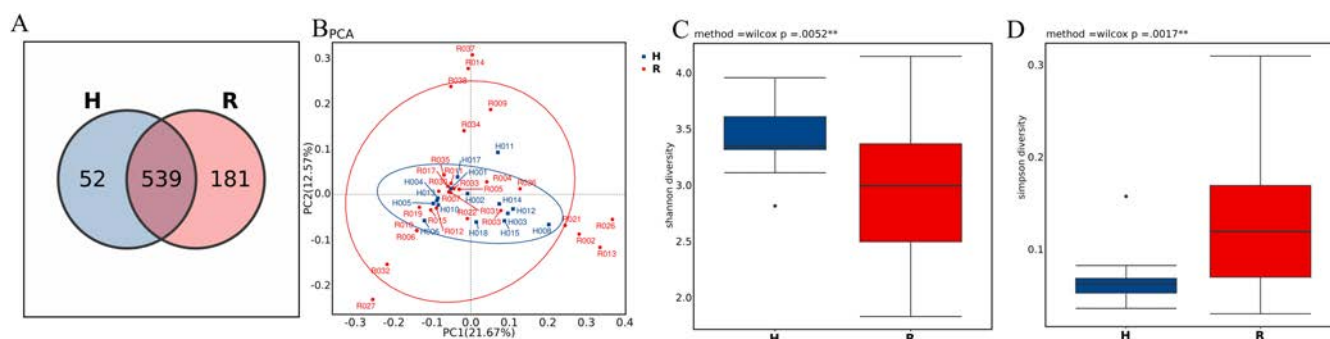


FIGURE 1 | Altered diversity and overall composition of the gut microbiota in Chinese patients with rheumatoid arthritis. (A) The Venn diagram showing shared and unique operational taxonomic units (OTUs) between groups. (B) The principal component analysis (PCA) based on OTUs. (C) The Shannon indices and (D) the Simpson indices of rheumatoid arthritis patients and healthy controls based on OTUs. H group = Healthy control group, *n* = 15; R group = Rheumatoid arthritis group, *n* = 28. ***p* < 0.01 (Wilcoxon test).

controls. Orthogonal projections to latent structures discriminant analysis (OPLS-DA) showed separation between patients with RA and healthy controls in both ion modes (Figure 3A), with the following model parameters: positive ion mode, $R^2X(\text{cum}) = 0.088$, $R^2Y(\text{cum}) = 0.969$, $Q^2(\text{cum}) = 0.338$; negative ion mode, $R^2X(\text{cum}) = 0.177$, $R^2Y(\text{cum}) = 0.983$, $Q^2(\text{cum}) = 0.369$. Permutation testing with 99 permutations supported model validity, with permuted Q^2 values lower than the observed values in both modes ($p = 0.020$ and $p = 0.010$ for positive and negative ion modes, respectively; Figure 3B).

Using the criteria specified in the Methods ($VIP \geq 1$ and raw $p \leq 0.05$), 31 and 36 differentially abundant metabolites were identified in positive and negative ion modes, respectively (Figure 3C,D; Figure S4A,B). Key differential metabolites included 5-aminolevulinic acid (5-ALA), a porphyrin metabolism intermediate; L-valine, a branched-chain amino acid; flavin mononucleotide (FMN), a riboflavin derivative; corticosterone, a steroid hormone; ascorbic acid, an essential antioxidant; and docosahexaenoic acid (DHA), an omega-3 polyunsaturated fatty acid with established anti-inflammatory

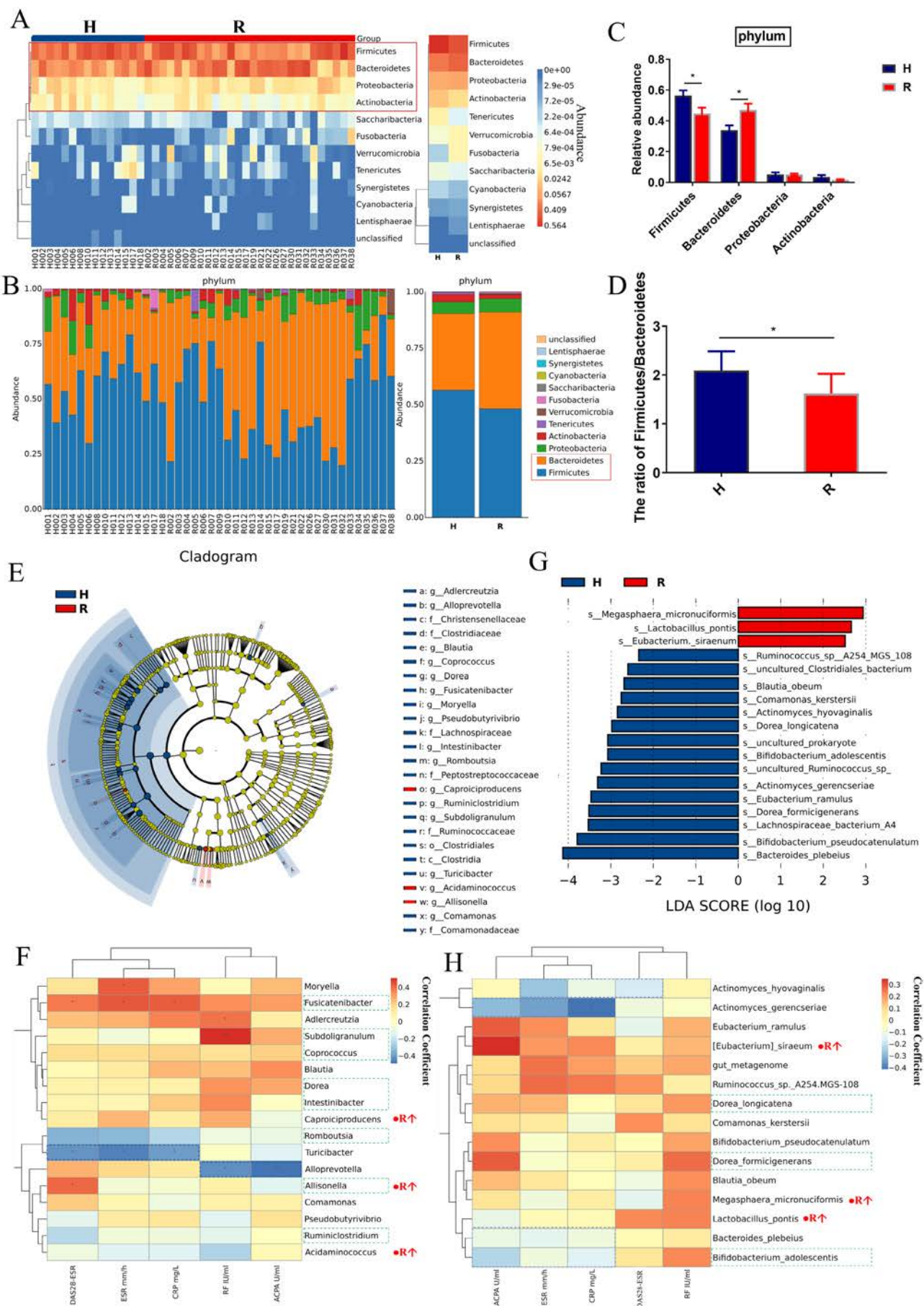


FIGURE 2 | Legend on next page.

FIGURE 2 | Differential gut microbial taxa associated with rheumatoid arthritis. (A) The heatmap and (B) the stacked histogram of species distribution at phylum level. (C) The histogram of relative abundance of four major gut microbiota phyla. (D) The Firmicutes/Bacteroidetes ratio. (E) Linear discriminant analysis effect size cladogram from class to genus levels. (F) Spearman correlation heatmap between clinical indicators and differential genera. (G) Linear discriminant analysis bar chart at species taxonomic levels. (H) Spearman correlation heatmap between clinical indicators and discrepant species. H group = Healthy control group, $n = 15$; R group = Rheumatoid arthritis group, $n = 28$. * $p < 0.05$, ** $p < 0.01$ (Wilcoxon test).

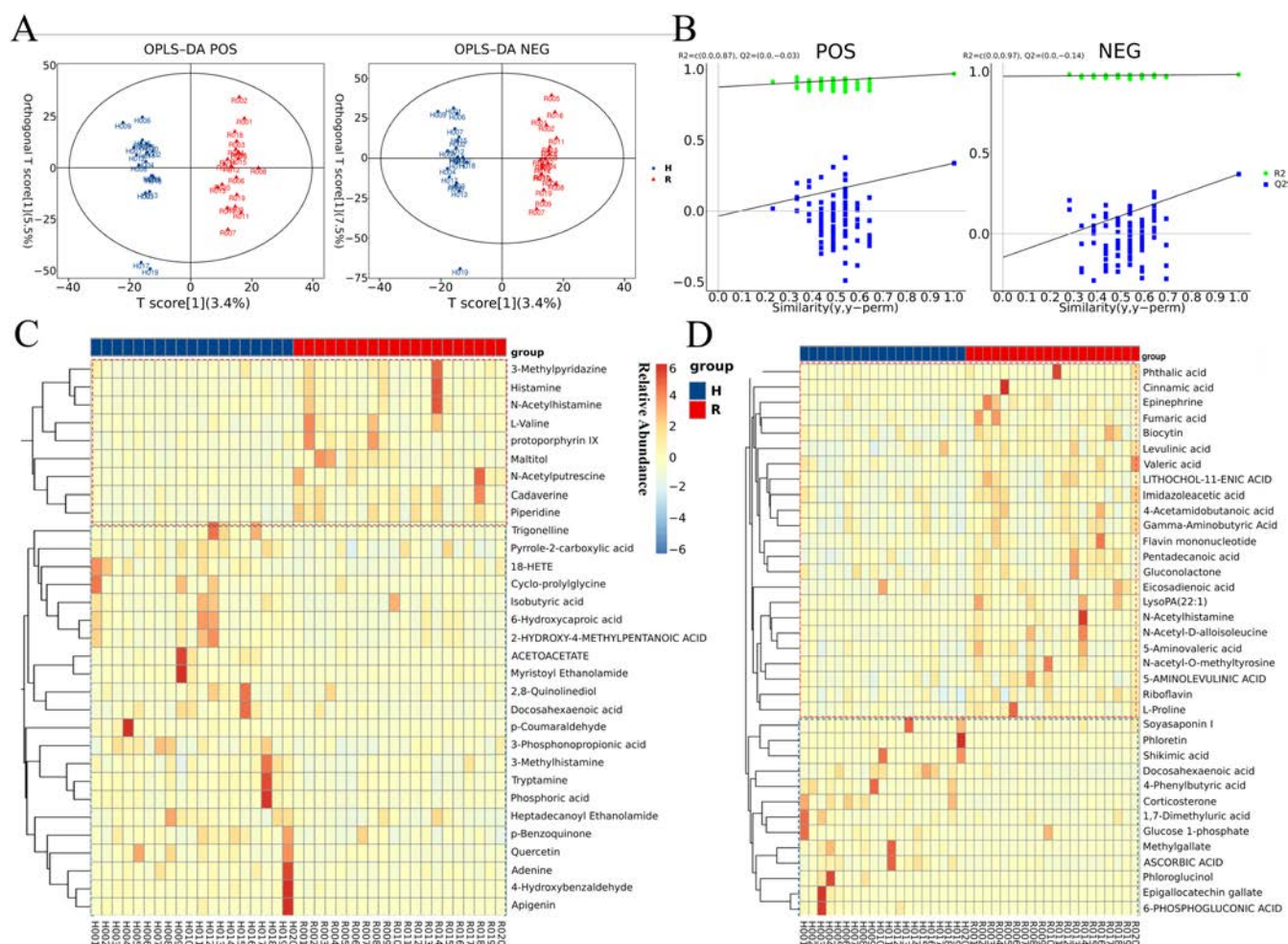


FIGURE 3 | Differential fecal metabolites between rheumatoid arthritis and healthy controls. (A) The orthogonal projections to latent structures discriminant analysis (OPLS-DA) maps in positive and negative ion modes. (B) The validation models in positive and negative ion mode. (C) Heatmap of metabolite relative abundance in positive-ion mode. (D) Heatmap of metabolite relative abundance in negative-ion mode. H group, $n = 19$; R group, $n = 20$ (after quality control filtering).

properties. Several of these metabolites have known immunomodulatory or antioxidant functions, suggesting potential functional links to the observed gut microbial alterations.

3.4 | Integrated Microbiota-Metabolite Signatures and Disease Activity

In positive mode (Figure 4A), 5-ALA and corticosterone correlated negatively with disease activity indices, whereas FMN and ascorbic acid correlated positively; group comparisons confirmed elevated 5-ALA, reduced corticosterone, and increased FMN in RA (Figure S5A-C). KEGG annotation mapped these

mainly to porphyrin/chlorophyll metabolism, glycine/serine/threonine metabolism, steroid hormone biosynthesis, and riboflavin metabolism.

In negative ion mode (Figure 4C), L-valine and DHA were negatively associated with ACPA titers; RA showed increased L-valine and decreased DHA (Figure S5D,E), with L-valine mapped to valine-leucine-isoleucine biosynthesis/degradation, aminoacyl-tRNA biosynthesis, and pantothenate/CoA biosynthesis, and DHA to unsaturated fatty acid biosynthesis.

Figure 4B,D present pathway enrichment bubble plots in positive and negative ion modes, respectively. Collectively, these

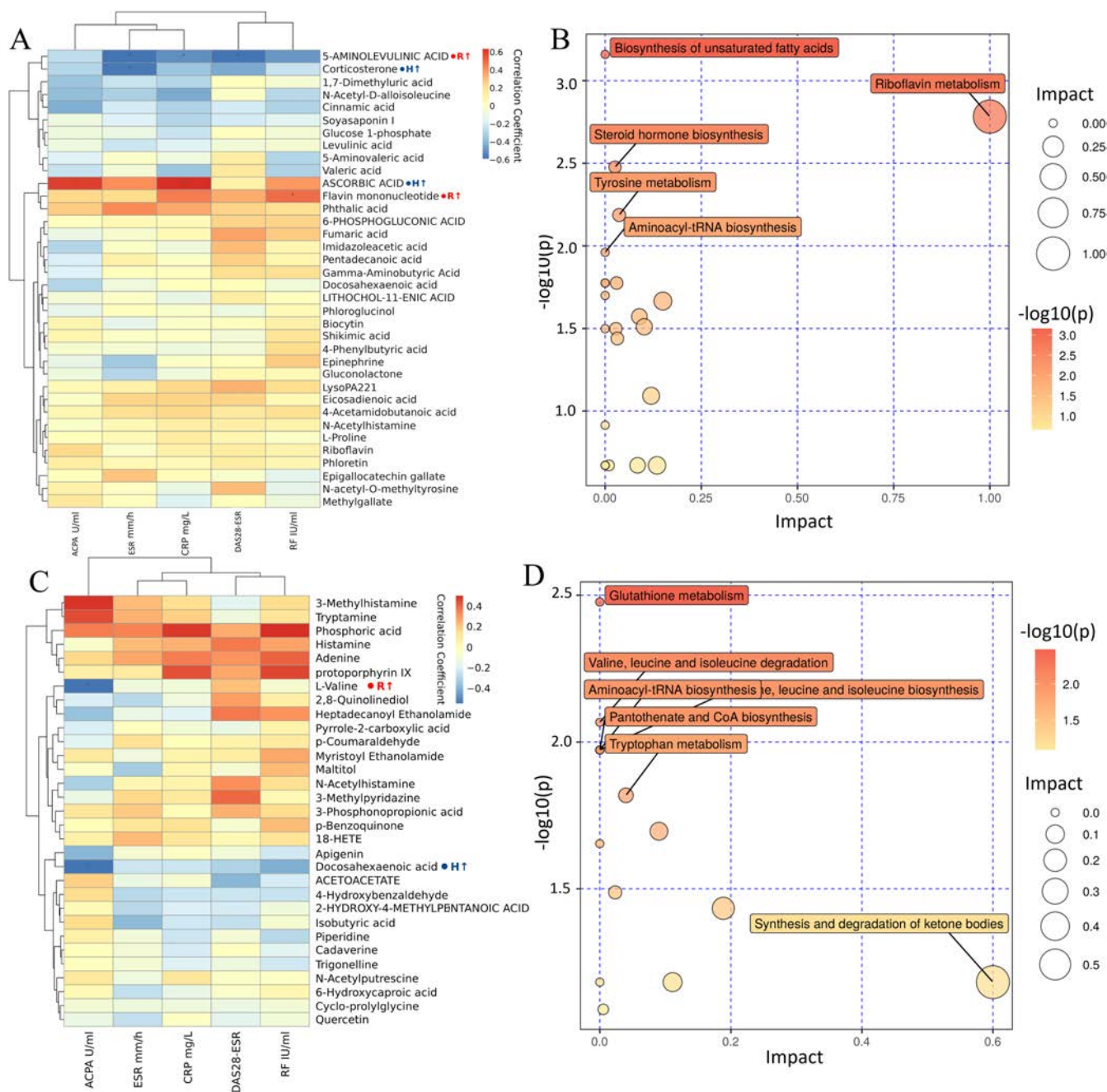


FIGURE 4 | Differential fecal metabolites and enriched pathways associated with disease activity in RA. (A) The Spearman correlation heatmaps of differential metabolites and clinical indicators in positive ion mode. (B) The Kyoto Encyclopedia of Genes and Genomes pathway enrichment bubble plots in positive ion mode. (C) The Spearman correlation heatmaps of differential metabolites and clinical indicators in negative ion mode. (D) The KEGG pathway enrichment bubble plots in negative ion mode. Pathway maps are adapted from KEGG (Kanehisa Laboratories). * $p < 0.05$, ** $p < 0.01$ (Spearman correlation).

analyses corroborated dysregulation of interconnected metabolic pathways. Notably, tryptophan metabolism emerged as an enriched pathway, suggesting a potential role of microbial tryptophan metabolism in RA pathogenesis.

We therefore examined whether taxa within this pathway associated with altered metabolites. Taxa belonging to genera with reported tryptophan-metabolizing capacity (Figure 5A) showed reduced *Bifidobacterium adolescentis* and *Bacteroides plebeius* in RA. Because 16S rRNA sequencing profiles taxonomy rather

than function and published evidence for microbial tryptophan metabolism is largely genus-level, we regard these taxa as candidate, rather than established, participants in this pathway. Circular heatmaps (Figure 5B,C) further illustrate that metabolites enriched in RA fecal samples, including N-acetylputrescine, epinephrine, and 5-ALA, were negatively correlated with *B. adolescentis* abundance. Conversely, metabolites depleted in RA, including 3-phosphonopropionic acid, methyl gallate, glucose 1-phosphate, and 4-phenylbutyric acid, were positively correlated with *B. adolescentis*.

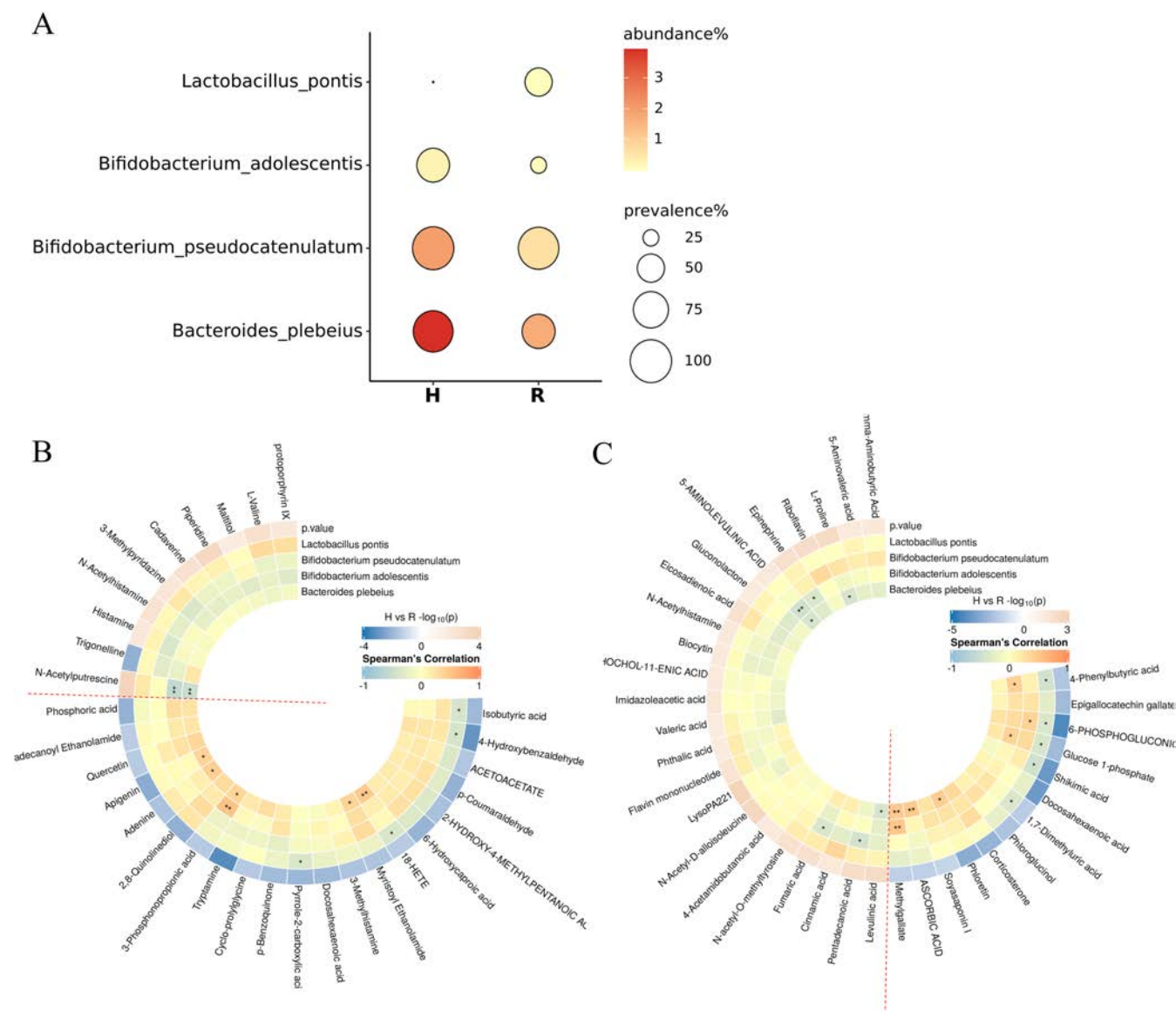


FIGURE 5 | Gut taxa belonging to genera with reported tryptophan-metabolizing capacity, and their associations with fecal metabolites in RA. (A) Relative abundance and detection of taxa belonging to genera with reported tryptophan-metabolizing capacity (Wilcoxon test). Species-level function was not assessed. (B) Circular heatmap of differential metabolites correlating with taxa belonging to genera with reported tryptophan-metabolizing capacity in positive ion mode. (C) Circular heatmap of differential metabolites correlating with taxa belonging to genera with reported tryptophan-metabolizing capacity in negative ion mode. * $p < 0.05$, ** $p < 0.01$ (Spearman correlation).

4 | Discussion

In this study, we profiled the fecal microbiome and metabolome in a cohort of 28 Chinese patients with rheumatoid arthritis and 19 healthy controls. Our investigation revealed altered gut microbial diversity and composition together with disease activity-associated metabolic dysregulation, nominating microbial and metabolic candidates as biomarkers or therapeutic targets pending validation.

Our findings in a treatment-naïve RA cohort empirically lend further support to this framework. Notably, while previous studies have documented *Bifidobacterium* depletion in RA patients [16, 17], many included patients receiving disease-modifying antirheumatic drugs (DMARDs) or glucocorticoids, which confound microbiome composition [18]. The

consistency of *B. adolescentis* reduction in our drug-naïve patients suggests that this signature may reflect disease-intrinsic dysbiosis rather than treatment alterations. The enrichment of *Allisonella* and its positive correlation with DAS28-ESR represent a novel candidate fecal marker associated with disease activity in early RA, though this requires validation in larger longitudinal cohorts.

In agreement with previous reports [16, 19, 20], we observed altered alpha diversity in RA patients (reduced Shannon, increased Simpson indices). The F/B ratio was lower in RA, as in inflammatory bowel disease and systemic lupus erythematosus [21, 22]. Our contribution lies in linking specific microbial and metabolic signatures to disease activity through dual-omics integration rather than merely confirming general dysbiosis. The genus-level enrichment of *Allisonella* and its

positive correlation with DAS28-ESR extend prior links to osteopenia and radiation proctitis to RA [23, 24], nominating a novel fecal marker of disease activity; this exploratory association requires validation.

Several RA-associated species were reduced, including *B. adolescentis*, *D. formicigenerans*, and *D. longicatena*. Although *B. adolescentis*—a commensal with immunomodulatory properties in preclinical models [25, 26]—correlated negatively with ACPA, ESR, and CRP without reaching significance. Because 16S rRNA sequencing cannot resolve strains and *B. adolescentis* shows strain-specific functional heterogeneity (some promote Th17, others are anti-inflammatory [25, 26]), extrapolation of preclinical findings to our cohort requires caution and strain-resolved metagenomic validation.

Metabolomic analysis revealed alterations in fecal 5-ALA, L-valine, FMN, corticosterone, ascorbic acid, and DHA, several correlating with disease activity; however, fecal levels reflect diet, microbial metabolism, and host absorption and are not direct surrogates of serum or tissue concentrations. Elevated fecal 5-ALA, negatively correlated with disease activity, upregulates heme oxygenase-1 via porphyrin augmentation and confers anti-inflammatory and immune-modulatory properties [27], suggesting relevance to RA inflammation; its unexpected increase may reflect a compensatory response to intestinal inflammation, warranting experimental verification.

Elevated fecal L-valine contrasts with reduced valine in RA serum and synovial fluid [28], indicating differential regulation of fecal versus systemic pools; its negative correlation with ACPA titers hints at a compensatory mucosal-protective role, though speculative. FMN and its precursor riboflavin were elevated in RA and FMN correlated positively with RF; yet, given riboflavin's anti-inflammatory effects in Crohn's models [29, 30], fecal FMN elevation appears incompatible with those protective roles, a discrepancy warranting blood-based validation.

Corticosterone was reduced in RA feces and negatively correlated with disease activity indices. Emerging evidence indicates that intestinal mucosa can synthesize corticosterone locally as a negative-feedback brake on immune activation [31, 32]. Reduced fecal corticosterone in our RA patients may reflect disturbed local immune-metabolic homeostasis, although this interpretation remains speculative.

DHA—an omega-3 fatty acid with established anti-inflammatory and immunomodulatory properties [33, 34]—was reduced in RA fecal samples and negatively correlated with ACPA titers. Population-based dietary surveys have associated higher DHA intake with lower RA risk [35]. Similarly, fecal ascorbic acid—an essential water-soluble antioxidant—was lower in RA patients. The concurrent depletion of these anti-inflammatory, antioxidant metabolites is consistent with, but does not establish, reduced protective potential in the RA gut, though whether this reflects dietary insufficiency, impaired absorption, or increased metabolic consumption cannot be determined cross-sectionally.

KEGG pathway enrichment analysis highlighted tryptophan metabolism as an enriched pathway, consistent with recent reports implicating it in RA pathogenesis [36]. At the

microbial level, reduced *B. adolescentis*—a taxon involved in tryptophan metabolism—was observed in RA. However, our untargeted metabolomics platform detected only tryptamine from this pathway; downstream indoles were not identified. Consequently, while gut microbial tryptophan metabolism appears altered in RA, we cannot conclude that specific derivatives such as indoles are directly pathogenically relevant, and targeted metabolomics are warranted to clarify its functional output.

Integration revealed correlations between *B. adolescentis* abundance and differential metabolites: RA-enriched metabolites (e.g., N-acetylputrescine, 5-ALA) were negatively correlated with *B. adolescentis*, whereas RA-depleted ones (e.g., methyl galate and 4-phenylbutyric acid) were positively correlated, suggesting a microbiota-metabolite interaction of undetermined functional relevance. The mechanistic interpretations discussed above should therefore be regarded as hypotheses to be tested in longitudinal cohorts, targeted metabolomic studies, and experimental models.

Several limitations should be acknowledged. First, the modest sample size (28 RA patients and 19 healthy controls) and cross-sectional design preclude causal inference; although age, sex, BMI, and smoking were balanced, residual confounding by these and by habitual diet cannot be excluded, and the sample size also precluded multivariable adjustment and sensitivity analyses. Second, the single-center Chinese cohort limits generalizability to populations with different genetic backgrounds, diets, and lifestyles; the observed variability in disease duration may reflect delayed access to specialist rheumatology care, which could introduce selection bias. Third, 16S rRNA sequencing resolves taxonomy only to genus or species level and permits no strain-level or functional profiling; strain-specific functional heterogeneity therefore remains unconfirmed, and assignment of taxa to roles such as tryptophan metabolism was inferred from published genus-level evidence rather than demonstrated. Fourth, untargeted metabolomics yields relative quantification requiring targeted validation to confirm the identity and absolute concentrations of the reported metabolites, and metabolite-host-microbe interactions remain uncharacterized; differential metabolites were identified using nominal *p* values without multiple-comparison adjustment. Finally, fecal metabolites are influenced by diet, medication, and geography that were not fully controlled; although individuals with extreme dietary patterns were excluded by self-report (without a structured instrument) and supplement use was prohibited within 3 months, quantitative dietary data (e.g., food-frequency questionnaires) were not collected, so residual dietary confounding cannot be excluded and the findings should be interpreted cautiously. Overall, these results are preliminary and hypothesis-generating, requiring validation in larger, multi-center, longitudinal cohorts with standardized dietary assessment and targeted metabolomic follow-up.

5 | Conclusion

This study identifies distinct alterations in gut microbiota and fecal metabolites associated with rheumatoid arthritis disease

activity. The enrichment of *Allisonella* and altered abundance of *Bifidobacterium adolescentis*, together with dysregulation of related metabolic pathways, may provide candidate signatures for disease monitoring and potential targets for microbiota-directed interventions, pending validation of their clinical relevance. These findings lend further support to the gut–joint axis hypothesis in rheumatoid arthritis pathogenesis and warrant validation in larger, longitudinal cohorts.

Author Contributions

Concept: Jing Liang, Yile Ren; Design: Jing Liang, Yile Ren; Supervision: Yile Ren; Materials: Jing Liang, Xiangyu Wei, Chen Shang, Siqi Shao, Wenting Hao; Data Collection and/or Processing: Jing Liang, Xiangyu Wei, Chen Shang, Siqi Shao, Wenting Hao; Analysis and/or Interpretation: Jing Liang, Xiangyu Wei, Chen Shang, Siqi Shao, Wenting Hao; Literature Search: Jing Liang, Yile Ren; Writing Manuscript: Jing Liang, Yile Ren; Critical Review: Yile Ren.

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Ethics Statement

Ethical committee approval was received from the Ethics Committee of The Affiliated Xuzhou Municipal Hospital of Xuzhou Medical University (Approval no: xyy11[2021]021 and xyy11[2023]032) in accordance with the Declaration of Helsinki.

Consent

Written informed consent was obtained from all participants who agreed to take part in the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw microbiota sequencing data and metabolomics data are available in the NCBI Sequence Read Archive (BioProject PRJNA1338242) and OMIX (OMIX010686), respectively. Additional processed data are provided in the Data S1. All Figures S1–S5 and Table S1 are cited in the main text where appropriate.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** It contains the processed datasets supporting this study, including clinical metadata, OTU/ASV abundance matrices, alpha- and beta-diversity statistics, taxonomic differential-abundance results, LEfSe and OPLS-DA outputs, differential fecal metabolites with identification details, microbiota-metabolite correlations, and KEGG pathway-enrichment results, organized into 26 labeled sheets. **Figure S1:** STROBE-style participant flow diagram. A total of 28 patients with rheumatoid arthritis (RA) and 20 healthy controls (HCs) were assessed for eligibility. One HC was excluded because of diarrhea near the time of sample collection, resulting in a final clinical cohort of 28 RA patients and 19 HCs. After quality-control filtering, 28 RA and 15 HC samples were included in the microbiome analysis, and 20 RA and 19 HC samples were included in the metabolomic analysis. All 20 RA patients in the metabolomic dataset had paired qualified microbiome data and were therefore included in the integrated microbiome-metabolite correlation analysis. **Figure S2:** Altered diversity and overall composition of the gut microbiota based on amplicon sequence variants in rheumatoid arthritis. (A) The diagram of Venn based on ASV. (B) The diagram of ASV-based principal component analysis. (C, D) Shannon index and Simpson index of RA patients and healthy controls based on ASV. H group = Health control group, $n = 15$; R group = Rheumatoid arthritis group, $n = 28$. $*p < 0.05$ (Wilcoxon test). **Figure S3:** Differential gut microbial taxa based on amplicon sequence variant analysis from phylum to species levels. (A) The histogram of four major intestinal flora screened by ASV analysis at phylum level from healthy controls and RA patients. (B) The ratio of Firmicutes and Bacteroidetes from the above 2 groups. H group = Health control group, $n = 15$; R group = Rheumatoid arthritis group, $n = 28$. (C–G) The histogram of different intestinal flora from class to genus level between the two groups. (H) Histogram of relative abundance of three gut microbes screened based on both OTU cluster analysis and ASV methods in RA patients and healthy controls. (I) Heatmap of the correlation analysis between the relative abundances of tryptophan-metabolizing bacterial taxa in the fecal microbiome of patients with rheumatoid arthritis and clinical indicators (ns, $p > 0.05$). Red means positive correlation and blue means negative correlation. H group = Health control group, $n = 15$; R group = Rheumatoid arthritis group, $n = 28$. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ (Wilcoxon test). **Figure S4:** *apl70905-sup-0005-FigureS4.tif*. Z-score plots of differential metabolites in positive and negative ion modes. (A) Z-score chart of differential metabolites in positive ion mode. (B) Z-score chart of differential metabolites in negative ion mode. All measurements were standardized using Z-scores calculated from control group distributions (mean μ , standard deviation σ) according to the formula: $z = (x - \mu) / \sigma$. **Figure S5:** Relative abundance of selected differential metabolites and their annotated metabolic pathways. The boxplots and annotated metabolic pathways of (A) 5-aminolevulinic acid, (B) corticosterone, and (C) flavin mononucleotide. The boxplot and annotated metabolic pathways of (D) L-valine, and the boxplot and

annotated metabolic pathway of (E) docosahexaenoic acid. According to the conditions of p value ≤ 0.05 and $VIP \geq 1$, differential metabolites were screened. $*p < 0.05$ (Wilcoxon test). Pathway maps are adapted from KEGG (Kanehisa Laboratories). **Table S1:** PERMANOVA (Adonis) analysis of between-group differences in gut microbial community composition. PERMANOVA was performed based on Bray–Curtis and Jaccard distance matrices with 999 permutations per test, for both OTU- and ASV-based datasets. Each test involved a single two-group comparison; therefore, no multiple-testing adjustment was applied. ASV, amplicon sequence variant; HC, healthy controls; OTU, operational taxonomic unit; RA, rheumatoid arthritis.



LETTER TO THE EDITOR

Bilateral Reversed Palmaris Longus: An Unexpected Cause of Carpal Tunnel Syndrome in Sjögren's Syndrome

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Dear Editor,

A 58-year-old female patient presented with bilateral numbness and tingling in her hands, exacerbating nocturnally for 6 months. She denied any history of trauma. Her medical history included primary Sjögren's syndrome (pSS), for which she was receiving hydroxychloroquine (200 mg, twice daily). Physical examination revealed positive Phalen's and Tinel's signs bilaterally, without thenar atrophy or weakness. Schaeffer's test was positive bilaterally (Figure 1). Laboratory evaluations, including acute phase reactants, complement levels, and immunological parameters, were unremarkable. Notably, her underlying disease was clinically and serologically stable, with no new extraglandular involvement. Point-of-care ultrasound (US) revealed bilaterally enlarged, hypoechoic median nerves with increased cross-sectional area and an unexpected anatomical variation: bilateral reversed palmaris longus (PL) muscles (Figure 2, Video S1). Electrodiagnostic studies confirmed mild carpal tunnel syndrome (CTS), showing sensory conduction slowing of the median nerve across the wrist with preserved motor responses and no denervation changes, and excluded polyneuropathy. Magnetic resonance imaging was not performed. The patient was managed conservatively with resting splints and median nerve gliding exercises achieving significant symptomatic relief at one-month follow-up without the need for surgical intervention. At the six-month follow-up, laboratory results, including acute-phase reactants, complement levels, and immunological parameters, were normal. Phalen's and Tinel's tests were negative, and passive stretching of the PL muscle did not elicit tingling or paresthesia. The patient was advised to continue using the resting splint and performing the prescribed exercises.

Peripheral neuropathy is a common manifestation of pSS; CTS occurs in approximately 12.8% of patients, often reflexively attributed to systemic inflammation or tenosynovitis [1]. Autoimmune factors, complement deficiency, hypergammaglobulinemia, and disease duration may contribute to this increased prevalence [1]. Furthermore, the PL is a superficial forearm muscle known for considerable anatomical variability. One rare variant is the reversed PL, characterized by a proximal tendon and a distal muscle belly located at the wrist. Previously, the prevalence of a reversed palmaris longus (RPL) was reported as 0.2% in 1600 dissected upper limbs, while more recent data from 335 distal forearm MRI examinations demonstrated a prevalence of 0.6% [2]. Bilateral occurrence of RPL appears to be exceedingly rare. The PL has been implicated in carpal tunnel mechanics; loading of the flexor tendons increases intracarpal canal pressure, and cadaveric studies suggest that the PL may exert a significant effect on hydrostatic pressure within the tunnel [2]. This effect is likely mediated through traction on the flexor retinaculum, which alters the shape and volume of the carpal tunnel, thereby increasing pressure [2]. In the case of a reversed PL, the distally located muscle belly may further contribute to volume-related effects at the wrist [2]. Conversely, the absence of the PL tendon a common variation with a global prevalence of ~20% has been associated with a lower frequency of CTS in case-control studies, indirectly supporting the PL's role in carpal tunnel dynamics [3]. On the other hand, presentation time and worsening at night of the patient is raising questions. We believe the anomalous muscle belly acted as a static space-occupying lesion that was tolerated for years, with symptoms emerging

Berkay Yalçınkaya and Hilmi Berkan Abacıoğlu contributed equally and are co-first authors.

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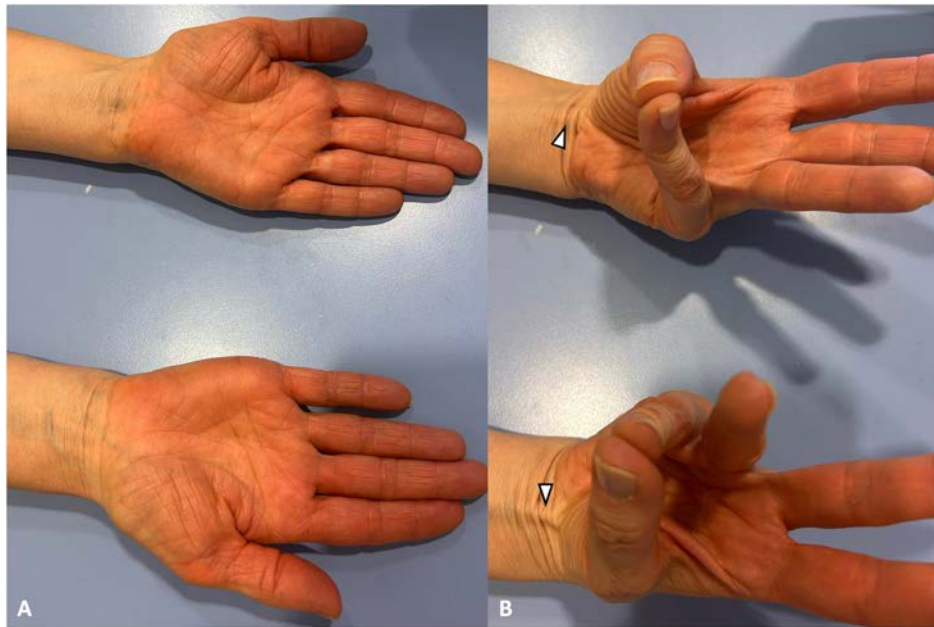


FIGURE 1 | Both hands of the patient in the neutral position (A) and during the Schaeffer's test (B). Arrowheads indicate the palmaris longus tendons.

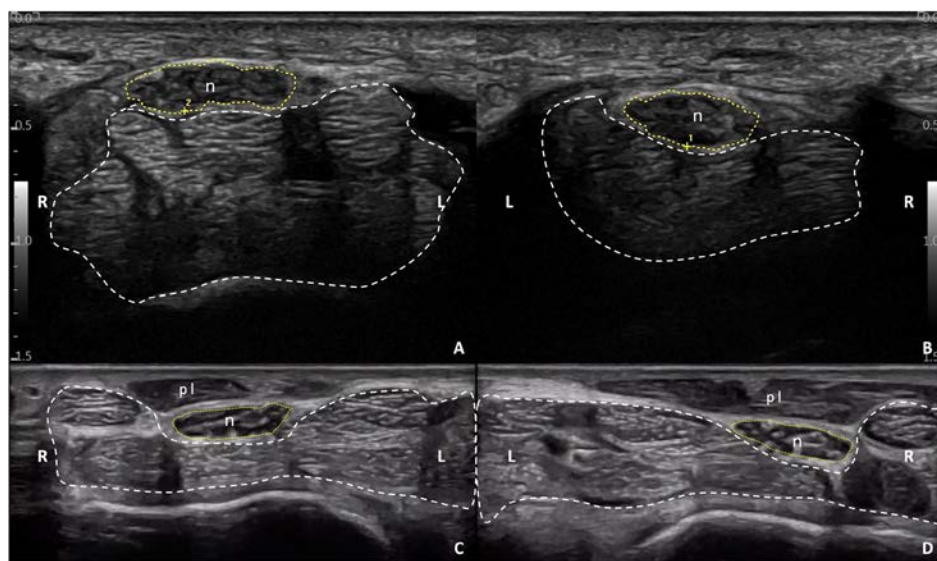


FIGURE 2 | Short-axis ultrasound images of the right (A,C) and left (B,D) wrists demonstrate bilateral reversed palmaris longus muscles (*pl*), potentially contributing to median nerve (*n*, yellow dashed lines) entrapment. The median nerve cross-sectional area measures 12 mm² on both sides at the carpal tunnel level above the flexor tendons (white dashed lines). L: Left; R: Right.

once age-related changes (tenosynovial thickening, reduced tunnel compliance etc.) lowered the threshold for median nerve compression; reduced muscle bulk does not necessarily reduce the space occupied, given fatty infiltration and the fixed position of the belly [4]. Regarding nocturnal worsening, we agree that contraction may aggravate symptoms, but the anomalous muscle primarily raises baseline carpal tunnel pressure, and nocturnal factors such as sustained wrist flexion and fluid redistribution during sleep push the pressure beyond the tolerance of the nerve [5]. However, these inferences are drawn from the existing CTS literature, as studies specifically addressing reversed PL are lacking.

Given the patient's stable autoimmune profile, the reversed palmaris longus (PL) likely served as the primary mechanical driver of the nerve entrapment, rather than active pSS-related inflammation. To the best of our knowledge, CTS associated with bilateral reversed PL in a systemic autoimmune condition has not been previously reported in the English literature. Ultimately, when patients with systemic inflammatory diseases present with entrapment neuropathies, clinicians should avoid prematurely attributing symptoms solely to the underlying condition. This case highlights that the routine use of point-of-care ultrasound is invaluable for identifying unexpected anatomical anomalies, ensuring accurate diagnosis,

and preventing unnecessary escalation of immunosuppressive treatment.

Author Contributions

Hilmi Berkan Abacıoğlu: writing the original text, investigation, conceptualization. **Berkay Yalçınkaya:** writing the original text, investigation, conceptualization. **Ahmet Furkan Çolak:** writing the original text, investigation. **Gamze Kılıç:** supervision, editing.

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The authors have nothing to report.

Ethics Statement

Informed consent was obtained from the patient.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Video S1:** Short-axis sonographic tracking from distal (wrist) to proximal (forearm) illustrates the close anatomical relationship between the reversed palmaris longus muscle (*pl*) and the median nerve (*n*). Arrowhead; proximal palmaris longus tendon.



LETTER TO THE EDITOR

Case Report: Sequential Autoimmune Hemolytic Anemia, Acquired Hemophilia A, and Pure Red Cell Aplasia in Systemic Lupus Erythematosus

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Dear Editor,

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that frequently involves the hematopoietic system [1]. Autoimmune hemolytic anemia (AIHA) is relatively common, whereas acquired hemophilia A (AHA) and pure red cell aplasia (PRCA) are rare and reported mainly as isolated cases [2–4]. To our knowledge, the sequential occurrence of AIHA, AHA, and PRCA—encompassing hematologic autoimmune manifestations attributed in the literature to antibody-mediated (AIHA, AHA) and predominantly T-cell-associated (PRCA) mechanisms—in a single patient with SLE has not been previously reported. We report a patient with SLE who developed AIHA, AHA, and PRCA in succession over 14 years, followed by lupus nephritis and describe this sequence in the context of the heterogeneous, multi-layered immune dysregulation that characterizes SLE.

In March 2012, a 22-year-old man presented with jaundice, fatigue, and polyarthralgia; his medical, family, and psychosocial histories were unremarkable. Investigations showed leukopenia ($2.2 \times 10^9/L$), anemia (hemoglobin 79 g/L), reticulocytosis ($106 \times 10^9/L$), elevated total and indirect bilirubin (31.4 and $21.5 \mu\text{mol/L}$), mildly elevated lactate dehydrogenase (278.0 U/L), and positive direct and indirect Coombs tests; haptoglobin, peripheral blood smear, and direct antiglobulin test subtyping (IgG/C3) were not performed. Antinuclear antibody was 1:320; anti-SS-A, anti-Ro-52, and anticardiolipin antibodies were positive; and complement C3 was low. SLE with concurrent AIHA was diagnosed per the 2012 SLICC criteria. High-dose glucocorticoids and hydroxychloroquine induced remission, and glucocorticoids were tapered off by November 2014.

In January 2016, he was admitted with 3 days of gross hematuria and a painful, ecchymotic swelling of the left upper arm. The activated partial thromboplastin time was markedly prolonged (116.3 s), factor VIII activity was 4.4%, and the factor VIII inhibitor titer was 19.2 Bethesda units/mL, confirming AHA; lupus anticoagulant and antiphospholipid antibodies were now negative. Bleeding resolved within 3 days with intravenous methylprednisolone (80 mg/day), fresh frozen plasma, and cryoprecipitate, and oral cyclophosphamide (100 mg/day) was added with gradual improvement. In July 2017, cyclophosphamide was switched to tacrolimus because of thrombocytopenia ($35 \times 10^9/L$), which resolved after the glucocorticoid dose was increased.

On February 22, 2018, he presented with 7 days of exertional dyspnea, and pallor was the only physical finding. Hemoglobin was 70 g/L with normal platelets and a markedly reduced reticulocyte count ($4.60 \times 10^9/L$); the Coombs test was negative and serum erythropoietin exceeded 762 mIU/mL. Bone marrow examination showed erythroid hypoplasia with giant pronormoblasts, consistent with PRCA (Figure 1). Secondary causes were excluded: serology for Epstein-Barr virus, cytomegalovirus, hepatitis B and C, HIV, Toxoplasma, rubella, herpes simplex virus, and *Treponema pallidum* was negative or unremarkable (isolated HBsAb positivity, consistent with prior immunization); chest computed tomography showed no thymoma; marrow cytology revealed no atypical cells or tumor/lymphoma infiltration; ferritin, folate, and vitamin B12 were normal; and there was no exposure to drugs known to cause PRCA. Parvovirus B19 testing was not performed at the time of PRCA diagnosis. Flow cytometry showed lymphopenia ($0.79 \times 10^9/L$) with a reduced CD4/CD8 ratio (0.46): CD4⁺ T cells were decreased both

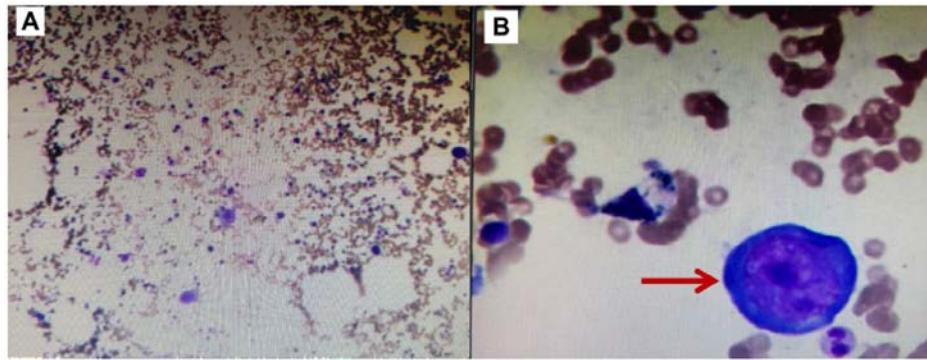


FIGURE 1 | Bone marrow aspirate smear. (A) Low-power view ($\times 200$) showing erythroid hypoplasia with scattered erythroid cells (Wright-Giemsa stain). (B) High-power view ($\times 1000$) showing a giant pronormoblast (arrow); erythroid precursors at other stages are reduced (Wright-Giemsa stain).

proportionally (26.5%) and in absolute terms ($0.21 \times 10^9/L$), whereas the $CD8^+$ T-cell fraction was 57.6% (absolute count $0.45 \times 10^9/L$); $CD3^+$ T cells accounted for 87.08% ($0.69 \times 10^9/L$). He received high-dose methylprednisolone (240 mg/day, tapered by response), cyclosporine A (150 mg twice daily), intravenous immunoglobulin, and red-cell transfusions, with improvement, and tacrolimus was discontinued. In October 2019, bilateral avascular necrosis of the femoral heads prompted glucocorticoid withdrawal, and cyclosporine A was continued.

In March 2021, proteinuria developed and renal biopsy showed Class IV + V lupus nephritis (LN). Because the initial regimen (prednisone, tacrolimus, and cyclophosphamide) yielded an inadequate response, telitacept (80 mg subcutaneously once weekly, combined with prednisone and tacrolimus) was initiated in March 2022 and maintained through May 2026; 24-h urinary protein decreased from 1 g/24 h to below 0.5 g/24 h from March 2024 onward. As of May 2026, no further hematologic episodes had occurred, and the patient remained asymptomatic with a satisfactory quality of life. The full longitudinal course is summarized in Table 1.

The pathogenesis of SLE involves multilayered immune dysregulation—including epigenetic changes, Tfh/Treg imbalance, and aberrant B-cell activation [5]—that produces highly heterogeneous manifestations ranging from single-organ to multisystem disease. The sequential development of AIHA, AHA, and PRCA in this patient may reflect how polyclonal immune dysregulation in SLE can affect different hematopoietic compartments, possibly through distinct mechanisms.

The patient first developed AIHA, which occurs in 3%–14% of adults with SLE [2]. In our patient, total and indirect bilirubin levels were elevated and lactate dehydrogenase was mildly elevated. This predominantly indirect hyperbilirubinemia, together with the elevated LDH, is compatible with hemolysis and supports the diagnosis of AIHA. AIHA is mediated chiefly by IgG autoantibodies against erythrocyte antigens such as Band 3, glycophorin A, and Rh antigens [6, 7], causing hemolysis through Fc receptor-mediated opsonophagocytosis and complement activation [8]. Complement activation in SLE-associated AIHA persists into remission and is modulated by IgG subclass and complement regulatory proteins [5, 8]. Glucocorticoids remain first-line, with response rates of about 70%–85% [9]; rituximab is

effective for refractory or relapsed disease [10]. Our patient improved markedly with glucocorticoids and hydroxychloroquine alone, without additional immunosuppression.

The emergence of AHA 4 years after AIHA remission may reflect broadening of autoimmune targeting beyond erythrocyte-surface antigens to circulating coagulation factors. SLE-associated AHA is rare and arises from loss of tolerance with production of neutralizing autoantibodies, most often against factor VIII [3, 5]; these inhibitors disrupt the intrinsic coagulation cascade and cause potentially life-threatening bleeding [3]. For inhibitor eradication, glucocorticoids plus cyclophosphamide achieve higher response rates than glucocorticoids alone [11], as in our patient. At the time of the bleeding episode in 2016, bypassing agents (activated prothrombin complex concentrate and recombinant activated factor VII) and factor VIII concentrates were not available at our center, and the patient's financial circumstances also precluded these costly therapies; fresh frozen plasma and cryoprecipitate therefore served as an accessible source of factor VIII while immunosuppressive therapy was being established. Hemostatic management has since evolved substantially: the European Acquired Hemophilia Registry (EACH2) showed that bypassing agents are now recommended as first-line therapy for acute bleeding [12]. More recently, emicizumab, a bispecific monoclonal antibody that mimics the cofactor function of activated factor VIII, has emerged as an option: the 2023 GTH-AHA-EMI study found that prophylactic emicizumab markedly reduced bleeding [13], and real-world data showed that 87.1% of patients remained free of breakthrough bleeding and discontinued bypassing agents early [14]. Emicizumab represents a potential contemporary therapeutic option for AHA, particularly in selected patients with significant or recurrent bleeding. Treatment decisions should be individualized based on bleeding severity, inhibitor titer, drug availability, thrombosis risk, and local protocols.

Despite control of AHA, immune dysregulation persisted and culminated in PRCA in 2018. SLE-associated PRCA is primarily T-cell-mediated: T lymphocytes from affected patients suppress erythropoiesis in vitro [4]. Giannouli et al. [15] reported that interferon- γ and tumor necrosis factor- α enhance Fas-induced apoptosis and antagonize erythropoietin (EPO) signaling. In our patient, the proportionally elevated $CD8^+$ T-cell fraction and the therapeutic response to cyclosporine A are consistent with, but

TABLE 1 | Clinical course, key laboratory findings, treatment, and outcomes (2012–2026).

Date	Clinical event	Key laboratory findings	Diagnosis	Treatment	Outcome
Mar 2012	Jaundice, fatigue, polyarthralgia	WBC $2.2 \times 10^9/L$; Hb $79 g/L$; Ret $106 \times 10^9/L$; TBil 31.4 and IBil $21.5 \mu mol/L$; LDH $278.0 U/L$; direct and indirect Coombs tests positive; ANA $1:320$; anti-SS-A, anti-Ro-52, aCL positive; C3 decreased; haptoglobin, blood smear, and DAT subtyping not performed	SLE with AIHA (2012 SLICC criteria)	High-dose glucocorticoids + hydroxychloroquine	Remission; glucocorticoids tapered off by Nov 2014
Jan 2016	Gross hematuria; painful echymotic swelling of the left upper arm	APTT $116.3 s$; FVIII:C 4.4% ; FVIII inhibitor $19.2 BU/mL$; LA and aPL negative	AHA	IV methylprednisolone $80 mg/day$ + FFP + cryoprecipitate (bypassing agents and FVIII concentrates unavailable at our center); oral cyclophosphamide $100 mg/day$ added	Bleeding resolved within 3 days; gradual improvement
Jul 2017	Thrombocytopenia during cyclophosphamide therapy	Platelets $35 \times 10^9/L$	Drug-associated thrombocytopenia	Cyclophosphamide switched to tacrolimus; glucocorticoid dose increased	Platelet count recovered
Feb 2018	Exertional dyspnea for 7 days; pallor	Hb $70 g/L$; Ret $4.60 \times 10^9/L$; Coombs negative; EPO $> 762 mIU/mL$; marrow: erythroid hypoplasia with giant pronormoblasts; lymphopenia $0.79 \times 10^9/L$; CD4/CD8 ratio 0.46 (CD4 $^+$ $0.21 \times 10^9/L$, decreased; CD8 $^+$ $0.45 \times 10^9/L$) ^a	PRCA	IV methylprednisolone $240 mg/day$ (tapered by response) + cyclosporine A $150 mg$ twice daily + IVIG + red-cell transfusion; tacrolimus discontinued	Anemia improved
Oct 2019	Bilateral hip pain	Imaging-confirmed bilateral osteonecrosis of the femoral heads	Glucocorticoid-associated osteonecrosis	Glucocorticoids withdrawn; cyclosporine A continued	Stable
Mar 2021	Proteinuria	24-h urinary protein $1 g/24 h$; renal biopsy: Class IV + V lupus nephritis	Lupus nephritis (Class IV + V)	Induction with prednisone + tacrolimus + cyclophosphamide	Suboptimal disease control
Mar 2022	Persistent disease activity	—	—	Telitacicept initiated at $80 mg$ subcutaneously once weekly, combined with prednisone + tacrolimus	24-h urinary protein consistently $< 0.5 g/24 h$ from Mar 2024 onward
May 2026	Follow-up	No further hematologic episodes	Stable disease	Maintenance therapy (telitacicept $80 mg$ weekly + prednisone + tacrolimus)	Asymptomatic; satisfactory quality of life

Abbreviations: aCL, anticardiolipin antibodies; AHA, acquired hemophilia A; AIHA, autoimmune hemolytic anemia; ANA, antinuclear antibody; aPL, antiphospholipid antibodies; APTT, activated partial thromboplastin time; BU, Bethesda units; DAT, direct antiglobulin test; EPO, erythropoietin; FFP, fresh frozen plasma; FVIII:C, factor VIII activity; Hb, hemoglobin; IBil, indirect bilirubin; IV, intravenous; IVIG, intravenous immunoglobulin; LA, lupus anticoagulant; LDH, lactate dehydrogenase; NK, natural killer; PRCA, pure red cell aplasia; Ret, reticulocyte count; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics; TBil, total bilirubin; WBC, white blood cell count.

^aSecondary causes of PRCA were excluded: EBV, CMV, HBV, HCV, and HIV infections; thymoma (chest CT, February 27, 2018); malignancy/lymphoproliferative disease (marrow cytology); nutritional deficiencies (ferritin, folate, and vitamin B12 normal); and culprit-drug exposure. Parvovirus B19 testing was not performed.

do not by themselves establish, a predominantly cell-mediated process. A recent immune-repertoire study found that PRCA is characterized by T-cell receptor abnormalities and AIHA by B-cell receptor disturbances [16]; that cohort, however, consisted of patients with primary PRCA and AIHA without SLE, and whether such mechanisms account for the sequence observed in our patient remains a conceptual interpretation that cannot be established from a single case. PRCA is not monolithic, however, and humoral injury may coexist: anti-EPO antibodies can neutralize endogenous EPO and impair erythropoiesis, reported in 15%–21% of patients with SLE [17]; these were not measured in our patient, so humoral mechanisms cannot be excluded. Parvovirus B19 testing was likewise not performed at the time of PRCA diagnosis, so B19-associated erythroid suppression cannot be completely excluded, although the absence of fever, rash, arthralgia, or respiratory symptoms at presentation argues against an acute B19 infection. Cyclosporine A is first-line for PRCA, with a response rate of up to 75%; combined with glucocorticoids, it resolved the anemia in our patient, and this treatment response is consistent with—though it does not prove—an immune-mediated mechanism.

Each lineage-specific attack was transiently controlled, but durable immune homeostasis was not restored, allowing new target-organ damage. SLE-associated AHA [18, 19] and SLE cases in which AIHA was followed by or coincided with PRCA [20] have each been reported; the sequential occurrence of all three conditions in a single patient with SLE, however, has not been previously reported.

In summary, the sequential development of AIHA, AHA, and PRCA in this patient with SLE has not been previously reported. Although conventional immunosuppression achieved remission of each hematologic complication, the underlying immune dysregulation persisted and eventually involved the kidney. This case suggests that immune dysregulation in SLE may sequentially target different hematopoietic lineages and even other organ systems over the entire disease course. Therefore, long-term, dynamic hematologic and multisystem surveillance is essential in patients with SLE.

Author Contributions

Xingjie Dong collected the clinical data, performed the literature review, and drafted the manuscript. Luying Gong assisted with data collection and contributed to manuscript revision. Shujie Li and Chunfeng Hou supervised the clinical management, conceived the study, critically revised the manuscript for important intellectual content, and approved the final version. All authors read and approved the final manuscript.

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The authors have nothing to report.

Consent

Written informed consent was obtained from the patient for publication of this case report and the accompanying images.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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LETTER TO THE EDITOR

High Prevalence of Dysglycemia and Sleep-Disordered Breathing Risk in Inflammatory Back Pain A Cross-Sectional Study of 500 Patients With Inflammatory Back Pain Without Classifiable Axial Spondyloarthritis

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Dear Editor,

Inflammatory back pain (IBP), the hallmark of axial spondyloarthritis (axSpA) [1, 2], is frequently encountered in orthopedic and rheumatology practice, yet metabolic comorbidities in this population remain poorly characterized. Dysglycemia and obstructive sleep apnea (OSA) have each been associated with systemic inflammation involving pathways such as TNF- α , IL-6, and endoplasmic reticulum stress [3, 4]; whether such pathways could contribute to enthesal disease is speculative and was not examined in this study. We report a cross-sectional study of 500 strictly defined IBP patients with a contemporaneous trauma control group, systematically screening both conditions. Although IBP is the cardinal symptom of axSpA, not all patients presenting with IBP fulfill classification criteria for axSpA, and metabolic comorbidities in patients with IBP who lack a classifiable axSpA diagnosis (hereafter “non-SpA IBP”, an operational label that does not imply that axSpA has been definitively excluded) remain largely unexplored.

Five hundred consecutive IBP patients and 85 consecutive trauma patients (controls) were enrolled at SKIMS Medical College, Srinagar (January 2024–October 2025; IEC protocol #283/2025). Our hospital is located on a national highway and receives patients from across Kashmir and from Jammu; patients in both groups presented directly, without referral. IBP patients were recruited from the orthopedic outpatient department, which includes an orthopedic-rheumatology service and sees a large volume of patients with osteoarthritis and low back ache; patients

presenting there with low back ache were screened for IBP, and those meeting the IBP criteria were enrolled. Controls were consecutive trauma patients presenting to our hospital during the same period with ankle, forearm, wrist, calcaneal, olecranon, patellar or clavicle fractures, or ankle sprain; patients with compartment syndrome were excluded. They were assessed against the IBP criteria with reference to the period before the trauma, and the eight who met these criteria were excluded, leaving 85 controls for analysis. Neither group was randomized or matched, and the same exclusion criteria were applied to both groups (see below). Inclusion required fulfillment of at least two of the three validated IBP criteria sets (Calin, Berlin and ASAS) and ≥ 2 MRI-confirmed corner inflammatory lesions. MRI scans were interpreted independently by two radiologists at our institution, each blinded to the other's reading and to the clinical findings; where readings differed, the lower score was taken, and agreement between the readers was near complete (kappa 0.91). Sacroiliac joint involvement was assessed using the ASAS MRI definition [5]: active inflammatory sacroiliitis required clear bone marrow edema (BME) or osteitis in typical subchondral or periarticular bone marrow locations; a single BME lesion had to be visible on at least two consecutive slices, whereas multiple BME lesions on a single slice sufficed. A corner inflammatory lesion (bone marrow edema at an anterior or posterior vertebral corner, as described by the ASAS/OMERACT MRI study group [6]) was counted as genuine only when it was present on three sagittal sections, and inclusion required two or more such lesions. Definition of the non-SpA IBP population: all 500 patients underwent sacroiliac (SI) joint

MRI and HLA-B27 testing as part of the diagnostic workup, and were formally assessed against the ASAS axSpA classification criteria [7]; patients with sacroiliitis on MRI, or any HLA-B27 positivity, were excluded. The cohort therefore comprises patients who were HLA-B27 negative, had no sacroiliitis on MRI and did not fulfill the ASAS axSpA classification criteria at the time of assessment; axSpA was not definitively excluded, and very early or pre-classification axSpA may have been present in some patients, particularly as spinal corner inflammatory lesions can themselves occur in axSpA [6]. Exclusion criteria for both groups included ESR > 25 mm/h or CRP > 6 mg/L, known diabetes or OSA, and current corticosteroid/NSAID/DMARD use; no patient in either group was receiving corticosteroids, NSAIDs or disease-modifying agents at enrolment. Dysglycemia was defined by HbA1c $\geq 5.7\%$ (ADA criteria) [8] and sleep-disordered breathing risk by a STOP-Bang score ≥ 3 (score range 0–8) [9], hereafter described consistently as STOP-Bang-defined high SDB risk; for the gradient analysis, scores were categorized as low (0–2), intermediate (3–4) and high (5–8) risk [9]. In a BMI-free sensitivity analysis, the BMI item was removed, giving a modified score of 0–7; the ≥ 3 cutoff was retained, and low-, intermediate- and high-risk categories were defined as 0–2, 3–4 and 5–7. Alternative STOP-Bang scoring models have been evaluated against polysomnography in surgical patients [10], but we are not aware of published validation of a BMI-free version of the summed score, and the modified score and its cutoffs have not been independently validated here. As an internal consistency check, we compared the BMI-free score with the four-item STOP score (high risk ≥ 2) [11] in the IBP group: the two scores were strongly correlated (Spearman $\rho = 0.88$), high-risk classification agreed in 95.2% of patients ($\kappa = 0.85$), and dysglycemia was more prevalent with a STOP score ≥ 2 than < 2 (79.0% vs. 64.5%; $p = 0.002$), with a similar gradient across STOP scores (χ^2 for trend = 14.96; $p < 0.001$) (Table S5). Because the two scores share the four STOP items, this is a consistency check rather than independent validation. This analysis is nonetheless exploratory and is presented as an unvalidated sensitivity analysis. A subset of 129 IBP patients—all of whom had a STOP-Bang score ≥ 3 and were therefore already at higher prior risk of OSA—underwent polysomnography (PSG). The OSA confirmation rate in this selected high-risk subset therefore represents the positive predictive value of the STOP-Bang ≥ 3 threshold in this population, not the prevalence of OSA in the IBP cohort as a whole. Proportions were compared with chi-square tests, continuous variables with two-sample *t*-tests, and between-group associations were additionally examined in multivariable logistic regression adjusting for age, sex and BMI, with crude and adjusted odds ratios (OR/aOR) and 95% confidence intervals.

The IBP group was older (mean age 48.8 ± 11.5 vs. 41.6 ± 17.0 years) and heavier (BMI 28.8 ± 4.1 vs. 26.6 ± 8.9 kg/m²), but had a lower proportion of men (35.4% vs. 58.8%) than the control group because the groups differed on these characteristics, the adjusted analyses were performed. Missing data were minimal: BMI was unavailable for 10 of 500 IBP patients (2.0%) and sex was unrecorded for 1 of 85 controls; no participant had missing age, HbA1c or STOP-Bang score. Complete-case multivariable models therefore included 490 IBP patients and 84 controls ($n = 574$). Only 12 IBP patients (2.4%) and 4 controls had mild ESR/CRP elevations, all below exclusion thresholds.

The prevalence of dysglycemia was significantly higher in IBP patients: 75.8% (379/500) vs. 40.0% (34/85) in controls ($p < 0.001$), including prediabetes (54.8% vs. 34.1% [29/85]) and previously undiagnosed diabetes (21.0% vs. 5.9% [5/85]). STOP-Bang-defined high SDB risk was also significantly more prevalent: 82.8% (414/500) vs. 28.2% (24/85) ($p < 0.001$). After adjustment for age, sex, and BMI, IBP remained independently associated with dysglycemia (aOR 3.37, 95% CI 1.96–5.77; $p < 0.001$) and with STOP-Bang-defined high SDB risk (aOR 14.49, 95% CI 7.36–28.53; $p < 0.001$). We caution, however, that the STOP-Bang score itself incorporates age, sex, BMI, and blood-pressure-related items, so the adjusted OR quantifies the association between IBP status and STOP-Bang-defined SDB risk independent of these covariates and should not be interpreted as an independent increase in objectively measured OSA risk; the exploratory BMI-free sensitivity analysis (aOR 15.57, 95% CI 7.92–30.60) mitigates, but does not eliminate, this overlap. PSG confirmed OSA in 108/129 high-risk patients tested (83.7%). Key findings are summarized in Table 1 (see Tables S1–S5).

A gradient of STOP-Bang-defined SDB risk category was associated with dysglycemia prevalence in IBP patients: 60.5% (52/86) in low-risk, 76.7% (217/283) in intermediate-risk, and 84.0% (110/131) in high-risk patients (χ^2 for trend = 14.61; $p < 0.001$). In the control group, prevalence varied across strata (31.1%, 64.7%, 57.1%) but did not rise monotonically; although the linear-trend test was nominally significant (χ^2 for trend = 5.39; $p = 0.020$), we do not interpret the control-group pattern as a dose–response relationship. The IBP gradient persisted when the BMI item was removed from the score in the exploratory analysis ($n = 499$; 61.2% $\rightarrow$ 77.4% $\rightarrow$ 82.9%; χ^2 for trend = 11.73; $p = 0.001$; Table S5), which suggests, but does not establish, that the relationship is not simply an artifact of shared anthropometric variables. Comorbidity data are shown in Table S3.

TABLE 1 | Prevalence of dysglycemia and STOP-bang-defined SDB risk in IBP vs. comparison group (see Tables S1–S5).

Finding	IBP ($n = 500$)	Comparison group ($n = 85$)	<i>p</i>
Dysglycemia (HbA1c $\geq 5.7\%$), <i>n</i> (%)	379 (75.8)	34 (40.0)	<0.001
Prediabetes (HbA1c 5.7%–6.4%), <i>n</i> (%)	274 (54.8)	29 (34.1)	—
Undiagnosed diabetes (HbA1c $\geq 6.5\%$), <i>n</i> (%)	105 (21.0)	5 (5.9)	—
STOP-Bang-defined high SDB risk (≥ 3), <i>n</i> (%)	414 (82.8)	24 (28.2)	<0.001
PSG-confirmed OSA (high-risk subset), <i>n</i> (%)	108/129 (83.7)	—	—

Note: High SDB risk denotes STOP-Bang-defined risk, not polysomnography-confirmed OSA. Abbreviations: IBP, inflammatory back pain; OSA, obstructive sleep apnea; PSG, polysomnography; SDB, sleep-disordered breathing.

These findings—over three-quarters with dysglycemia and four-fifths at elevated STOP-Bang-defined SDB risk in a single-center cohort of patients with IBP without classifiable axSpA, compared with a contemporaneous trauma control group—indicate an association between IBP and a substantial metabolic burden that is unlikely to be explained by steroid/NSAID use or overt systemic inflammation, since patients with these factors were excluded by design rather than by statistical demonstration; the excess persisted after adjustment for age, sex, and BMI. The 21% rate of previously undiagnosed diabetes supports further evaluation of targeted HbA1c screening strategies in this population. Because this was a cross-sectional study, our

data show association rather than causation; prospective and interventional studies are needed to clarify whether treatment of dysglycemia or OSA influences IBP outcomes. Additional limitations include the single-center design; the use of trauma patients as controls, who may differ from IBP patients in lifestyle, physical activity, smoking, socioeconomic, and other metabolic characteristics not captured by adjustment for age, sex, and BMI, so residual confounding cannot be excluded; the use of PSG only in a selected high-risk subset; the exploratory, unvalidated nature of the BMI-free STOP-Bang analysis, which tests the robustness of the association rather than screening accuracy and may perform less well than the full score because BMI was

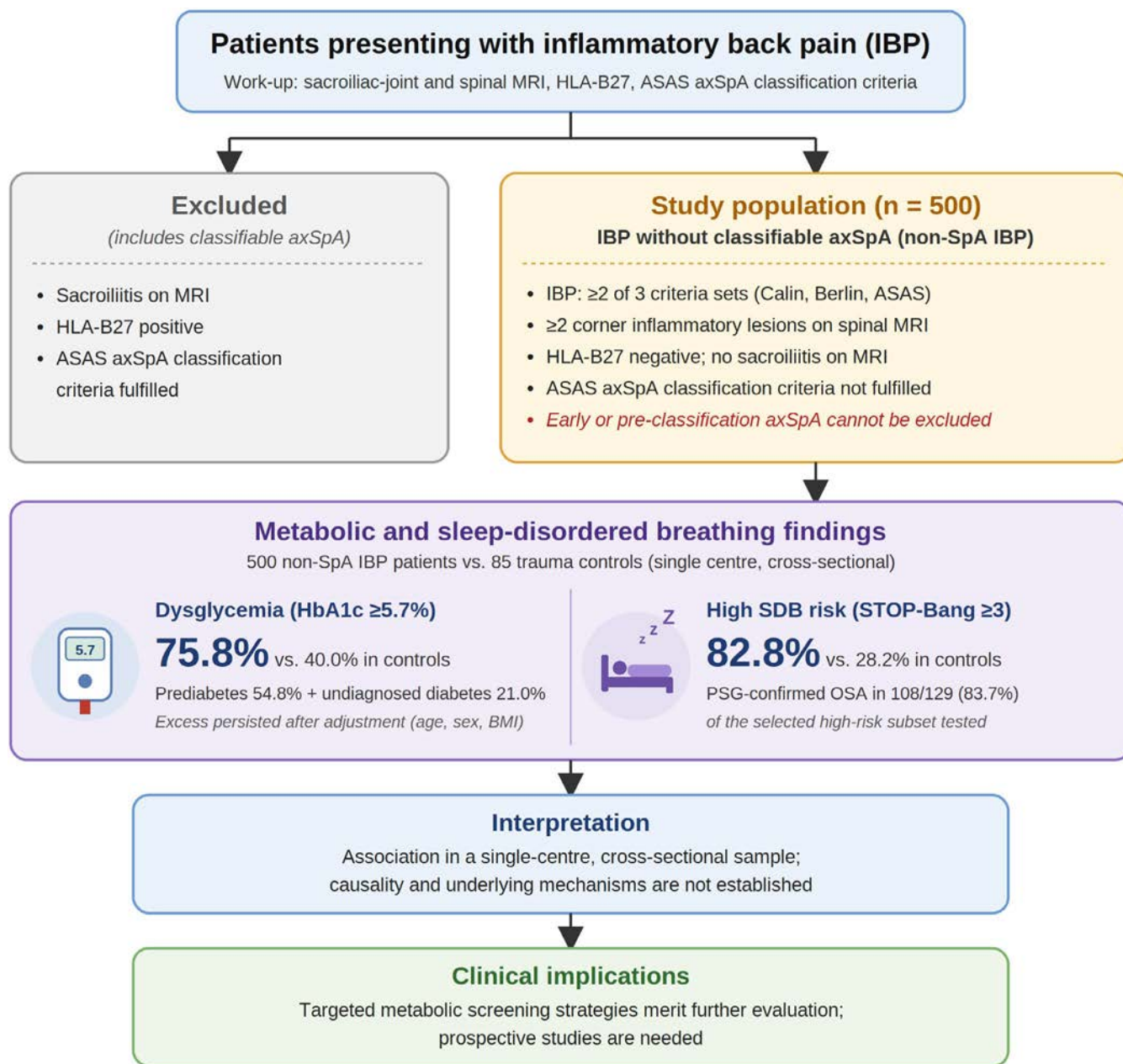


FIGURE 1 | Conceptual framework of inflammatory back pain (IBP) without classifiable axial spondyloarthritis (non-SpA IBP) as defined in this study. Patients presenting with IBP underwent sacroiliac-joint MRI, HLA-B27 testing and assessment against the ASAS classification criteria; those with sacroiliitis on MRI or HLA-B27 positivity were excluded. The remaining patients did not have a classifiable axSpA diagnosis at the time of assessment, and early or pre-classification axSpA cannot be excluded. In this single-center, cross-sectional sample, dysglycemia and STOP-Bang-defined sleep-disordered breathing risk were more prevalent than in trauma controls; these findings are associations, and targeted metabolic screening strategies merit further evaluation.

among the more predictive of the “Bang” items in a surgical cohort [10]; and the possibility of early or pre-classification axSpA in some patients. Our conclusions are accordingly limited to associations within this single-center sample.

We hope these findings prompt broader metabolic awareness in the management of inflammatory back pain (Figure 1).

Author Contributions

S.A.D., S.F., A.M., T.A.D., A.Q. conceived, researched, collected the data, did the statistical analysis, wrote and revised the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Baseline characteristics. **Table S2:** Adjusted associations (IBP vs. control). **Table S3:** Recorded comorbidities (controls). **Table S4:** Missing data by group and variable. **Table S5:** Dysglycemia across STOP-Bang, BMI-free STOP-Bang and STOP score categories, and agreement between the BMI-free and STOP scores (IBP group).